TRANSLATIONAL REVIEW

Matrix Metalloproteinases as Therapeutic Targets for Idiopathic Pulmonary Fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a restrictive lung disease that is associated with high morbidity and mortality. Current medical therapies are not fully effective at limiting mortality in patients with IPF, and new therapies are urgently needed. Matrix metalloproteinases (MMPs) are proteinases that, together, can degrade all components of the extracellular matrix and numerous nonmatrix proteins. MMPs and their inhibitors, tissue inhibitors of MMPs (TIMPs), have been implicated in the pathogenesis of IPF based upon the results of clinical studies reporting elevated levels of MMPs (including MMP-1, MMP-7, MMP-8, and MMP-9) in IPF blood and/or lung samples. Surprisingly, studies of gene-targeted mice in murine models of pulmonary fibrosis (PF) have demonstrated that most MMPs promote (rather than inhibit) the development of PF and have identified diverse mechanisms involved. These mechanisms include MMPs: (*1*) promoting epithelial-to-mesenchymal transition (MMP-3 and MMP-7); (*2*) increasing lung levels or activity of profibrotic mediators or reducing lung levels of antifibrotic mediators (MMP-3, MMP-7, and MMP-8); (*3*) promoting abnormal epithelial cell migration and other aberrant repair processes (MMP-3 and MMP-9); (*4*) inducing the switching of lung macrophage phenotypes from M1 to M2 types (MMP-10 and MMP-28); and (*5*) promoting fibrocyte migration (MMP-8). Two MMPs, MMP-13 and MMP-19, have antifibrotic activities in murine models of PF, and two MMPs, MMP-1 and MMP-10, have the potential to limit fibrotic responses to injury. Herein, we review what is known about the contributions of MMPs and TIMPs to the pathogenesis of IPF and discuss their potential as therapeutic targets for IPF.

Keywords: matrix metalloproteinase; interstitial lung disease; idiopathic pulmonary fibrosis; lung; fibrosis

Clinical Relevance

In this *Translational Review*, we describe the molecular and cell biology of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases, and review the evidence that links MMPs to idiopathic pulmonary fibrosis (IPF), the cellular sources of MMPs, and the mechanisms involved. Although initial studies of randomized clinical trials for nonselective MMP inhibitors as new therapies for cancer produced disappointing results, since then, newer approaches to target metalloproteinases more selectively have been developed for other diseases. We have included a discussion of the advantages (and potential limitations) of these new therapeutic approaches targeting MMPs and their potential as therapeutics for IPF.

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Idiopathic Pulmonary Fibrosis

In the United States, approximately 50,000 patients are newly diagnosed with idiopathic pulmonary fibrosis (IPF) each year. The median survival of patients with IPF is only 3–5 years (1). Although numerous medical therapies have been evaluated in patients with IPF, the only therapies that slow the progression of this disease, pirfenidone (2) and nintedanib (3), are associated with side effects and are not fully effective at reducing mortality. Thus, there is an urgent need to identify novel therapeutic targets for IPF. Herein, we review the evidence linking members of the matrix metalloproteinase (MMP) family to the pathogenesis of IPF, identify knowledge gaps in the field of MMPs and IPF, and discuss potential approaches to target MMPs as novel therapeutics for IPF.

IPF is characterized by the deposition of excessive amounts of extracellular matrix (ECM) proteins in the lungs, thereby replacing the normal architecture of the lung. IPF is the most common type of idiopathic interstitial pneumonia, and is characterized pathologically by the pattern of usual interstitial pneumonitis. Although the etiology of IPF is still unclear, several pathogenic mechanisms have been implicated in its development, including aberrant repair of injured epithelium, fibroblast activation, epithelial-to-mesenchymal transition (EMT), collagen deposition, and immune cell dysfunction. MMPs are expressed by most of the cellular culprits and pathologic processes implicated in IPF pathogenesis.

MMPs

MMPs are zinc-dependent endopeptidases that, together, degrade all components of the ECM. Consequently, it was initially thought that MMPs would limit lung fibrosis by degrading ECM proteins in the lung. However, recent studies have implicated MMPs in regulating the activities of proteins other than ECM proteins, including mediators of inflammation, latent growth factors, antifibrotic growth factors, and cleaving cell surface molecules and receptors. However, most studies of MMP-deficient mice in pulmonary fibrosis (PF) models have shown the opposite—that MMPs promote pulmonary fibrotic responses to injury.

MMP Structure

MMPs are multidomain proteins (Figure 1). The signal peptide at the amino terminus targets the protein to the cell's secretory pathway. The propeptide domain, containing the highly conserved cysteine switch motif, PRCGXPD, is cleaved during activation of the latent proenzyme by yet-to-be identified peptidases. The catalytic domain contains the highly conserved Zn^{2+}-binding motif, HEXXHXXGXXH, in which the three histidines (H) bind to the active site zinc, and the nucleophilic glutamate (E) attacks the substrate's peptide bond. The proline-rich hinge domain connects the catalytic domain to the C-terminal domain with a flexible segment of up to 75 residues. The carboxyterminal hemopexin-like domain regulates substrate binding and specificity. Some MMPs contain additional domains.

MMP Classification

Mice express 23 MMPs, and 24 MMP genes have been identified in humans, including two duplicated genes encoding MMP-23 (4). MMPs are usually classified by their substrate specificity *in vitro* into seven main groups: (*1*) interstitial collagenases (MMP-1, -8, -13, and -18 [in *Xenopus laevis*]), which cleave types I–III interstitial collagens; (*2*) gelatinases (MMP-2 and -9), which cleave denatured collagens (gelatins) and basement membrane proteins; (*3*) stromelysins (MMP-3, -10, and -11), which cleave laminin and other basement membrane proteins; (*4*) membrane-type MMPs (MT-MMPs), which are expressed on cell surfaces and linked to plasma membranes either by a glycophosphatidylinositol anchor (MMP-17 and -25) or a transmembrane domain (MMP-14, -15, -16, and -24); (*5*) matrilysins (MMP-7 and -26), which lack the carboxyterminal domain and cleave proteoglycans, laminin, elastin, and type IV collagen; (*6*) metalloelastase (MMP-12), which cleaves elastin and some basement membrane proteins; and (*7*) other MMPs (MMP-19, -20, -23, and -28).

The different domains of MMPs generally determine their substrate specificity. For some subclasses of MMPs, the contributions of individual domains or residues to the substrate specificity has been

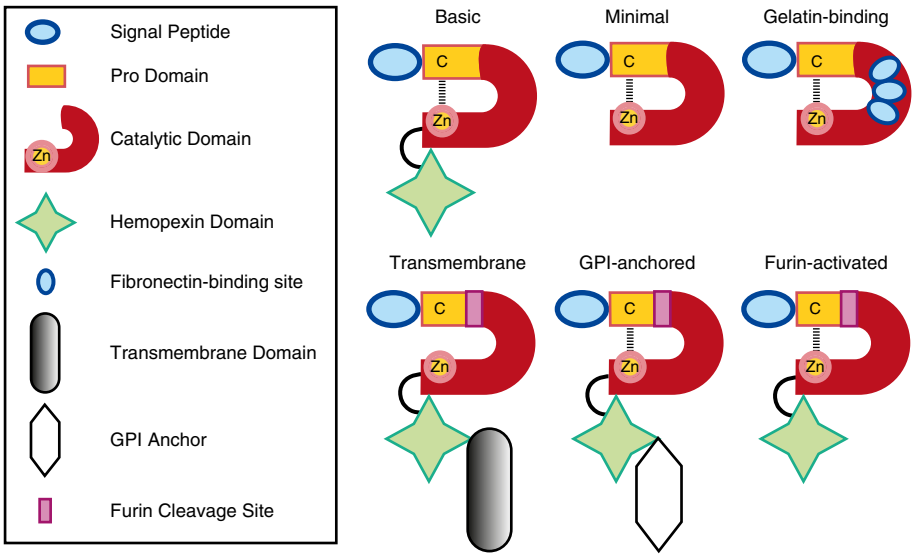

Figure 1. Matrix metalloproteinase (MMP) classification by protein domain structure. MMPs can be categorized according to their protein domain structure. All MMPs share: (*1*) an N-terminal signal peptide and (*2*) a prodomain containing the consensus sequence in which a cysteine residue binds to the zinc ion in the catalytic domain to maintain enzyme latency. MMPs with the basic/simple domain structure also have a flexible linker region followed by a hemopexin-like domain (four-bladed β propeller structure), which helps determine substrate specificity. Minimal MMPs lack this linker and the hemopexin-like domain. Membrane-type MMPs are anchored to plasma membrane by either a transmembrane domain or a glycophosphatidyl inositol (GPI) anchor. Gelatin-binding MMPs contain fibronectin-binding sites in the catalytic domain. Furin-activated MMPs contain a furin cleavage site in their prodomain, allowing them to be activated intracellularly in the *trans*-Golgi network. C, carboxy terminus; Zn, zinc atom.

determined. For example, for the interstitial collagenases, the hemopexin-like domains, hinge regions, and active sites together are crucial for cleavage of the collagen triple helix (5–7). Before active site cleavage of the individual α-chains of collagen can occur, the triple helical structure must be opened (a process termed triple helicase activity). This is mediated by binding of the C-terminal hemopexin domain (along with the hinge region and active site) to the interstitial collagen substrate. This process induces localized conformational changes in native collagen that enables the individual α-chains of collagen to then be cleaved by the active sites of the MMPs (8). MMP-2 and -9 have three fibronectin type II domain repeats inserted into the catalytic domain just ahead of the zinc-binding region. These fibronectin repeats facilitate binding of MMP-2 and-9 to gelatin substrates, and are required for MMP-9 to cleave types V and X1 collagens (9). The amino acid at the S(2) pocket of the substrate-binding cleft of MMP-2 and -9 (which is highly variable among the MMP family) also contributes to substrate selectivity (10).

Regulation of MMPs

Most MMPs are not expressed constitutively by cells, and most cells (except polymorphonuclear neutrophils [PMNs]) need to be activated to express MMPs. MMP activity is tightly regulated at the transcriptional and post-translational levels, and by cellular localization.

Transcriptional Regulation

MMP expression is regulated by numerous transcription factors (Table 1 and Ref. 11). MMPs have been classified into three main categories based upon the cis-acting element binding sites in their promoters: (*1*) MMPs with a TATA box located −30 base-pairs (bp) from the transcription start site, an activator protein (AP) -1 binding site at −70 bp, and a polyoma enhancer A binding protein (PEA) -3 site adjacent to the AP-1 site; (*2*) MMPs with a TATA box, but no AP-1 site (MMP-8, -11, and -21); and (*3*) MMPs lacking a TATA box that are largely constitutively expressed (MMP-2, -14, and -28) (12). However, there is overlap between these three categories. For example, MMP-9 (a gelatinase), MMP-13 (an interstitial collagenase), MMP-3 (a stromelysin), and MMP-12 (an elastase) are all regulated by both AP-1 and PEA-3 transcription factors (Table 1).

Post-Translational Regulation

MMPs are synthesized as latent proenzymes that require activation to attain full catalytic activity. Reactive oxygen and nitrogen species, proteinases, and organomercurials can disrupt the bond between the sulfhydryl group of the conserved cysteine in the propeptide domain and the active site zinc ion—the so-called "cysteine switch" mechanism of pro–MMP activation (13) *in vitro*. Various proteinases cleave the propeptide to expose the active site (e.g., active MMPs, serine proteinases, and proteinases involved in coagulation). However, activators of pro-MMPs *in vivo* are not known.

Cellular Localization

In addition to membrane-type MMPs, other MMPs lacking transmembrane domains or glycophosphatidylinositol anchors are expressed on cell surfaces (MMP-2, -8, and -9) by binding to adapter proteins or yet-to-be identified molecules after MMPs are released by cells (14–16). Localization of MMPs on cell surfaces prevents widespread proteolysis and/or protects active MMPs from inhibition by tissue inhibitors of MMPs (TIMPs) (14, 15).

MMPs Implicated in the Pathogenesis of PF by Studies of Gene-Targeted Mice

MMP expression levels in blood and lung samples are altered in patients with IPF compared with normal subjects (Table 2). Studies of MMP gene–targeted mice have shown that a number of MMPs regulate processes implicated in IPF pathogenesis (Table 3 and Figure 2). Subsequently here, we outline the expression patterns for individual MMPs linked to IPF in cells in fibrotic lungs. We also describe the potential roles of each MMP in pathologic processes in IPF lungs based upon: (*1*) the phenotypes of mice lacking or overexpressing individual MMPs in models of PF; or (*2*) activities of MMPs that have been described in human or murine cells *in vitro* that are pertinent to IPF. However, it is important to note that different cell types have different roles in PF, such that increased expression of an MMP (or MMPs) in one cell type might be "profibrotic," whereas increased expression in another cell type might serve an "antifibrotic" function. Similarly, MMPs might have one function early during early wound repair processes and an entirely different function in the maintenance or propagation of fibrosis. Accordingly, it is almost impossible to assign a given MMP to exclusively a "pro-" or "anti-" fibrotic category. Nevertheless, for MMP gene–targeted mice that have been studied in models of PF, we have divided these MMPs into those that are "pro-" or "anti-" fibrotic based upon the overall effect of the MMP on lung collagen levels 14–21 days after delivering bleomycin to the lungs (Table 3 and Figure 2).

MMP-3

MMP-3 (stromelysin-1) is expressed by epithelial cells, fibroblasts, endothelial cells, alveolar macrophages, and monocytes, and cleaves type IV collagen and basement membrane proteins *in vitro*. MMP-3 levels are increased in IPF lungs (17, 18) mainly in bronchial and alveolar epithelial cells, interstitial fibroblasts, alveolar macrophages, and other leukocytes (18).

MMP-3 activities during PF. *Mmp3*$^{-/-}$ mice are protected from bleomycin-induced PF, and overexpression of MMP-3 in rat lungs promotes PF (18). Mmp-3 promotes PF by: (*1*) activating β-catenin signaling in lung epithelial cells to increase E-cadherin cleavage and EMT (18); (*2*) activating latent transforming growth factor (TGF)-β by releasing latent TGF-β homodimer from both latency-associated peptide and latent TGF-β–binding protein-1 (19); and (*3*) inhibiting distal epithelial repair by releasing endostatin bound to type XVIII collagen, a proteoglycan located in alveolar capillary and epithelial basement membranes (20), permitting endostatin to induce lung epithelial cell apoptosis. Interestingly, endostatin levels are increased in IPF plasma and bronchoalveolar lavage fluid (BALF) samples and inversely correlate with lung function (21).

MMP-7

MMP-7 (matrilysin) is expressed by lung epithelial cells, mononuclear phagocytes, and fibrocytes (22). Plasma and BALF MMP-7 levels are increased in patients with IPF, and plasma MMP-7 levels have been validated as a biomarker for IPF (23). MMP-7 is expressed by airway epithelial cells and macrophages in IPF lungs (24). MMP-7 expression is increased by osteopontin in A549 cells, and osteopontin colocalizes with MMP-7 in IPF lung

Table 1. Transcriptional Regulation of Matrix Metalloproteinases

MMP	Transcription Factors and Promoter Regulatory Regions	Other Mechanisms of Regulation	References
MMP-9	NF-κB, PEA-3, AP-1, TIE	mRNA stability, p38, Rac-1	183-186
MMP-8	C/EBP-β	TGF-β, TNF-α, IL-1β	35, 36, 187
MMP-13	AP-1, PEA-3, Ets, OSE-2, TIE	BMP-2, FGF-2, p38, PTH, vitamin D, mechanical strain	73, 188-191
MMP-3	AP-1, PEA-3, SPRE	p38, EMMPRIN (CD147)	192, 193
MMP-7	AP-1, Tcf-4, PEA-3, TIE	β-catenin	194-196
MMP-12	AP-1, TRF, PEA-3, Tcf-4, LBP	PAR-1, GM-CSF, IL-1β, MCP-1	197, 198
MMP-19	AP-1, PEA-3		170
MMP-28	SOX-like		199
MMP-2	AP-2, p53/AP-2	UV radiation, EMMPRIN, MAPK	193, 200
MMP-1	SBE, AP-1, PEA-3, TIE, C/EBP-β, FGF, hyperoxia	UV radiation, EMMPRIN, $\alpha_2\beta_1$ integrin	101, 107, 193, 201 202
MMP-10	AP-1, PEA-3	TGF-α, KGF, IFN-γ, TGF-β	203
MMP-11	PEA-3, NF-1, RARE, AP-1–like	TSH	143, 204
MT1-MMP	SAF-1, HBS, Tcf-4	Fibronectin, soluble E-cadherin	205, 206

Definition of abbreviations: AP-1, activator protein-1; AP-2, activator protein-2; BMP-2, bone morphogenetic protein 2; C/EBP-β, CCAAT/enhancer-binding protein-β; EMMPRIN, extracellular matrix metalloproteinase inducer; Ets, E26 transformation-specific; FGF, fibroblast growth factor; GM-CSF, granulocyte/macrophage colony–stimulating factor; HBS, hypoxia-inducible factor–binding site; KGF, keratinocyte growth factor; LBP, leader-binding protein; MAPK, mitogen-activated protein kineases; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MT1, membrane-type 1; NF-1, nuclear factor-1; OSE-2, osteoblast-specific element-2; p53/AP-2, p53/AP-2 combination binding site; PAR-1, protease-activated receptor-1; PEA-3, polyoma enhancer A binding protein-3; PTH, parathyroid hormone; Rac-1, Ras-related C3 botulinum toxin substrate 1; RARE, retinoic acid–responsive element; SAF-1, serum amyloid A activating factor-1; SBE, STAT-binding element; SOX, Sry-related high mobility group box; SPRE, stromelysin-1 platelet-derived growth factor (PDGF)-responsive element; Tcf-4, T cell factor-4/β-catenin binding site; TGF, transforming growth factor; TIE, TGF-β inhibitory element; TRF, octamer binding protein; TSH, thyroid stimulating hormone; UV, ultraviolet.

epithelium (25). Two single-nucleotide polymorphisms in the MMP-7 promoter region (which both increase MMP-7 transcription) have been associated with IPF (26).

MMP-7 activities during PF. Mmp-7 promotes regeneration of ciliated airway epithelial cells after epithelial injury *in vitro* (27). However, Mmp-7 promotes PF in mice, as *Mmp7*$^{-/-}$ mice are protected from bleomycin-mediated PF (28, 29). MMP-7 may promote PF by cleaving E-cadherin to activate epithelial cells (30) and/or proteolytically activating heparin-binding epidermal growth factor precursor (pro-HB-EGF) to release active HB-EGF, which promotes human lung fibroblast proliferation (31).

MMP-8

MMP-8 (neutrophil collagenase or collagenase-2) is expressed by PMNs and at lower levels by activated monocytes, macrophages, lymphocytes, lung epithelial cells, fibroblasts, fibrocytes, dendritic cells (32), natural killer (NK) cells (33), and mesenchymal stem cells (22, 34–37). Pro-MMP-8 is stored in PMN-specific granules and released upon PMN activation. In other cells, MMP-8 is regulated at the transcriptional level (Table 1) by TGF-β1 and TNF-α in fibroblasts (35, 36), and IL-1β and CD40 ligand in mononuclear phagocytes (37).

MMP-8 protein levels are increased in IPF BALF (17, 38–40) and lung homogenates (38), and MMP-8 is up-regulated in macrophages and bronchial epithelial cells, but down-regulated in alveolar epithelial cells in IPF lungs (38). Plasma MMP-8 levels are increased in patients with IPF, and *MMP8* steady-state mRNA levels are increased in IPF peripheral blood monocytes (38). However, MMP-8 plasma and BALF levels do not correlate with decline in lung function or mortality in patients with IPF (38).

MMP-8 activities in PF. *Mmp8*$^{-/-}$ mice are protected from bleomycin-mediated PF (35, 41), but have increased accumulation of macrophages in the lung during the acute inflammatory phase of this model (35, 41). Mmp8's profibrotic activities are linked to Mmp-8 reducing lung levels of macrophage inflammatory protein-1α and IFN-γ–inducible protein-10 (Ip-10 or CXCL10) (35), which are both chemotactic for mononuclear phagocytes. In addition, Ip-10 and its receptor, CXCR3, have antifibrotic activities (42, 43), and Ip-10 inhibits fibroblast chemotaxis (43). Another study reported that Mmp-8 cleaves il-10 in the murine lung to increase PF (41). MMP-8 may also promote PF by regulating increasing fibrocyte migration into the lung. Fibrocytes are circulating bone marrow–derived cells expressing CD45 and collagen, and promote fibroproliferative responses to injury when recruited to the lungs (44). Fibrocytes express MMP-8 (22), and incubating fibrocytes with an MMP-8 inhibitor decreased their migration *in vitro*, suggesting that MMP-8 promotes fibrocyte migration (22).

MMP-9

MMP-9 (gelatinase B) is expressed by all leukocytes, fibroblasts, and epithelial and endothelial cells (45). Pro-MMP-9 protein is stored in the tertiary granules of PMNs. In other cells, MMP-9 expression is regulated by transcription factors (Table 1). MMP-9 levels are increased in IPF BALF samples (39) and localized to alveolar and interstitial macrophages, metaplastic airway epithelial cells, and PMNs in IPF lungs (46, 47).

MMP-9 activities during PF. Although membrane-bound MMP-9 on tumor cells activates latent TGF-β1 (48), wild-type (WT) and *Mmp9*$^{-/-}$ mice have similar lung collagen levels when treated with bleomycin. However, MMP-9 likely

Table 2. Matrix Metalloproteinases That Are Up-Regulated in Patients with Idiopathic Pulmonary Fibrosis

MMP	Peripheral Blood Samples*	Lung Samples*
MMP-9	• Increased protein in plasma (23)	• Increased protein levels in BALF and alveolar macrophages (39)
MMP-8	• Increased protein in plasma (23) • Increased steady-state mRNA in peripheral blood monocytes (38)	• Increased protein levels in BALF (17, 39, 40) • Increased protein levels in BALF, whole lung, lung macrophages, and epithelial cells (38)
MMP-13	• Not known	• Increased protein levels in whole lung (75) • Protein levels not increased in BALF (39)
MMP-3	• Increased protein in plasma (23)	• Increased protein levels in BALF (17) • Increased protein and steady-state mRNA levels in whole lung (18) • Increased protein levels in lung macrophages, epithelial cells, and intravascular leukocytes (18)
MMP-7	• Increased protein in plasma (23)	• Increased protein levels in BALF and lung tissue (23) • Increased steady-state mRNA levels in whole lung (29) • Increased protein levels in lung epithelial cells (29)
MMP-19	• Not known	• Increased protein levels in BALF, whole lung, and dysplastic lung epithelial cells overlying fibrosis (83)
MMP-2	• Not known	• Increased protein levels in whole lung and reactive epithelium and myofibroblasts (105, 112, 113)
MMP-1	• Increased protein in plasma (23)	• Increased protein in BALF and whole lung (23)
MT1, 2, 3, 5-MMP	• Not known	• Increased protein and mRNA levels in whole lung (146) • MT1- and MT2-MMPs expressed in alveolar epithelial cells (146) • MT3-MMP expressed in fibroblasts in fibrotic foci and alveolar epithelial cells (146) • MT5-MMP expressed in basal bronchiolar epithelial cells and areas of squamous metaplasia (146)

Definition of abbreviations: BALF, bronchoalveolar lavage fluid; MMP, matrix metalloproteinase; MT, membrane-type.
*Expression of MMPs (either at the protein or steady-state mRNA level) was measured and found to be increased in peripheral blood samples, BALF, or whole-lung tissue from patients with idiopathic pulmonary fibrosis versus control subjects.

promotes abnormal epithelial repair processes in fibrotic lungs, as: (*1*) MMP-9 is predominantly expressed in metaplastic alveolar epithelial cells in IPF lungs and bleomycin-treated mice; and (*2*) bleomycin-treated *Mmp9*$^{-/-}$ mice are protected from alveolar bronchiolization (49), which is the abnormal proliferation of bronchiolar cells in the alveoli occurring in experimental PF (50–53), and in areas of severe fibrosis in IPF lungs (54, 55). However, the activity of MMP-9 in regulating lung fibrotic responses is cell context specific. MMP-9 does not regulate the lung fibrotic response in transgenic mice overexpressing profibrotic IL-13 in airway Club cells (56). However, bleomycin-treated transgenic mice that overexpress human MMP-9 in macrophages have reduced PF, and this is preceded by significant reductions in PMN and lymphocyte counts in BAL samples and lower TIMP-1 levels in BALF samples (57). Overexpressing MMP-9 in alveolar macrophages limits bleomycin-induced PF in mice by MMP-9 cleaving insulin-like growth factor (IGF)–binding protein-3 (IGFBP-3). IGFBPs are carrier proteins that exert their function through IGFs. However, IGFBP-3 also has IGF-independent effects mediated by binding to TGF-β receptors. IGFBP-3 has been strongly linked to the pathogenesis of IPF and other fibrosing conditions (58). TGF-β increases IGFBP-3 secretion by fibroblasts, and IGFBP-3 serves as a downstream modulator of TGF-β by inducing fibroblast production of syndecan-2 and regulating TGF-β induction of syndecan in human fibroblasts (59). Syndecan-2 promotes cell signaling, proliferation, migration, and cytoskeletal organization, cell–matrix interactions, and ECM assembly (60–62).

MMP-12

MMP-12 (macrophage metalloelastase) is expressed by macrophages, but can also be expressed by lung stromal cells (63). *MMP-12* expression is increased during classical activation of macrophages with LPS, and even more so during IL-4–induced alternative macrophage activation (64).

MMP-12 lung and serum levels are increased in patients with systemic sclerosis with interstitial lung disease, and levels correlate with severity of restriction in pulmonary function testing (65). Furthermore, the rs2276109 A/G functional polymorphism in the *MMP12* locus is associated with the presence of interstitial lung disease in patients with systemic sclerosis (66). Mmp-12 expression is also increased in the lungs of animals with PF (67, 68).

Mmp-12 activities during PF. There are conflicting reports on the activities of Mmp-12 in regulating PF in mice. MMP-12 does not regulate bleomycin-mediated PF (69), nor does it regulate PF resulting from overexpression of IL-13 in airway Club cells in mice (56). However, MMP-12 promotes PF that is induced in mice by antibody-mediated cluster of differentiation 95 (CD95) or apoptosis antigen-1 (FAS) activation in the lung, as *Mmp12*$^{-/-}$ mice are protected in this model (70). *Mmp12*$^{-/-}$ mice with antibody-mediated FAS activation in their lungs have decreased expression of early growth response factor-1 (a zinc-finger transcription factor involved in pulmonary responses to TGF-β) and Cyr-61 (a cysteine-rich ECM protein involved in fibroblast adherence to ECM). Transgenic mice overexpressing TGF-β have increased lung levels of Mmp-12, and TGF-β–driven PF is dependent upon Mmp-12, as genetic deletion of *Mmp12* in these mice ameliorates PF (67). In a model of pulmonary and hepatic fibrosis induced by *Schistosoma mansoni* infection, Mmp-12

Table 3. Studies of Matrix Metalloproteinase Gene–Targeted Mice in Models of Pulmonary Fibrosis

MMP*	Pulmonary Fibrosis Model Studied	Pulmonary Fibrosis versus WT mice	Other Pulmonary Phenotype versus WT Mice*
Mmp3$^{-/-}$	Bleomycin	↓	Overexpression of MMP-3 promotes fibrosis (18)
Mmp7$^{-/-}$	Bleomycin	↓	Decreased pulmonary inflammation after 14 days (29)
Mmp8$^{-/-}$	Bleomycin	↓	Increased accumulation of lung macrophages (35, 41)
Mmp9$^{-/-}$	Bleomycin	No change	Protected from alveolar bronchiolization (49)
Transgenic mice overexpressing MMP-9 in alveolar macrophages	Bleomycin	↓	Reduced neutrophil and lymphocyte counts in bronchoalveolar lavage and lower lung levels of Timp-1
Mmp12$^{-/-}$	Bleomycin	No change	Similar pulmonary inflammation (69)
Mmp12$^{-/-}$	FAS Antibody	↓	Similar pulmonary inflammation (70)
Mmp13$^{-/-}$	Bleomycin	↑	Increased pulmonary inflammation (75)
Mmp13$^{-/-}$	Radiation	↓	Decreased pulmonary inflammation (76)
Mmp19$^{-/-}$	Bleomycin	↑	Associated with decreased PTGS2 (83)
Mmp28$^{-/-}$	Bleomycin	↓	Decreased macrophage transition to the M2 phenotype (94)

Definition of abbreviations: FAS, cluster of differentiation 95 [CD95] or apoptosis antigen-1; MMP, matrix metalloproteinase; PTGS2, prostaglandin-endoperoxide synthase 2; Timp, tissue inhibitors of MMP; WT, wild type.
*Mice genetically deficient in MMPs by gene targeting (or mice overexpressing MMP-9) were evaluated in different models of pulmonary fibrosis (bleomycin-, radiation-, or FAS-activating antibody–mediated pulmonary fibrosis). Pulmonary fibrosis was measured (usually as total lung collagen levels) and compared with that in WT mice.

has profibrotic activities in the lung and liver (71). MMP-12 may also contribute to IPF pathogenesis by cleaving ECM proteins, as BALF levels of a type IV collagen fragment generated by MMP-12 are increased in patients with IPF (72), and human MMP-12 can cleave a number of human ECM proteins *in vitro* (45).

MMP-13

MMP-13 (collagenase-3) is expressed by fibroblasts (73) and regulated at the transcriptional level by TGF-β, IL-1β, IL-6, and TNF-α (Table 1). MMP-2 and MT1-MMP (MMP-14) cleave and activate pro–MMP-13 (74). MMP-13 protein levels are increased in IPF lung samples (75), but not in IPF BALF samples (39).

MMP-13 activities in PF. MMP-13 has varying effects on lung fibrotic response to injury. *Mmp13*$^{-/-}$ mice have increased bleomycin-mediated PF associated with an increased early inflammatory response in the lung (75), but reduced pulmonary inflammation and fibrosis when irradiated (76). Thus, the effects of MMP-13 on the pulmonary inflammatory response to the inciting agent may determine the extent to which PF develops. However, Mmp-13 does not regulate PF during hyperoxic lung injury (77). In a murine model of hepatic fibrosis, MMP-13 promotes fibrosis (78), but macrophage expression of MMP-13 in this model contributes to resolution of hepatic fibrosis (79).

MMP-19

MMP-19 has a unique stretch of residues in the linker region between the pro domain and the Zn^{2+}-binding domain, and was initially thought to represent a new subfamily of MMPs (80). MMP-19 is expressed by monocytes, macrophages, fibroblasts, and endothelial cells. MMP-19 associates noncovalently via its hemopexin domain with the cell surface of macrophages, and this process is dependent upon cell adhesion (81). MMP-19 expression in fibroblasts depends on the ERK1/2 and p38 signaling pathways (82).

MMP-19 activities in PF. MMP-19 is highly expressed in dysplastic epithelium overlying fibrotic areas in IPF lungs (83). MMP-19 has antifibrotic activities, as *Mmp19*$^{-/-}$ mice have greater bleomycin-mediated PF than WT mice (83). MMP-19 mediates its antifibrotic activities, in part by inducing expression of prostaglandin-endoperoxide synthase 2 (83), a key regulatory enzyme in the synthetic pathway of prostaglandin E2, an antifibrotic mediator (84–87). MMP-19 may also mediate its protective activities by regulating adaptive immune responses, as: (*1*) unchallenged *Mmp19*$^{-/-}$ mice have impaired thymocyte maturation and T cell–mediated contact hypersensitivity (88); and (*2*) reduced expression of T cell costimulatory molecules predicts decreased transplant-free survival in patients with IPF (89). However, MMP-19 has profibrotic activities in mice with hepatic fibrosis (90). Thus, the activities of MMP-19 in regulating fibrotic responses to injury depend on the site of injury and the inciting agent.

MMP-28

MMP-28 (epilysin) is unusual among MMPs as it: (*1*) contains a furin activation sequence and is activated intracellularly by a furin-like proprotein convertase (91); and (*2*) is expressed constitutively in the lung and other organs (92). In the adult murine lung, MMP-28 is predominantly expressed in airway Club cells and lung macrophages. MMP-28 gene expression is increased in IPF lungs (93).

MMP-28 activities during PF. MMP-28 promotes PF in bleomycin-treated mice by inducing macrophages to switch from a classically-activated (M1) phenotype to an alternatively-activated (M2) phenotype (94). Macrophage phenotype has been strongly implicated in regulating fibroproliferative responses, as the M2-macrophage phenotype promotes fibroblast proliferation and collagen synthesis (95). In bleomycin-treated mice, depletion of M2 macrophages during the fibrogenic phase reduces PF (96). PF developing in TGF-β1 over-expressing transgenic mice is also dependent upon activation of M2 macrophages (97). Although MMP-28 induces TGF-β–mediated EMT in cancer cells (98),

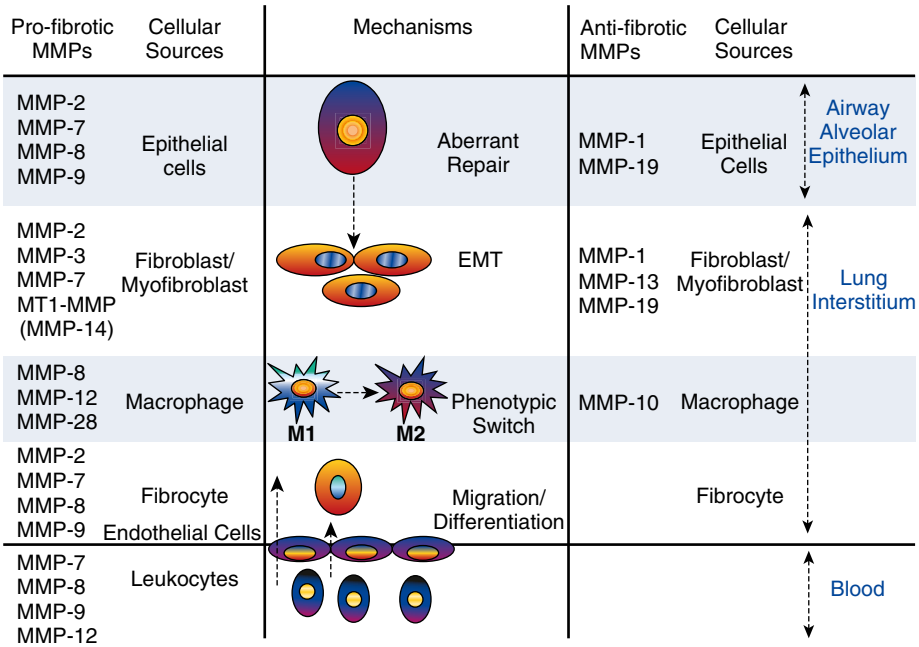

Figure 2. Cells that express MMPs and mechanisms or potential mechanisms by which MMPs regulate pulmonary fibrosis (PF) in murine model systems. MMPs are highly expressed in lung tissues during fibrosis but vary in their dominant cellular expression (epithelial cell, fibroblast, macrophage, fibrocyte, or peripheral blood leukocyte), compartmentalization in the lung (airway and airway/alveolar epithelium, lung interstitium, or blood), and their activity (profibrotic or antifibrotic). MMPs having overall profibrotic activities (assessed as increased total lung collagen levels) in murine models of PF include MMP-3, -7, -8, -9, -12, -13, and -28 (shown in the *left panel*). Based upon their *in vitro* activities or activities in other organs, MMP-1, -2, and -11 have potential to promote PF. MMPs having overall antifibrotic activities (assessed as decreased total lung collagen levels) in models of PF include MMP-13 and -19. Based upon its *in vitro* activities or activities in other organs, MMP-1 and -10 have potential to inhibit PF. Antifibrotic MMPs (or potentially antifibrotic MMPs) are shown in the *right panel*. The mechanisms by which MMPs promote or inhibit PF are illustrated in the *middle panel*. EMT, epithelial mesenchymal transition; MT1, membrane type 1 metalloprotease.

whether this is the case during the development of PF is not known.

MMPs Having Potential to Contribute to PF, but Has Not Yet Been Studied in Animal Models of PF

For MMPs that have not been evaluated using gene-targeted mice in PF models, we have speculated on their potential activities based upon what is known about their activities in other diseases or in *in vitro* systems.

MMP-1

MMP-1 (collagenase-1 or fibroblast collagenase in humans) degrades type I–III collagens *in vitro*. Initially, it was thought that there was no murine homolog of MMP-1, but subsequently the *McolA* (*Mmp1A*) and *McolB* (*Mmp1B*) genes were identified, and are now thought to be the murine homologs of MMP-1 (99). Mmp1a and Mmp1b proteins are 82% identical to each other. They resemble human MMP-1 with 74% identity at the nucleotide level, and Mmp1a has 58% amino acid similarity with MMP-1, making it the least homologous of any mammalian MMP-1 homolog. MMP-1 is expressed by fibroblasts, macrophages, bronchial epithelial cells, and endothelial cells. MMP-1 binds to α2β1-integrin on cell surfaces (100), which induces signaling via α2β1-integrin to increase MMP-1 expression (101). Activation of pro–MMP-1 is mediated by interactions between a serine proteinase (urokinase-type plasminogen activator) and MMP-3 (102).

A single-nucleotide polymorphism in the *MMP1* promoter region at the AP-1 binding site (which increases MMP-1 transcription) has been associated with IPF (103). In addition, MMP-1 levels are elevated in plasma samples from patients with IPF (104), MMP-1 expression in IPF lung is increased, and MMP-1 is localized mainly in dysplastic epithelial cells overlying fibrotic interstitium (105).

MMP-1 activities during PF. The activities of MMP-1 in regulating lung fibrotic responses are not clear. *Mmp1A*$^{-/-}$ mice have been generated (106), but, to our knowledge, have not yet been studied in models of PF. *In vitro* studies suggest that MMP-1 has protective activities in IPF. First, overexpression of human MMP-1 in lung epithelial cells increases their proliferation, migration, and expression of hypoxia-inducible factor-1α, and inhibits their oxidant production and apoptosis (107). Second, MMP-1 promotes normal re-epithelialization of acute wounds (108). Third, osteopontin, a mediator that is significantly up-regulated in IPF lungs, reduces MMP-1 expression by lung fibroblasts and increases their migration and proliferation *in vitro* (25). In addition, one *in vivo* study reported that MMP-1 has antifibrotic activities, as plasmid-mediated expression of human MMP-1 in the livers of rats with hepatic fibrosis reversed the fibrosis (109). Whether MMP-1 limited collagen accumulation in rat livers by degrading interstitial collagens was not determined in this study.

MMP-2

MMP-2 (or gelatinase A) cleaves denatured collagens and basement membranes *in vitro*, and is expressed by airway epithelial cells, macrophages, endothelial cells, lung fibroblasts, and fibrocytes (22). Pro–MMP-2 is activated by forming ternary complexes with members of the MT-MMP subfamily and TIMP-2 on the surfaces of fibroblasts and macrophages (16, 110). Pro–MMP-2 binds by its hemopexin domain to the COOH terminus of TIMP-2, which, in turn, binds via its NH_2-terminal inhibitory domain to a member of the MT-MMP subfamily. The pro domain of MMP-2 is then cleaved by an adjacent TIMP-free MT-MMP molecule, generating active MMP-2 anchored to the cell surface (111). MMP-2 expression is increased in IPF lungs mainly in reactive airway epithelial cells and myofibroblasts (105, 112, 113), and close to fibroblastic foci.

MMP-2 potential activities during PF. *Mmp2*$^{-/-}$ mice are abnormal in the unchallenged state, having a 15% slower growth rate from Postnatal Day 3 to

adulthood (114). To date, *Mmp2*$^{-/-}$ mice have not been studied in models of PF. However, MMP-2 has potential to promote IPF pathogenesis by: (*1*) degrading lung ECM proteins; (*2*) promoting EMT; and (*3*) regulating Wnt/β-catenin signaling.

MMP-2 is localized close to breaks in epithelial basement membranes in IPF lungs, and has been linked to degradation of basement membranes (115), which induces angiogenesis (116, 117) and thereby promotes lung fibroproliferative responses (118, 119). Fibrotic responses are linked to endothelial-derived MMP-2. Vascular endothelial growth factor, an endothelial cell growth factor and profibrotic mediator contributing to bleomycin-mediated PF in mice (120), induces endothelial release of MMP-2 (121). Endothelial cells shed plasma membrane–derived vesicles (~300–600 nm in diameter) containing pro- and active MMP-2 (along with MMP-9, MT1-MMP, TIMP-1, and TIMP-2) (122). In a model of canine renal fibrosis, endothelial-derived extracellular vesicles expressing MMP-2 degraded renal basement membranes (123).

Several studies link MMP-2 to EMT. Transgenic mice overexpressing MMP-2 in kidney proximal tubular epithelium develop glomerulosclerosis, largely due to EMT (124). MMP-2 also promotes EMT in lens epithelial cells (125) and cardiac endocardial cushion–derived cells (126).

MMP-2 expression has been linked to aberrant activation of the Wnt/β-catenin signaling pathway (127), and Wnt signaling has been implicated in IPF pathogenesis (128–130). Silencing of β-catenin in mice using small interfering RNA decreases bleomycin-mediated PF, and reduces pulmonary TGF-β and Mmp-2 levels (131). It is also noteworthy that IPF typically develops in aging patients. Old mice have higher baseline lung levels of MMP-2 (and MMP-9) and are more susceptible to bleomycin-mediated PF than young mice (132). Whether age-related changes in lung levels of MMP-2 (and MMP-9) contribute to IPF is not known. There is one report suggesting that MMP-2 may have protective activities during fibrotic responses, as bleomycin-treated mice that received human umbilical cord endothelial cells intravenously were protected from PF and this was associated with increased lung levels of MMP-2 (133).

MMP-10

MMP-10 (stromelysin-2) is expressed by endothelial cells, fibroblasts, and macrophages. MMP-10 expression is increased in IPF lungs, but the cell types expressing MMP-10 were not identified (134). However, *in vitro* studies show that human lung fibroblast MMP-10 expression is up-regulated in human lung fibroblasts by their binding to type I collagen (135), but down-regulated as the stiffness of ECM to which they adhere increases (85). MMP-10 expression increases during endothelial cell activation (136, 137).

MMP-10 potential activities in IPF. *Mmp10*$^{-/-}$ mice are normal in the unchallenged state (138), but have not yet been studied in the bleomycin PF model. However, MMP-10 expression is increased in the fibrotic areas of the lungs of rats treated with cerium oxide (component of diesel exhaust) (139). MMP-10 promotes macrophage migration *in vitro* (140), and induces polarization of macrophages toward an M2 phenotype associated with increased collagenase activity in a skin wound-healing model (141). Thus, MMP-10 may have antifibrotic activities in the lung by increasing macrophage-mediated collagen degradation.

MMP-11

MMP-11 (stromelysin-3) is expressed by fibroblasts and activated intracellularly by a furin-like proconvertase enzyme. The promoter region of the MMP-11 gene is organized differently from that of other MMPs (Table 1). MMP-11 expression has not been assessed in IPF.

Mmp-11 potential activities during PF. *Mmp11*$^{-/-}$ mice have been generated (142), but have not yet been evaluated in models of PF. However, MMP-11 has potential to contribute to IPF, as MMP-11 activates Notch signaling (143), and Notch activation promotes myofibroblast differentiation (144).

MT-MMPs

MT1-MMP (MMP-14) is responsible for most of the cleavage of type I collagen that is associated with human and murine pulmonary fibroblasts (145). Whether this is the most important type I collagenase produced by pulmonary fibroblasts in the setting of IPF is not known.

MT-MMP expression in patients with IPF. MT1-MMP (MMP-14) is the most highly expressed MT-MMP in IPF lungs (146). MT1-MMP (MMP-14) and MT2-MMP (MMP-15) are localized in alveolar epithelial cells, MT3-MMP (MMP-16) in fibroblastic foci and alveolar epithelial cells, and MT5-MMP (MMP-24) in basal bronchiolar epithelial cells and areas of squamous metaplasia in IPF lungs (146).

Potential activities of MT-MMPs in IPF. *MT1-Mmp*$^{-/-}$ mice develop severe skeletal abnormalities and have impaired alveolar development at 1 month of age with an approximately 40% decreases in alveolar surface area, and increased mortality, which precludes their analysis in models of PF (147, 148). However, MT1-MMP has the potential to limit the development or progression of PF as MT1-MMP is a potent collagenase in other organs (149) and the dominant collagenase expressed by fibroblasts (145). MT1-MMP may also lead to reduced bleomycin-mediated PF and promote lung repair by increasing the recruitment and engraftment of mesenchymal stem cells in the murine lung (150). Alternatively, MT1-MMP may promote fibrotic responses to injury by activating latent TGF-β by cleaving latency-associated peptide (151) and/or inhibiting normal repair processes in the injured lung.

MT2-Mmp$^{-/-}$, *MT3-Mmp*$^{-/-}$, *MT4-Mmp*$^{-/-}$, and *MT5-Mmp*$^{-/-}$ mice (152) have been generated, but there are currently no published reports on their phenotype in PF models. To our knowledge, *MT6-Mmp*$^{-/-}$ mice (*Mmp25*$^{-/-}$ mice) have not yet been generated.

TIMPs

The four members of the TIMP family (TIMPs 1–4) are the most important endogenous inhibitors of MMPs. The aminoterminal domain of TIMPs binds to and inhibits the active site of MMPs. Each TIMP inhibits most MMPs, suggesting redundancy in their function. However, the MT-MMPs are inhibited predominantly by TIMP-2 and TIMP-3. In addition, the carboxyterminus of TIMP-1 forms a complex with the hemopexin domain of pro–MMP-9 to prevent pro–MMP-9 activation by stromelysin (153).

Timp-1$^{-/-}$, *Timp-2*$^{-/-}$, and *Timp3*$^{-/-}$ mice have similar pulmonary fibrotic responses to bleomycin as WT mice (154). Although *Timp4*$^{-/-}$ mice are normal in the unchallenged state (155), there are no published studies of their phenotype in PF models. Studies of TIMP compound–null mice in PF models are needed to better

understand the contributions of these inhibitors to lung fibrotic responses to injury.

Caveats of Interpreting Results of Studies of MMP Gene–Targeted Mice

There are caveats when interpreting results of studies of MMP gene–targeted mice in models of PF. The most commonly studied model of PF in mice (instillation of bleomycin into the lungs) has limitations as a model system for IPF. In particular, bleomycin induces a robust acute pulmonary inflammatory response in mice (35) whereas most patients with IPF do not have substantial pulmonary inflammation. Thus, the lack of a specific MMP might influence the results of the bleomycin model due to its role in the acute inflammatory phase of the model. In addition, unlike human IPF, bleomycin-mediated PF is self-limiting and/or resolves over time in some strains of mice (141). Ideally, MMP gene–targeted mice should be studied in other models of PF (156) and better models of IPF when these are developed.

The results of studies of MMP gene–targeted mice in murine models should be interpreted with caution as some MMPs that have been shown to be pro-fibrotic in mice have antifibrotic activities when tested in human cell culture systems. Also, given the functional redundancy that might exist within the MMP family (157) studies of MMP-deficient mice in models of PF must also consider the possibility that functional compensation occurs and that this could hinder our understanding of the roles of individual MMPs during PF. To address these issues, studies will need to alter the expression of individual MMPs in a cell-type and temporally-conditional manner. Also, in contrast to MMP-deficient mice, complete loss of expression of an MMP gene in humans is unlikely. Similarly, studying transgenic mice that over express an MMP also has limitations as an IPF model system as the expression level achieved may be higher or lower than that occurring in IPF lungs and/or in a different cell type from the main MMP-expressing cell(s) in IPF lungs. The response of an MMP gene–targeted mouse to a profibrotic stimulus may depend on how the animal was generated, other functions of the MMP that contribute to the observed phenotype, and potentially different expression patterns or functions of murine versus human MMPs. Thus, the functions of MMPs in injury processes may be cell, organ, and species specific.

Therapeutic Targeting of MMPs for IPF

The evidence from studies of MMP gene–targeted mice in animal models of PF indicates that many MMPs have potential as new therapeutic targets for IPF. Although relatively nonselective MMP inhibitors (MMPIs) had efficacy at limiting PF in murine models (158, 159), MMPIs had poor efficacy in randomized clinical trials for various cancers, and were associated with limiting side effects (160). In particular, dose-limiting musculoskeletal pain was a problematic off-target effect, although this was partly due to inhibition of other metalloproteinases, including proteinases with a disintegrin and a metalloproteinase domain (ADAMs) and ADAMs with a thrombospondin domain (ADAMTSs) (161). Thus, the development of more selective MMPIs, selective lung targeting, or other approaches to reduce MMP levels or activity in the lung might be a more effective therapeutic approach for IPF (162).

Inhibition of Profibrotic MMPs in the Lung

Potential rational targets for specific MMP inhibition based upon studies of gene-targeted mice in PF models include MMP-3, -7, -8, and -28. However, the beneficial activities of MMP-7 in host defense (163) and MMP-8 in inhibiting the growth of experimental tumors in mice (164) may make direct systemic inhibition of these two MMPs problematic as a therapeutic strategy for IPF. Direct MMP inhibition options include small-molecule hydroxymate inhibitors that chelate the Zn^{2+} ion at the active site. However, thus far, the specificity of many of these analogs is limited. Monoclonal antibodies blocking MMP activity are very specific and well tolerated (165, 166), but expensive and require parenteral administration. Using antisense nucleic acids that bind and silence mRNA molecules or ribosomes are another potential approach to inhibiting MMPs. Other approaches having potential as IPF therapeutics include: (*1*) activity-based probes that bind and only inhibit active MMPs (167); (*2*) novel biomaterials, such as injectable hydrogels that release specific inhibitors upon proteolytic release by the specific active MMP being targeted (168); and (*3*) interfering with upstream inducers of MMP activity.

Augmenting the Expression of Antifibrotic MMPs in the Lung

Augmenting the expression of antifibrotic MMP-13 and -19 may have therapeutic potential in IPF. In chondrocytes, two transcription factors, the CCAAT enhancer binding protein and runt-related transcription factor 2 induce MMP-13 promoter activity (169). It may be possible to inhibit lung fibrosis by increasing expression of CCAAT enhancer binding protein β and runt-related transcription factor 2 to increase MMP-13 expression in pulmonary cells. The MMP-19 promoter region is very similar to that of other MMPs, as it contains a TATA box, an AP-1 binding site, and a putative PEA-3 site. However, a region further upstream in the MMP-19 promoter has a large effect on transcriptional activity (170), and, unlike other MMPs, many cytokines (e.g., TNF-α, IL-6, TGF-β, IL-8, IL-15, IL-8, and CCL5) do not increase transcription of *MMP19* in keratinocytes. Thus, increasing the activity of the as-yet-unidentified transcription factors that bind to this upstream promoter element could selectively up-regulate the expression of MMP-19 and limit the progression of fibrotic responses to injury.

Epigenetic Approaches

Mechanisms of epigenetic regulation of gene expression include DNA methylation, histone acetylation or methylation, RNA methylation, and microRNA regulation. MMP expression is regulated by epigenetic mechanisms in cells and also in organs in diseases other than IPF. IPF is a disease associated with aging and a history of cigarette smoke exposure, which both influence epigenetic modifications of genes. There is a growing body of evidence that epigenetic regulation of genes contributes to IPF (171–177). DNA methylation occurs at CpG sites (in which cytosine nucleotide occurs next to a guanine nucleotide) in promoter regions, leading to silencing of gene expression. However, MMPs that are clustered in the 11q22.3 chromosomal region (MMP-1, -3, -7, -8, -10, -12, -13, and -20) have relatively few CpG islands, and DNA methylation is unlikely to play a large role in regulation of their expression.

Nonetheless, analysis of global changes in DNA methylation in IPF tissue demonstrated hypomethylation of the *MMP7* promoter, associated with increased MMP-7 expression (176).

Histone acetylation/deactetylation and microRNA regulation are linked to MMP regulation. Histone deacetylase 2 reduces histone-3 and -4 acetylation in cell lines, thereby reducing MMP-9 expression (178). Hepatic stellate cells (a source of myofibroblasts during hepatic fibrosis) suppress MMP-9 and -13 expression by up-regulating histone deacetylase-4 during their differentiation (179). In aortic smooth muscle cells, oxidized low density lipoprotein up-regulates microRNA 29b expression, which inhibits transcription of DNA methyltransferase 3B to increase MMP-2 and -9 expression (180). If MMP expression is shown to be regulated by epigenetic mechanisms in IPF lungs, manipulating the epigenetic control of MMP gene expression (increasing the expression of protective MMPs or silencing the expression of profibrotic MMPs) could be another promising therapeutic avenue for IPF.

Exosite Targeting

Exosite-masking therapies bind and protect the cleavage site of the specific MMP substrate being targeted. For example, the recombinant hemopexin domain of MT1-MMP efficiently competes with full-length MT1-MMP for exosite binding, and thereby inhibits collagen cleavage by full-length MT1-MMP (181). By altering the noncatalytic domains of ADAMTS, the capacity of ADAMTS to recognize and cleave exosites can be modified (182). It is also possible to block the interaction of a specific MMP with a key substrate that promotes IPF pathogenesis while preserving its capacity to cleave other substrates. This may be an important approach, as individual MMPs generally have several key substrates, and MMP-mediated cleavage of some of these substrates may have beneficial activities for the host (including antitumor and host defense activities). However, many of the crucial substrates for many MMPs implicated in IPF have yet to identified, and this information is needed before exosite targeting can advance as a therapeutic approach for IPF.

Future Directions

Because of the complexity of the expression and regulation of MMPs, there is still much to learn about MMP activity during pulmonary fibrotic responses to injury. MMPs are expressed in different tissues and various cell types, and can have beneficial and/or deleterious activities in different organs. Future investigations should focus on tissue-specific regulation of MMP expression. The crucial activators of most pro-MMPs are not known, and these activators may also prove to be important therapeutic targets. Activation of MMPs *in vivo* often involves cell surface adapter proteins that bring together an MMP and its substrate to increase their effective concentration. In many cases, these adapter proteins are unknown and may also prove to be highly specific therapeutic targets. The mechanism of epigenetic changes in MMP expression and activity in humans is another important area ripe for future investigation. The development of more specific MMPIs is also crucial for developing effective therapies lacking unwanted off-target effects. However, the tight regulation of MMP expression and minimal contribution to normal tissue homeostasis make MMPs promising targets for manipulation in IPF. ■

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