RECENT ADVANCES IN UNDERSTANDING THE ROLE OF MATRIX METALLOPROTEINASES IN PULMONARY ARTERIAL HYPERTENSION: A REVIEW

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ABSTRACT :

Pulmonary arterial hypertension (PAH) is a multifactorial life-threatening disease, characterized by high pulmonary artery pressure, with an ultimate right heart failure. Without treatment, death occurs within 3 years of diagnosis. PAH is characterized by abnormal remodelling of small, peripheral resistance vessels in the lung involving proliferation and migration of vascular smooth muscle, endothelial cell and fibroblasts. Current therapeutic approaches in controlling PAH largely depend on symptomatic relief while the prognosis rate is lower due to the lack of specific molecular targets and the involvement of several factors in the development of PAH. Several studies have indicated direct involvement of matrix metalloproteinase (MMPs) during development and disease progression and associated matrix remodelling in vasculature. PAH can be present in an idiopathic form, usually called idiopathic pulmonary arterial hypertension (IPAH), viral infections, portal hypertension with or without cirrhosis, and anorectic drug ingestion. Increased MMP activity has been demonstrated in experimental animal models of PAH, and MMP inhibition has been shown to either attenuate or enhance vascular remodelling. In the present article recent advancement in the areas of MMPs in progressive PAH has been reviewed with possible therapeutic intervention for MMP inhibition. The present review also addresses the impact of therapeutic strategies on achieving possible PAH reversals and scopes for future research.

Key words: Pulmonary Arterial Hypertension, Matrix metalloproteinases, Tissue inhibitor of matrix metalloproteinases, Therapeutic interventions

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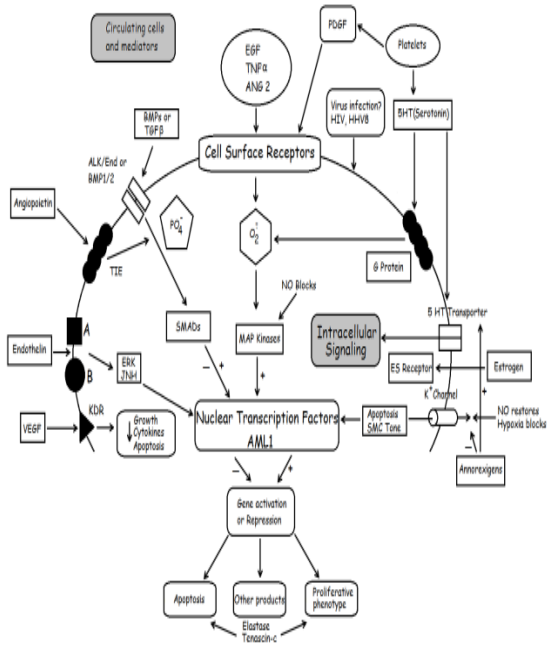
Introduction:

Pulmonary arterial hypertension (PAH) is a debilitating and life-threatening disease, often affecting young people. PAH is marked by a persistent elevation of pulmonary artery pressure with normal pressure in the left ventricle (Lykouras et al., 2008; Raiesdana & Loscalzo, 2006). An elevation of mean pulmonary arterial pressure above 25 mmHg at rest or 30 mmHg with exercise is considered diagnostic for PAH. PAH is characterized by persistent vasoconstriction, in situ thrombosis, intimal lesions and extracellular matrix (ECM) remodeling, inducing increased pulmonary arterial pressure (Boutet et al., 2008; Davies & Morrell, 2008; George, Sun, & Jeanine, 2012). Figure 1 summarizes some of the cellular processes implicated in the pathogenesis of PAH.

Matrix metalloproteinases (MMPs) are matrix-degrading enzymes involved in ECM turnover in smooth muscle cell (SMC) and endothelial cell migration and proliferation.

Figure 1



Tissue inhibitor of metalloproteinases (TIMPs) is the endogenous inhibitors of MMPs. MMP expression and activity are found to be increased in experimental PAH (Lepetit et al., 2005). The MMP–TIMP imbalance may lead to matrix accumulation, and increased MMP activity may contribute to

SMC migration and proliferation. The process of vascular remodeling can occur as a primary response to injury, or stimulus such as hypoxia, within the resistance vessels of the lung. Alternatively, the changes seen in more proximal vessels may arise secondary to a sustained increase in intravascular pressure. To withstand the chronic increase in intraluminal pressure, the vessel wall becomes thickened and stronger. This "armouring" of the vessel wall with extra-smooth muscle and extracellular matrix leads to a decrease in lumen diameter and reduced capacity for vasodilatation (Jeffery & Morrell, 2002).

Homoeostatic imbalances between vasodilators and vasoconstrictors, growth inhibitors and mitogenic factors and antithrombotic and prothrombotic determinants are the underlying pathogenetic mechanisms associated with PAH (Rabinovitch, 2008; Tuder, Marecki, Richter, Fijalkowska, & Flores, 2007). The molecular mechanisms regulating collagen deposition during pathogenesis of PAH has been poorly understood (George, Sun, & Jeanine, 2012). This maladaptive response results in increased pulmonary vascular resistance and consequently, sustained pulmonary hypertension. The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by the finding that cellular heterogeneity exists within the traditional compartments of the vascular wall: intima, media, and adventitia. In addition, the developmental stage of the organism greatly modifies the response of the pulmonary circulation to injury (Jeffery & Morrell, 2002).

Matrix Deposition & Role of Matrix Metalloproeinases (MMPs)

Small changes in the rate of collagen synthesis and metabolism was reported to cause a marked alteration in collagen content and elastin and collagen deposition are increased during the development of PAH (McKenzie, Clancy, & Klein, 1984). Endothelial cells in the microvasculature secrete type IV collagen and elastin. In larger arteries, smooth muscle cells and fibroblasts produce collagen and elastin within the media and adventitia, respectively. This appears to be in response to increased wall tension because isolated stretched vessels also do this (Tozzi, Poiani, Harangozo, Boyd, & Riley, 1989). The degradation of matrix proteins is in large part caused by the activity of matrix metalloproteinases (collagenases and elastases), the activity of which is in turn regulated by tissue inhibitors of matrix metalloproteinases (TIMPs) (Jeffery & Morrell, 2002).

MMPs are a family of proteases that have implications in cell migration and regulating the composition and the content of extracellular matrix (ECM). Members of this family are secreted in a latent form and are cleaved by other proteases to active forms. Several MMPs (including MMP-1, -2, -3, and -9) are produced by vascular smooth muscle cells in the arterial wall (Jeffery & Morrell, 2002). The activities of MMPs in vivo are thought to be counterbalanced by their common inhibitors, TIMPs. It has been demonstrated that MMPs play important roles in the degradation of extracellular matrix macromolecules associated with tissue destruction in pulmonary vascular diseases (Fujimoto, Tokai, Iwata, Okada, & Hayakawa, 1995). In both monocrotaline (MCT) and hypoxic models of PAH, MMP-2 activity is increased (Frisdal et al., 2001) probably as a result of involvement in the matrix turnover associated with vascular remodeling during PAH. In addition, MMP activity is increased in rat pulmonary artery after removal from a hypoxic environment, accompanying active resorption of collagen, and resolution of remodeling, suggesting that MMPs may also mediate the breakdown of excess collagen during recovery (Thakker-Varia et al., 1998). Furthermore, inhibition of MMP by TIMP-1 gene transfer or administration of doxycycline (MMP inhibitor) worsened the development of pulmonary hypertension, including increased muscularization and periadventitial collagen accumulation, in rats exposed to hypoxia.219 Inhibition of MMP activity also suppresses tenascin-C expression (Cowan, Jones, & Rabinovitch, 2000 ; Cowan, Jones, & Rabinovitch, 1999).

It is unclear what the cellular and molecular mechanisms are underlying the transition from compensated hypertrophy to dilatation and failure that occurs during the pathogenesis of PAH. Marked increases in MMP-2 and MMP-9 have been observed in pulmonary hypertension (Frisdal et al., 2001; Zaidi, You, Ciura, Husain, & Rabinovitch, 2002). The up-regulation of MMPs in PAH contributes to the degradation of the adventitial extracellular matrix, which promotes proliferation and migration of vascular smooth muscle cells into small peripheral, normally non-muscular, pulmonary arteries within the respiratory acinus (Tajsic & Morrell, 2011). The complete cellular processes underlying the muscularization of the pulmonary arterial tree are not clear. This process is normally accompanied by the deposition of mature collagen fibres surrounding the small pulmonary arteries, which contribute to the constriction of pulmonary vessels and pathogenesis of PAH. The up-regulation of TIMP-1 (Wright, Tai, Wang, Wang, & Churg, 2007) in cigarette-smoke-induced vascular remodeling and pulmonary hypertension could inhibit interstitial collagenases, especially MMP-1, and promote the deposition of mature collagen fibers. It has been demonstrated that transgenic expression of MMP-1 inhibits myocardial fibrosis and prevents transition to heart failure in a pressure overload mouse model (Foronjy, Sun, Lemaitre, & D'Armiento, 2008).

Medial hypertrophy of pulmonary arterioles during PAH in humans is associated with enhanced proliferation of smooth muscle cells (SMCs). Elevated MMP-2 has been found in pulmonary artery SMCs (PA-SMCs) in humans with IPAH, leading to the hypothesis that MMP-2 contributes to the proliferation and migration of vascular SMCs in the pathogenesis of PAH. Rapidly growing meat-type (broiler) chickens provide a model of spontaneous PAH. Cultured PA-SMCs from normal birds were used to evaluate the effect of MMPs on cell proliferation. Gelatin zymography showed that endothelin (ET)-1-induced proliferation of PA-SMCs was concomitant with increased pro- and active MMP-2 production. Reverse transcription PCR demonstrated up regulation of MMP-2 mRNA. However, PA-SMC proliferation was inhibited by the MMP inhibitors doxycycline and cis-9-octadecenoyl-N-hydroxylamide. In vivo experiments revealed a significant increase of MMP-2 expression in hypertrophied pulmonary arterioles of PAH broiler chickens, which was positively correlated with wall thickness and medial hypertrophy (Cantini-Salignac, Lartaud, Schrijen, Atkinson, & Chabot, 2006). MMP-2 may contribute to medial hypertrophy in pulmonary arterioles during PAH in broiler chickens by enhancing the proliferation of vascular SMCs. This study demonstrated MMP-2 stimulates proliferation of PA-SMCs, supporting the hypothesis that increased expression of MMP-2 contributes to pulmonary vascular remodeling in broiler chickens with PAH. The pro-MMP-9 content of circulating monocytes was lower in the more severe forms of PAH this showed heart failure suggesting that such MMP enzymatic activity reflects heart failure following pulmonary vascular and myocardial remodeling in PAH. Study showed a link between the degree of severity of PAH and pro-MMP-9 content of circulating monocytes in patients with PAH-induced cardiac failure (Cantini-Salignac, Lartaud, Schrijen, Atkinson, & Chabot, 2006).

Aging

Aging is the major risk factor for the development of vascular diseases, such as hypertension and atherosclerosis (Benetos, Laurent, Hoeks, Boutouyrie, & Safar, 1993). Aged aorta has been shown to exhibit a significant increase in intimal width compared with that in young rats which is mainly composed of molecules, including collagen, proteoglycan, and actin. It contains markedly higher levels of MMP-2, fibronectin, TGF-β, and ICAM-1. Recent studies have demonstrated that chemotactic invasion of a reconstituted basement requires MMP-2 activity in smooth muscle cells (Pauly et al., 1994). Additionally, the expression and activity of MT-MMP (MMP-14), MMP-2, and MMP-9 increase during mechanical injury to arteries (Jenkins et al., 1998). MMP-2 has also been shown to be present within

atherosclerotic lesions. The proteolytic activity, conceivably, weaken the fibrous cap, resulting in its rupture (Galis, Sukhova, Lark, & Libby, 1994; Z. Li et al., 1996). Age-associated disorganization of the internal elastic lamina has been observed in the aorta in the absence of externally imposed experimental injury (Zhihe Li, Froehlich, Galis, & Lakatta, 1999). Both MMP-2 and MMP-9 exhibit elastase activity (Senior et al., 1991), as does a metalloelastase of macrophages (Shapiro et al., 1992). It has been demonstrated that both latent and active forms of MMP-2 are greater in the aortas of old than in young rats (Li, Froehlich, Galis, & Lakatta, 1999). A significant amount of MMP-2 activity has been found to be localized to the intima and elastic lamellae. MMP-2 has been shown to accumulate in the area surrounding SMC located just beneath the broken internal elastic lamina and MMP-2 may have roles in the fragmentation of the elastic laminae with aging (Li, Froehlich, Galis, & Lakatta, 1999). Importantly, SMCs are potentially a source of age-associated increase in MMP-2 in the aortic wall in situ, as early passage SMC from aged aorta secrete more MMP than those from young aorta. MMP-2 production in SMCs of aged aorta has been shown to be triggered by stimulation with cytokines, including interleukin-1, TNF-α and TGF-β which suggest that enhanced MMP-2 levels in the thickened intima of aortas from aged rats may reflect a chronically enhanced level of cytokine stimuli in vivo (Li, Froehlich, Galis, & Lakatta, 1999). The novel findings that increased MMP-2, TGF-β, and ICAM-1 levels are chronically elevated and localized to the thickened intima of aged rats not only provide insights into possible mechanisms of age-associated vascular remodeling but also illuminate new links between senescence markers in vitro and in cell senescence in vivo (Mandal, Mandal, Das, Chakraborti, & Chakraborti, 2003). The ability of smooth muscle to activate MMPs under stimulated conditions suggest that they participate in regulating physiological and pathological turnover of ECM molecules in human arterial wall. Such degradation may be one of the key requirements for smooth muscle to migrate from the media to the intima upon stimulation (Yanagi, Sasaguri, Sugama, Morimatsu, & Nagase, 1991). Since the activities of MMPs are inhibited by TIMPs in a 1:1 stoichiometry, the imbalance between MMPs and TIMPs resulting from a decrease in the level of antiprotease(s) has been proposed as one of the events responsible for injury to vascular bed (Fujimoto, Tokai, Iwata, Okada, & Hayakawa, 1995).

Meprin β

The metalloproteinase meprin β is a novel activator protein-1 (AP-1) effector molecule in PAH. Research has shown that Fra-2 transgenic (TG) mice model exhibited increased right ventricular systolic pressure (RVSP), accompanied by vascular remodeling and activation of the pro-proliferative and pro-fibrotic AKT pathway (Biasin et al., 2014). Microarray studies revealed meprin β as the most upregulated gene in Fra-2 TG mice. Its expression, increased at all investigated time points, preceded the decreased expression of MMPs and increased TGFβ, followed by collagen deposition. This study delineates a novel molecular mechanism underlying PASMCs proliferation and ECM deposition by identifying meprin β as an important mediator in regulating vascular remodeling processes. Thus, meprin β, a newer member of MMPs may represent a new molecule that can be targeted in pulmonary hypertension (Biasin et al., 2014).

Bone Morphogenetic Protein Receptors (BMPRs)

Bone morphogenetic protein receptors (BMPRs) are members of the transforming growth factor β superfamily of receptors (de Caestecker, 2004; Mehra & Wrana, 2002). Heteromeric complexes form between BMPR1 and BMPR2 (Gilboa et al., 2000). Aberrant BMP signaling has been linked to PAH. Various germline mutations in BMPR2 have been identified in familial and even sporadic forms of the disease (Deng et al., 2000; Thomson et al., 2001). Moreover, independent of a mutation, expression of BMPR1A (Du et al., 2003) and BMPR2 (Atkinson et al., 2002) is reduced in lungs of PAH patients. The

pathological features associated in PAH account for the progressive increase in pulmonary vascular resistance culminating in right-side heart failure (Humbert et al., 2004; Rubin, 1997). Mice homozygous null for Bmpr2 (Beppu et al., 2000), Bmpr1a (Mishina, Suzuki, Ueno, & Behringer, 1995), the ligand Bmp4 (Winnier, Blessing, Labosky, & Hogan, 1995) and the effector Smad4 (Sirard et al., 1998) die early in embryonic life owing to a lack of mesodermal induction. In mice with Flk1-targeted deletion of Bmpr1a (Flk1-Cre;Bmpr1aflox/flox) (Flk1 is also known Kdr – Mouse Genome Informatics) (Park et al., 2006), lethality occurs between E10.5 and E11.5, in association with massive abdominal hemorrhage. These mice exhibit dilatation of large vessels owing to poor recruitment of VSMCs around the EC layer, but it is not clear whether the vascular phenotype is due to Bmpr1a deficient ECs or SMCs (Park et al., 2006). Defective brain development documented in the SM22α-Cre; R26R; Bmpr1aflox/flox mice was associated with impaired clearing of brain microvessels related to a resistance of pericytes to apoptosis and decreased levels of MMP2. Knock-down of BMPR1A by RNA interference in human pulmonary artery SMCs reduced MMP2 and MMP9 activity, attenuated serum-induced proliferation, and impaired PDGF-BB-directed migration. RNA interference of MMP2 or MMP9 recapitulated these abnormalities, supporting a functional interaction between BMP signaling and MMP expression. In human brain microvascular pericytes, knock-down of BMPR1A reduced MMP2 activity and knock-down of either BMPR1A or MMP2 caused resistance to apoptosis. Thus, loss of Bmpr1a, by decreasing MMP2 and/or MMP9 activity, can account for vascular dilatation and persistence of brain microvessels, leading to the impaired organogenesis documented in the brain (El-Bizri et al., 2008).

Transforming Growth Factor-β1 (TGF-β1)

Up regulation of TGF-β1 was reported in several studies relating PAH. BMP-4 and BMPR2 were also reported (Jonigk et al., 2011) to be significantly up-regulated in concentric lesions in patients with APAH who did not exhibit pulmonary lesions (PLs) compared with other groups. PLs in IPAH and APAH showed only minor differences in remodeling-associated gene regulation: cKIT, HIF1a, MMP9, TGF-β1, and THBS1 were up-regulated in PLs in APAH compared with the adjacent arteries, with a comparable but nonsignificant trend in PLs in IPAH. Hemodynamic variables, such as the height of the mean pulmonary arterial pressure or pulmonary vascular resistance measured before transplantation, had no effect on remodeling-associated gene expression in PLs (Jonigk et al., 2011).

Systemic Sclerosis

Systemic sclerosis (SSc) is an autoimmune disease that can potentially involve all tissues and organs of the human body. Inhibition of Rho-associated kinases (Rock) has been reported to prevent differentiation of resting fibroblasts into myofibroblasts and reduced the synthesis of ECM in vitro by SSc fibroblasts without toxic side effects. Significant quantities of research have additionally shown that inhibitors of Rock exert anti-inflammatory effects and provide beneficial effects in vasospastic disorders. Thus, inhibition of Rock might simultaneously target 3 cardinal features of SSc (Akhmetshina et al., 2008), namely (a) excessive collagen production and deposition, (b) vascular damage, and (c) inflammation or autoimmunity.

Based on the extent of the disease and organ involvement, different subsets of patients have been identified and several classifications proposed aiming to better stratify affected patients. ET-1 has been reported to modulate the activity of proteolytic enzymes, such as MMP-2 and MMP-9, which belong to the family of MMPs (Xu et al., 1998; Yao, Morioka, Li, & Oite, 2001). Both enzymes, secreted in a latent form and activated at the cellular surface by the complex membrane tissue 1 (MT1)-MMP together with the tissue inhibitor of MMP (TIMPs), have a proteolytic activity towards a

number of different extracellular matrix proteins (ECM) (Giannelli & Antonaci, 2002). In particular, TIMP-2 is a more specific inhibitor of MMP-2 while TIMP-1 is more specific for MMP-9. The imbalance between MMPs and TIMPs promoting tissue remodeling in patients with SSc could play a role in determining the fibrogenic events occurring in several different organs as well as in the blood vessels that contribute to induce vascular phenomena, including PAH. Compared with healthy subjects ECM protein turnover is reduced as a result of the high levels of TIMP-1 and TIMP-2 observed in patients with SSc. However, it is increased during bosentan therapy as a result of the up-regulated levels of MMP-9, as previously reported. More studies are needed to clarify the role of MMPs and TIMPs in determining blood vessel damage which severely affects the prognosis and survival of patients with SSc, and to ascertain how bosentan therapy may interfere with the proteolytic remodeling of the vessels (Giannelli, Iannone, Marinosci, Lapadula, & Antonaci, 2006). Recently, a dual inhibitor of ET-1, bosentan, has been successfully evaluated in clinical trials in PAH patients (Gianluigi Giannelli, Iannone, Marinosci, Lapadula, & Antonaci, 2005). This is the first time that MMP-9 serum levels are reported to be down-regulated in PAH patients and up-regulated following bosentan treatment. Whether or not this is a direct or an indirect effect of the therapy is not yet known, but MMP-9 might be useful as an indicator of disease activity and also as a marker of PAH occurrence and of the effectiveness of bosentan (Gianluigi Giannelli, Iannone, Marinosci, Lapadula, & Antonaci, 2005).

In a clinical study (Benisty et al., 2005) it has been found that at least one MMP species was significantly higher in the urine samples of associated PAH (APAH) group (75%) compared to normal control subjects (17%). The detection of MMPs in the urine was highest in IPAH (80%). Four MMP species were highly detected in patients with APAH compared to control subjects: 150- , 140- , 92- (MMP-9), and 72- kDa species (MMP-2). Significant differences in MMP species were observed between the IPAH and APAH-other groups, with MMP-2 observed in 60% of patients with IPAH but only 15% of APAH-other patients. Urinary MMPs are detected in a significantly higher proportion of patients with APAH compared to control subjects and are likely to reflect the pulmonary vascular remodeling process. Urinary MMP species show correlation with disease etiology subtype (Benisty et al., 2005).

Human Herpes Virus-8 (HHV8)

IPAH is associated with human herpes virus 8 (HHV8) infections and demonstrates pathological angiogenesis similar to that observed with another HHV8-linked disease, namely Kaposi Sarcoma (KS) (Shan et al., 2007). According to current line of thinking, vascular remodeling and dysregulated angiogenesis including endothelial cell (EC) proliferation, migration, survival, dysfunction and secretion of angiogenic growth factors and MMPs may lead to pulmonary arterial obstruction thus significantly contributing to the increase of pulmonary vascular resistance seen in PAH (Humbert et al., 2004). HIV infection is known to be associated with the development of PAH and, interestingly, the prevalence of HHV-8 is increased in HIV patients infected with HIV (Antman & Chang, 2000; Cool et al., 2003; Gandhi & Greenblatt, 2002; Mehta, Khan, Mehta, & Sepkowitz, 2000). Most recently, Gutierrez et al reported the case of an HIV-infected patient co-infected with HHV-8 who developed severe PAH coincident with occult KS possibly indicating a relationship between HHV-8 infection and HIV-associated PAH (Gutierrez et al., 2006). The report by Shan et al provided new insights into the mechanisms via which HHV-8 promotes angiogenesis and vascular pathology (Shan et al., 2007). Retrovirally transduced cultured human pulmonary arterial endothelial cells (HPAEC) with HHV-8-encoded viral G protein-coupled receptors (vGPCR) were made and examined the effects on matrix MMP-2 activity, Src kinase activity, and angiogenesis. HHV-8-encoded vGPCR are highly related to the interleukin-8 (IL-8) binding human chemokine receptors CXCR1 and CXCR2 but, unlike these, display ligand

independent constitutive signaling activity (Sodhi, Montaner, & Gutkind, 2004). However, the concept of HHV-8 encoding viral chemokine receptor homologues thereby hijacking intracellular signaling pathways such as Src kinase and MMP-2 resulting in angiogenesis and thus contributing to HHV-8-associated diseases is interesting and merits further investigations.

HHV8 vGPCR induces MT1-MMP-mediated MMP-2 activation in HPAEC. vGPCR achieves such activation through coordinating MT1-MMP and TIMP-2 expression post-transcriptionally. Furthermore, vGPCR activates Src and such activation plays a critical role in proMMP-2 activation and in vitro angiogenesis induced by vGPCR. Recent study has shown vGPCR expression selectively activated matrix metalloproteinase MMP-2. A membrane type 1 MMP (MT1-MMP) neutralizing antibody and the tissue inhibitor of metalloproteinases-2 (TIMP-2) independently blocked vGPCR-induced MMP-2 activation. vGPCR expression concordantly promoted MMP-2 activation by increasing MT1-MMP expression while decreasing TIMP-2 expression. vGPCR activated Src kinase as demonstrated by phosphorylation of Src and its substrate focal adhesion kinase (FAK). vGPCR promoted angiogenesis of HPAECs as demonstrated by a substantial increase in tubulogenesis in vitro. The Src inhibitors PP2 and SU6656 significantly diminished vGPCR-induced MMP-2 activation and tubulogenesis. Recent reseach has indicated that vGPCR induces MMP-2 activation in HPAECs through regulation of MT1-MMP and TIMP-2 expression. vGPCR activates Src and inhibition of such activation abrogates proMMP-2 activation and in vitro angiogenesis induced by vGCPR. The recent advances in this area also reported vGPCR as an etiological agent in IPAH and identified Src and MMP-2 as potential therapeutic targets in HHV8 associated KS and IPAH (Shan et al., 2007).

Transgenic expression of human MMP-1 and MMP-9

MMP-1expressed in mouse macrophages and was examined for its effects in attenuating the decompensating features of monocrotaline (MCT)-induced PAH. Measurement of RV (right ventricular) pressure revealed a 2.5-fold increase after treatment with MCT, which was reduced to 1.5-fold in MMP-1 transgenic mice (George, Sun, & Jeanine, 2012). There was conspicuous pulmonary inflammation with chronic infiltration of mononuclear cells after the administration of MCT, which was significantly diminished in transgenic mice. Furthermore, transgenic mice showed decreased collagen deposition compared with WT (wild-type). Staining for Mac-3 (macrophage-3) and α-SMA (α-smooth muscle actin) revealed extensive infiltration of macrophages and medial hypertrophy of large pulmonary vessels with complete occlusion of small arteries respectively. These changes were markedly reduced in MMP-1 transgenic mice compared with WT. Western blotting for molecules involved in cell multiplication and proliferation depicted a significant decrease in the lung tissue of transgenic mice after the treatment with MCT. This study demonstrated that transgenic expression of human MMP-1 decreased proliferation of smooth muscle cells and prevented excessive deposition of collagen in the pulmonary arterial tree. The results indicated that up-regulation of MMP-1 could attenuate the debilitation of human PAH and provide an option for therapeutic intervention (George, Sun, & Jeanine, 2012).

Interestingly recent study demonstrated that transgenic expression of human MMP-9 increases MCT-induced PAH in experimental mice. The MCT-induced PAH in mouse is a reproducible and potentially valuable animal model for the human disease. The results further demonstrated that MMP-9 plays a significant role in the pathogenesis of PAH and effective blocking of MMP-9 could provide an option in the therapeutic intervention of human PAH. The data further pointed out MMP-9 as an important molecule in the pathogenesis of PAH contributing to the fibrosis and remodelling of pulmonary vessels, and effective blockade of MMP-9 could be a possible potential therapeutic intervention of this chronic disease (George & D'Armiento, 2011).

IL-32 and MMPs

IL-32 is a multifaceted cytokine with a role in infections, autoimmune diseases, and cancer, and it exerts diverse functions, including aggravation of inflammation and inhibition of virus propagation. IL-32 had been previously identified as a critical regulator of endothelial cell (EC) functions, and revealed that IL-32 also possesses angiogenic properties (Nold-Petry et al., 2014). The hyperproliferative ECs of human pulmonary arterial hypertension and glioblastoma multiforme exhibited a markedly increased abundance of IL-32, and, significantly, the cytokine colocalized with integrin αVβ3. Vascular endothelial growth factor (VEGF) receptor blockade has been reported for , EC hyperproliferation and three-fold increase in IL-32 gene expression. Mechanistically, these proangiogenetic activities likely use regulation of IL-8, MMP-9, activin A, and endostatin, but not VEGF or TGF-β1. A second signal is required to render cells responsive to exogenous IL-32, and IL-32-induced angiogenesis is at least in part dependent on the integrin αVβ3. Therefore, IL-32 emerges as a key player in endothelial cell biology at which the pathways of inflammation and angiogenesis converge (Nold-Petry et al., 2014).

COPD, cigarette smoking and emphysema

Several recent studies have explored the prevalence and the functional implications of PAH for patients with chronic obstructive pulmonary diseases (COPD). These highlight the importance of clearly defining pulmonary hypertension that can be quite heterogeneous in this patient population. Furthermore, the concept that pulmonary hypertension in COPD is merely driven by hypoxic vasoconstriction has been called into question by several lines of investigation that suggest a much more complex pathogenesis potentially occurring independently of hypoxemia. Finally, there has been much interest in exploring pulmonary hypertension-specific therapies in patients with COPD, but available data to support their use are limited (Orr et al., 2012).

Cigarette smoke induced COPD and emphysema in relation to matrix remodeling and the role of MMPs had been extensively studied (Churg et al., 2008). In the last 15 years, there has been an increasing interest in MMPs as mediators of emphysema, which involves destruction of the lungs over time. This has stemmed in part from the recognition that a number of MMPS, including MMP-9 and MMP-12 (Parks & Shapiro, 2001), can degrade elastin; in part from reports of increased levels of MMPs including MMP-1, -2, -9, -14 (Finlay et al., 1997; Imai et al., 2001; Ohnishi et al., 1998; Segura-Valdez et al., 2000), and in some studies, MMP-12 (Demedts et al., 2006; Grumelli et al., 2004; Imai et al., 2001; Molet et al., 2005; Woodruff et al., 2005), in BAL fluid, alveolar macrophage supernatant, or whole lung tissue from smokers with emphysema compared with those without; and in particular, from the report (Hautamaki et al., 1997) that mice with a targeted deletion of MMP-12 (MMP-12 (-/-) failed to develop emphysema after cigarette smoke exposure. Cigarette smoke causes increased whole lung or alveolar macrophage levels of MMP-2, -9, -12, -13, and -14 in mice (Churg et al., 2004) and MMP-1 in guinea pigs (Selman et al., 1996). Cigarette smoke directly activates Toll-like receptor-4 (TLR4) and probably other (as yet unidentified) cell surface receptors that drive MMP-12 release. In addition, smoke causes leakage of plasma proteins into the alveolar spaces (Churg et al., 2008). Advanced research in this area had shown considerable amount of evidence that MMP inhibition or deletion can significantly or even totally abrogate the development of emphysema, indicating a clear role for MMPs in this process. Mice lacking MMP-12 are completely protected against emphysema (Hautamaki et al., 1997; Shapiro et al., 2003), whereas mice lacking MMP-9 show no protection at all (Mahadeva & Shapiro 2005. This latter observation is of particular interest, since it has been suggested from studies of cultured alveolar human macrophages that MMP-9 is the major mediator of emphysema in humans (Rubio et al., 2004; Russell et al., 2002), and some have denied a role for MMP-12 in humans (Imai et al., 2001). Selman et al.

(152) found little protection with a broad spectrum MMP inhibitor in guinea pigs, but we (Churg et al., 2006) found ~70% protection in guinea pigs given a combined MMP-9/-12 inhibitor, AZ11557272, indicating that MMPs are not just confined to murine emphysema models, and lending support to the idea that one or both of these MMPs may be central players in humans. Animals exposed to smoke and AZ11557272 had 70% protection against decreases in airflow compared with animals exposed to smoke alone, thus showing that prevention of anatomic changes in animal models confers a corresponding physiological benefit. However, as is true of serine proteases, the exact role of MMPs in the pathogenesis of emphysema is unclear, particularly since neutrophils/serine proteases and MMPS appear to interact, and MMP inhibition reduces smoke-induced neutrophil and macrophage influx (29, 34, 128, 155). In an acute smoke model using MMP-12 (-/-) mice, it had been shown (Churg et al., 2002) that MMP-12 was required for smoke-induced neutrophil influx and neutrophils were required for matrix breakdown. This latter idea was also supported by the finding that the broad spectrum MMP inhibitor RS113456 acutely inhibited neutrophil influx and matrix breakdown (Dhami et al., 2000). But in a 6-mo smoke model, Shapiro et al. (Shapiro et al., 2003) found that MMP-12 (-/-) mice developed a BAL neutrophilia comparable to that of wild type animals, implying that proinflammatory mechanisms change over time; we have seen a similar late neutrophil recruitment effect in animals treated with A1AT and in TNFreceptor (p55 p75-/-) knockout mice (Churg et al., 2003; Chrug et al., 2004). It is of interest in this regard that Stevenson et al. (Stevenson et al., 2007), using gene microarrays, recently reported that gene expression patterns in rats change from an early proinflammatory to a later pattern of enhanced acquired immunity, although these data must be viewed with great caution, since rats are extremely susceptible to (and the Stevenson paper indeed found) the phenomenon known as particle overload (Oberdorster et al., 1995), and particle overload leads to prolonged generation of inflammatory, fibrogenic, and probably mutagenic mediators by alveolar macrophages. Additional evidence for interactions of neutrophils and macrophages comes from Shapiro et al. (Shapiro et al., 2003) who showed that neutrophil elastase activates pro-MMP-12 and destroys tissue inhibitor of metalloprotease (TIMP)-1; conversely, MMP-12 degrades A1AT. Thus neutrophil elastase and MMP-12 cooperate to increase each other's proteolytic potential.

PAH, ROS, Ca2+ and MMPs

Development of PAH-induced right ventricular failure (RVF) is associated with an increased reactive oxygen species (ROS) production. Increase in oxidative stress lead to generation of nitro-tyrosine residues in TIMPs and liberate active MMPs. Research has been performed to test the hypothesis that an imbalance in MMP:TIMP ratio leads to interstitial fibrosis and RVF and whether the treatment with folic acid (FA) alleviates ROS generation, maintains MMP/TIMP balance, and regresses interstitial fibrosis, using a mouse model of pulmonary artery constriction (PAC) (Qipshidze, Tyagi, Metreveli, Lominadze, & Tyagi, 2012). Treatment with FA decreased the pressure to 35 mmHg from 50 mmHg in PAC mice. Similarly, RV volume in PAC mice was increased compared with the sham group. A robust increase of ROS was observed in RV of PAC mice, which was decreased by treatment with FA. The protein level of MMP-2, -9, and -13 was increased in RV of PAC mice in comparison with that in the sham-operated mice, whereas supplementation with FA abolished this effect and mitigated MMPs levels. The protein level of TIMP-4 was decreased in RV of PAC mice compared with the Sham group. Treatment with FA helped PAC mice to improve the level of TIMP-4. These results point that FA treatment improves MMP/TIMP balance and ameliorates mitochondrial dysfunction that results in protection of RV failure during pulmonary hypertension (Qipshidze, Tyagi, Metreveli, Lominadze, & Tyagi, 2012).

Oxidative stress has been implicated in many disease processes including reperfusion injury. In ischemia-reperfusion significant amount of H2O2 is produced (Frears, Zhang, Blake, O'Connell, & Winyard, 1996). Many workers have demonstrated that H2O2 readily oxidizes various biomolecules such as low density lipoproteins. H2O2 can react with the tyrosine residues of proteins to form 3-nitrotyrosine (Frears, Zhang, Blake, O'Connell, & Winyard, 1996). Under sustained pulmonary hypertension, excessive collagen degradation occurs within the wall of the vessel. Collagenases, with their unique ability to degrade native fibrillar collagen, have been implicated in several pathological states, for example, vascular diseases associated with collagen degradation (Galis, Sukhova, & Libby, 1995).

Ca2+ plays the role of a second messenger in many biochemical and physiological processes (Berridge, 1993; Chakraborti & Chakraborti, 1995; Chakraborti, Michael, & Sanyal, 1992). An increase in Ca2+ level in situ caused by a variety of agonists is due to an influx of extracellular Ca2+ and/or release of Ca2+ from its suborganelle stores (Baumhuter & Richter, 1982; Chakraborti, Gurtner, & Michael, 1989; Chelladurai, Seeger, & Pullamsetti, 2012). Oxidants have been shown to cause Ca2+ overload in cells and tissues (Bennett & Williams, 1993; Ghosh, Mullaney, Tarazi, & Gill, 1989; Llopis et al., 1993). In many systems, cell membrane associated Ca2+ATPase provide a major line of defense by counteracting agonists-induced increase in Ca2+ level in situ (Evers et al., 1988; Grover, 1985 ; Qu, Torchia, & Sen, 1992; Raeymaekers, Wuytack, & Casteels, 1985). Ca2+ATPases have been shown to be activated by a variety of proteases in different systems (Au, 1987 ; Carafoli, 1994). Activation of protease(s) under stimulated conditions have been shown to inactivate ambient protease inhibitors resulting in an alteration of protease(s)-antiprotease(s) balance in favor of the protease(s) (Bond & Butler, 1987; Mellgren, Mericle, & Lane, 1986). Recent research suggests that t-buOOH-mediated activation of Ca2+ATPase in pulmonary vascular smooth muscle plasma membrane occurs through the involvement of the MMP-2. In vitro experimental data provided evidence that treatment of bovine pulmonary vascular smooth muscle plasma membrane with tbuOOH increases MMP-2 activity which plays a pivotal role in stimulating Ca2+ATPase activity; and both basal and t-buOOH stimulated MMP-2 activity and Ca2+ATPase activity were found to be inhibited by EGTA and TIMP-2 in the membrane (S. Das, Chakraborti, Mandal, Mandal, & Chakraborti, 2002). Experimental evidence (Mandal, Das, Chakraborti, Mandal, & Chakraborti, 2003) showed (i) by adding low doses of MMP-2 or H2O2 to the smooth muscle membrane suspension caused submaximal increase in Ca2+ATPase activity, and pretreatment with TIMP-2 prevents the increase in Ca2+ATPase activity; (ii) combined treatment of the membrane with low doses of MMP-2 and H2O2 augments further the Ca2+ATPase activity caused by the respective low doses of either H2O2 or MMP-2; and (iii) pretreatment with TIMP-2 prevents the increase in Ca2+ATPase activity in the membrane caused by the combined treatment of MMP-2 and H2O2.

Treatment of smooth muscle with the superoxide (O .) generating system was reported to inhibit Na+ dependent Ca2+ uptake in the microsomes and that appears to be a potential mechanism for increase in Ca2+ overload under oxidant triggered condition (Chakraborti, Mandal, Das, & Chakraborti, 2005). A similar response on PKC activation, for example, by platelet activating factor-induced vasoconstriction has been demonstrated in pigs and that has been shown to be mediated by a pertussis toxin sensitive G protein (Murtha, Allen, & Orr, 1999). Thus, the role of MMP-2 on Na+ dependent Ca2+ uptake via PKCδ in association with RACK-1 and the involvement of a pertussis toxin sensitive G protein in microsomes isolated from bovine pulmonary artery smooth muscle upon treatment with the O . Generating system appears to be physiologically important (Chakraborti, Mandal, Das, & Chakraborti, 2005).

MMP Inhibition and PAH

The role of MMPs and their inhibitors as a therapeutic target for PAH has recently been reviewed extensively (Chelladurai, Seeger, & Pullamsetti, 2012). MMP inhibition has been shown to either attenuate or enhance vascular remodeling. Several pharmacological studies of MMP inhibition were mostly performed in two of the experimental models of PAH: hypoxia and MCT- induced PAH. Recent research (Cowan, Jones, & Rabinovitch, 2000 ; Cowan et al., 2000) has demonstrated therapeutic possibilities of elastase and MMP inhibitors in the MCT induced PAH model. In another important study (Vieillard-Baron et al., 2003) demonstrated that intratracheal instillation of the adenovirus-mediated overexpression of human TIMP-1 gene in the lungs of rats exposed to MCT reduced pulmonary vascular remodeling, right ventricular hypertrophy, gelatinase activity and muscularisation of peripheral pulmonary arteries, indicating possible reversal of MMP effects to mitigate PAH. Point to be noted that there are conflicting evidence available with MMP inhibition in the hypoxia-induced PAH model. A recent study (Kerr et al., 1987), employing collagen synthesis inhibitors (cis-4-hydroxy-L-proline) and crosslinking (β-aminopropionitrile) in the experimental rat model exposed to chronic hypoxia, resulted reduction of excess vascular collagen and reversal of PAH. However, in contrast, conflicting evidence (Vieillard-Baron et al., 2000) demonstrated that intratracheal instillation of the adenovirus-mediated overexpression of human TIMP-1 gene or administration of a broad-spectrum MMP blocker (doxycycline) in rats subjected to chronic hypoxia was associated with increased muscularisation and periadventitial collagen accumulation in distal arteries. Similarly, administration of a serine elastases inhibitor (SC-39026) to rats reduced hypoxia-induced PH (Maruyama et al., 1991). Furthermore, transgenic mice that overexpress the serine elastases inhibitor elafin when exposed to chronic hypoxia demonstrated reduced serine elastase and MMP activity compared to the nontransgenic mice. Importantly, elafin-transgenic mice displayed reduced right ventricular pressure, reducedmuscularisation and preservation of the number of distal vessels as compared with control or non-transgenic mice (Zaidi, You, Ciura, Husain, & Rabinovitch, 2002). Apart from MMP inhibitors, several pharmacological compounds are reported to reverse PAH via modulation of MMP/TIMPs (Pullamsetti et al., 2005). Lercanidipine, a vasoselective dihydropyridine calcium channel blocker, demonstrated beneficial effects in patients with PH by decreasing elevated circulating MMP-9 levels (Martinez et al., 2006; Tayebjee et al., 2004). This lercanidipine induced effect was associated with a significant decrease in MMP-9 activity without affecting proMMP-2 activity and TIMP-1 concentration besides a reduction in oxidative stress in patients with PH and with PAH and diabetes mellitus (Martinez et al., 2006). Therapeutic administration of a calcium channel blocker, amlodipine, immediately followed by MCT treatment suppressed the MCT-induced increase in MMP-2 activity, platelet activation, EC damage and SMC proliferation, and consequently inhibited the PAH progression (Mawatari et al., 2007). Administration of the FDA-approved drug for PAH, bosentan (dual endothelin receptor agonist, ETA/B), also attenuated the MCT-induced upregulation of MMP-2, TIMP-1, endothelial NO synthase expression and MCT-induced PAH (Koo, Kim, & Hong, 2011). The pharmacological studies of MMPinhibition in hypoxia- and MCT-induced experimental PH models have substantiated that selective or pan-MMP inhibition can be bidirectional: attenuates or exacerbates vascular remodelling and PAH. The differential outcome could partly suggest the existence of distinctive mechanisms involving ECM accumulation and MMP activity underlying the development of experimental PAH. Moreover, clinical studies performed in diseases such as cancer and arthritis pointed towards the possible dose-limiting side-effects of pan-MMP inhibition, such as musculoskeletal syndrome that manifests as pain and immobility in the shoulder joints, arthralgias, contractures in the hands and reduced overall quality of life in patients (Fingleton, 2008).

Statins

Statins, particularly simvastatin, have been reported to attenuate the development of pulmonary hypertension in experimental animal models [Nishimura T et al., 2003; Nishimura T et al., 2002,]. Controversy still surrounds the question of whether simvastatin has a beneficial effect on PAH, considering the variations between animal and clinical results [McMurtry et al., 2007; Zhao et al., 2009]. When simvastatin was added to conventional therapy, a small and transient reduction in right ventricular (RV) mass was observed [Wilkins et al., 2010]. However, this had no significant effect on the experimental PAH patients [Kawut et al., 2011]. Recent studies [Yao et al., 2012] confirmed that MMP-9 is involved in pulmonary vascular remodeling in a carotid artery–jugular vein (CA–JV) shunt PAH model, and first demonstrated that MMP-1 is also an important molecule that needs further investigation to better define its role and potential for therapeutic targeting in PAH. Simvastatin can prevent vascular remodeling in a CA–JV shunt PAH model in rats, which might be related to the inhibition of MMP-1, 9.

Future Directions

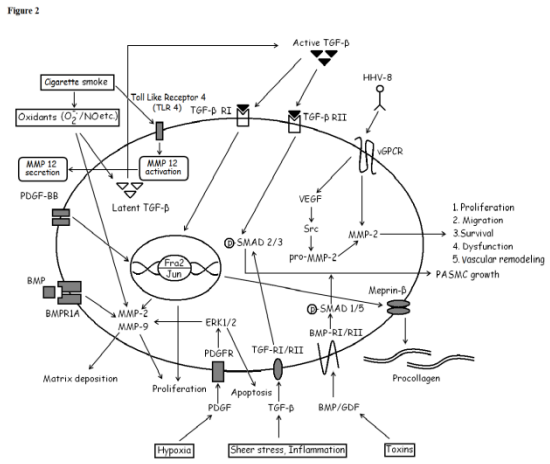


Figure 2 shows schematic diagram as a summary for the major pathways for MMP activation in relation to matrix remodeling, vascular homeostasis, and ECM deposition leading to PAH. Deregulated expression and activity profiles of MMPs/TIMPs have been detected in human PAH and experimental models of PAH. Conflicting studies have been published addressing MMP inhibition and possible PAH therapeutic intervention. Several issues need to be addressed before considering MMP inhibition as a clinical therapeutic strategy.

The conflicting outcomes of MMP inhibition studies in hypoxia and MCT model of PAH in terms of regressing pulmonary vascular lesions suggest that MMP inhibition mediated beneficial effects depend upon the primary insult involved, or the type of inhibitor used. In addition, this can also strongly support the existence of distinctive mechanisms underlying in the development of hypoxia-induced PH as compared with MCT-induced PAH and their differential responses to pharmacological agents.

A detailed characterization of MMP expression and activity in different sub-types of human PAH is lacking, especially in pulmonary vasculature and in different cell types of pulmonary vasculature. PAH caused by drugs or toxins induced ECM remodeling and MMP regulation need further investigation.

Additionally, neither chronic hypoxia- nor MCT-induced animal models of PAH perfectly resembles the complex human situation. Both models trigger only mild-to-moderate PAH and do not recapitulate neointimal proliferation and plexiform lesions that are important hallmarks of severe PAH (Jeffery & Morrell, 2002). This makes it extremely difficult to extrapolate the outcome of MMP inhibition in animal models to humans. Nevertheless, a greater understanding of the involvement of MMPs in experimental models of neointimal lesions or plexogenic lesions is indispensable to further explore the possibilities of therapeutic MMP inhibition in PAH.

Importantly, broad-spectrum MMP inhibitors, such as marimastat, failed to demonstrate clinical efficacy due to severe side-effects. The most frequent side-effect associated with the clinical trials of MMP inhibitors was a

musculoskeletal syndrome (Fingleton, 2008). Despite this, periostat is the only MMP inhibitor that has been approved by the FDA for the treatment of periodontal disease. Possible reasons for the low success rate of MMP inhibitors in the clinic include unwanted side-effects caused by their lack of selectivity, poor oral bioavailability and decreased potency in vivo.

Further studies are needed to explore the ET-1 antagonism and subsequent MMP regulations in PAH to better define the interaction between MMP-9 and ET levels in patients (Giannelli, Iannone, Marinosci, Lapadula, & Antonaci, 2005). More studies are needed to clarify the role of MMPs and TIMPs in determining blood vessel damage which severely affects the prognosis and survival of patients with SSc, and to ascertain how bosentan therapy may interfere with the proteolytic remodeling of the vessels (G. Giannelli, Iannone, Marinosci, Lapadula, & Antonaci, 2006). Furthermore, the value of specifically targeting HHV-8-encoded vGPCR, MMP-2, or Src kinase for the treatment of PAH should be evaluated in future studies (Friedrich & Bohm, 2007).

Third generation of MMP inhibitors is currently under investigation and may potentiate reverse remodeling process in PAH, without any detrimental off-target effects. The in vivo vascular cell matrix interactions are complex and reiterate the necessity to employ three-dimensional culture systems or organ culture models. In addition, it is important to identify the substrates/gene targets and crosstalk between MMPs and other signaling pathways relevant to PAH pathogenesis need to be investigated.

Clearly, assessing the pre-clinical efficacy of selective MMP inhibitors in severe animal models of PAH is warranted. Inhibitors of MMPs have been assessed in cancer trials. Toxicity was a problem early on, but newer agents have shown promise and merit (Vieillard-Baron et al., 2000; Srikala & Shepherd, 2003). In conclusion, MMPs plays an indispensible role in pulmonary vascular remodeling processes and may be an attractive target for the treatment of PAH (Chelladurai, Seeger, & Pullamsetti, 2012). However, in a hypoxic PAH model, MMP inhibition led to increased fibroproliferation, and the effects of proteases in human PAH need to be better understood in coming years (Carlin & Peacock, 2008).

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