Matrix Metalloproteinases as Therapeutic Targets for Idiopathic Pulmonary Fibrosis

Vanessa J. Craig^{1,2}, Li Zhang¹, James S. Hagood^{3,4}, and Caroline A. Owen^{1,5}

¹Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts; ²Division of Pulmonary, Critical Care, and Sleep Medicine, University of California–San Diego, La Jolla, California; ³Division of Pediatric Respiratory Medicine, University of California–San Diego, La Jolla, California, and ⁴Rady Children's Hospital of San Diego, San Diego, California; and ⁵Lovelace Respiratory Research Institute, Albuquerque, New Mexico

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.

(Received in original form January 14, 2015; accepted in final form June 29, 2015)

This work was supported by Public Health Service, National Heart, Lung, and Blood Institute grants HL063137, HL086814, HL111835, HL105339, HL114501, AI111475-01, T32 HL007633, and T32 HL007633, Flight Attendants Medical Research Institute grant CIA123046, and the Brigham and Women's Hospital–Lovelace Respiratory Research Institute Consortium.

Author Contributions: Conception and design—V.J.C. and C.A.O.; drafting the manuscript for important intellectual content—V.J.C., L.Z., J.S.H., and C.A.O.

Correspondence and requests for reprints should be addressed to Caroline A. Owen, M.D., Ph.D., Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Room 855B, HIM Building, Boston, MA 02115. E-mail: cowen@rics.bwh.harvard.edu

Am J Respir Cell Mol Biol Vol 53, Iss 5, pp 585–600, Nov 2015

Copyright © 2015 by the American Thoracic Society

Originally Published in Press as DOI: 10.1165/rcmb.2015-0020TR on June 29, 2015

Internet address: www.atsjournals.org

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 glycosylphosphatidylinositol 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 glycosylphosphatidylinositol (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 glycosylphosphatidylinositol 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). *Mmp3* 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- κ B, PEA-3, AP-1, TIE	mRNA stability, p38, Rac-1	183-186
MMP-8	C/EBP- β	TGF- β , TNF- α , IL-1 β	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-1 β , 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
MMP-10	AP-1, PEA-3	TGF- α , KGF, IFN- γ , TGF- β	202
MMP-11	PEA-3, NF-1, RARE, AP-1-like	TSH	203
MT1-MMP	SAF-1, HBS, Tcf-4	Fibronectin, soluble E-cadherin	143, 204
			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 kinases; 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- β 1 and

TNF- α in fibroblasts (35, 36), and IL-1 β 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-1 α 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- β 1 (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 protein levels in BALF (17, 39, 40)
	• Increased steady-state mRNA in peripheral blood monocytes (38)	• 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*
<i>Mmp3</i> ^{-/-}	Bleomycin	↓	Overexpression of MMP-3 promotes fibrosis (18)
<i>Mmp7</i> ^{-/-}	Bleomycin	↓	Decreased pulmonary inflammation after 14 days (29)
<i>Mmp8</i> ^{-/-}	Bleomycin	↓	Increased accumulation of lung macrophages (35, 41)
<i>Mmp9</i> ^{-/-}	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
<i>Mmp12</i> ^{-/-}	Bleomycin	No change	Similar pulmonary inflammation (69)
<i>Mmp12</i> ^{-/-}	FAS Antibody	↓	Similar pulmonary inflammation (70)
<i>Mmp13</i> ^{-/-}	Bleomycin	↑	Increased pulmonary inflammation (75)
<i>Mmp13</i> ^{-/-}	Radiation	↓	Decreased pulmonary inflammation (76)
<i>Mmp19</i> ^{-/-}	Bleomycin	↑	Associated with decreased PTGS2 (83)
<i>Mmp28</i> ^{-/-}	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-1 β , 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, *Mmp13* 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 Zn²⁺-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- β 1 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 metalloproteinase.

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 $\alpha 2\beta 1$ -integrin on cell surfaces (100), which induces signaling via $\alpha 2\beta 1$ -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-1 α , 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 NH₂-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% decrease 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).

Timp1^{-/-}, *Timp2*^{-/-}, 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 profibrotic 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 hydroxamate 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/deacetylation 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 be 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 *MMPi*s 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. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Schwartz DA, Helmers RA, Galvin JR, Van Fossen DS, Frees KL, Dayton CS, Burmeister LF, Hunninghake GW. Determinants of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1994;149:450-454.
- King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L, et al.; ASCEND Study Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2083-2092.
- Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, et al.; INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2071-2082.
- Puente XS, Sánchez LM, Overall CM, López-Otín C. Human and mouse proteases: a comparative genomic approach. *Nat Rev Genet* 2003;4:544-558.
- Clark IM, Cawston TE. Fragments of human fibroblast collagenase: purification and characterization. *Biochem J* 1989;263:201-206.
- Knäuper V, Osthus A, DeClerck YA, Langley KE, Bläser J, Tschesche H. Fragmentation of human polymorphonuclear-leucocyte collagenase. *Biochem J* 1993;291:847-854.
- Knäuper V, Cowell S, Smith B, López-Otín C, O'Shea M, Morris H, Zardi L, Murphy G. The role of the C-terminal domain of human collagenase-3 (*MMP-13*) in the activation of procollagenase-3, substrate specificity, and tissue inhibitor of metalloproteinase interaction. *J Biol Chem* 1997;272:7608-7616.
- Chung L, Dinakarandian D, Yoshida N, Lauer-Fields JL, Fields GB, Visse R, Nagase H. Collagenase unwinds triple-helical collagen prior to peptide bond hydrolysis. *EMBO J* 2004;23:3020-3030.
- O'Farrell TJ, Pourmotabbed T. The fibronectin-like domain is required for the type V and XI collagenolytic activity of gelatinase B. *Arch Biochem Biophys* 1998;354:24-30.
- Chen EI, Li W, Godzik A, Howard EW, Smith JW. A residue in the S2 subsite controls substrate selectivity of matrix metalloproteinase-2 and matrix metalloproteinase-9. *J Biol Chem* 2003;278:17158-17163.
- Haas TL, Stitelman D, Davis SJ, Apte SS, Madri JA. Egr-1 mediates extracellular matrix-driven transcription of membrane type 1 matrix metalloproteinase in endothelium. *J Biol Chem* 1999;274:22679-22685.
- Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. *J Cell Physiol* 2007;211:19-26.
- Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA* 1990;87:5578-5582.
- Owen CA, Hu Z, Barrick B, Shapiro SD. Inducible expression of tissue inhibitor of metalloproteinases-resistant matrix metalloproteinase-9 on the cell surface of neutrophils. *Am J Respir Cell Mol Biol* 2003;29:283-294.
- Owen CA, Hu Z, Lopez-Otin C, Shapiro SD. Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* 2004;172:7791-7803.
- Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GL. Mechanism of cell surface activation of 72-kDa type IV collagenase: isolation of the activated form of the membrane metalloprotease. *J Biol Chem* 1995;270:5331-5338.
- McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. *MMP* expression and abnormal lung permeability are important determinants of outcome in IPF. *Eur Respir J* 2009;33:77-84.

18. Yamashita CM, Dolgonos L, Zemans RL, Young SK, Robertson J, Briones N, Suzuki T, Campbell MN, Gauldie J, Radisky DC, *et al.* Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. *Am J Pathol* 2011;179:1733–1745.
19. Maeda S, Dean DD, Gomez R, Schwartz Z, Boyan BD. The first stage of transforming growth factor beta1 activation is release of the large latent complex from the extracellular matrix of growth plate chondrocytes by matrix vesicle stromelysin-1 (MMP-3). *Calcif Tissue Int* 2002;70:54–65.
20. Heljasvaara R, Nyberg P, Luostarinen J, Parikka M, Heikkilä P, Rehn M, Sorsa T, Salo T, Pihlajaniemi T. Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteinases. *Exp Cell Res* 2005;307:292–304.
21. Richter AG, McKeown S, Rathinam S, Harper L, Rajesh P, McAuley DF, Heljasvaara R, Thickett DR. Soluble endostatin is a novel inhibitor of epithelial repair in idiopathic pulmonary fibrosis. *Thorax* 2009;64:156–161.
22. García-de-Alba C, Becerril C, Ruiz V, González Y, Reyes S, García-Alvarez J, Selman M, Pardo A. Expression of matrix metalloproteinases by fibrocytes: possible role in migration and homing. *Am J Respir Crit Care Med* 2010;182:1144–1152.
23. Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, Lindell KO, Cisneros J, Macdonald SD, Pardo A, *et al.* MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. *PLoS Med* 2008;5:e93.
24. Fujishima S, Shiomi T, Yamashita S, Yogo Y, Nakano Y, Inoue T, Nakamura M, Tasaka S, Hasegawa N, Aikawa N, *et al.* Production and activation of matrix metalloproteinase 7 (matrilysin 1) in the lungs of patients with idiopathic pulmonary fibrosis. *Arch Pathol Lab Med* 2010;134:1136–1142.
25. Pardo A, Gibson K, Cisneros J, Richards TJ, Yang Y, Becerril C, Yousem S, Herrera I, Ruiz V, Selman M, *et al.* Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. *PLoS Med* 2005;2:e251.
26. Richards TJ, Park C, Chen Y, Gibson KF, Peter Di Y, Pardo A, Watkins SC, Choi AM, Selman M, Pilewski J, *et al.* Allele-specific transactivation of matrix metalloproteinase 7 by FOXA2 and correlation with plasma levels in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L746–L754.
27. Gharib SA, Altemeier WA, Van Winkle LS, Plopper CG, Schlesinger SY, Buell CA, Brauer R, Lee V, Parks WC, Chen P. Matrix metalloproteinase-7 coordinates airway epithelial injury response and differentiation of ciliated cells. *Am J Respir Cell Mol Biol* 2013;48:390–396.
28. Manicone AM, Huizar I, McGuire JK. Matrilysin (matrix metalloproteinase-7) regulates anti-inflammatory and antifibrotic pulmonary dendritic cells that express CD103 (alpha(E)beta(7)-integrin). *Am J Pathol* 2009;175:2319–2331.
29. Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, *et al.* Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 2002;99:6292–6297.
30. McGuire JK, Li Q, Parks WC. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am J Pathol* 2003;162:1831–1843.
31. Zhang L, Rice AB, Adler K, Sannes P, Martin L, Gladwell W, Koo JS, Gray TE, Bonner JC. Vanadium stimulates human bronchial epithelial cells to produce heparin-binding epidermal growth factor-like growth factor: a mitogen for lung fibroblasts. *Am J Respir Cell Mol Biol* 2001;24:123–131.
32. Chapoval SP, Lee CG, Tang C, Keegan AD, Cohn L, Bottomly K, Elias JA. Lung vascular endothelial growth factor expression induces local myeloid dendritic cell activation. *Clin Immunol* 2009;132:371–384.
33. Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R, Kämmerer U. Human decidua and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases. *Mol Hum Reprod* 2011;17:637–652.
34. Akhavan MA, Madden L, Buysschaert I, Sivakumar B, Kang N, Paleolog EM. Hypoxia upregulates angiogenesis and synovial cell migration in rheumatoid arthritis. *Arthritis Res Ther* 2009;11:R64.
35. Craig VJ, Quintero PA, Fyfe SE, Patel AS, Knolle MD, Kobzik L, Owen CA. Profibrotic activities for matrix metalloproteinase-8 during bleomycin-mediated lung injury. *J Immunol* 2013;190:4283–4296.
36. Hanemaaijer R, Sorsa T, Kontinen YT, Ding Y, Sutinen M, Visser H, van Hinsbergh VW, Helaakoski T, Kainulainen T, Rönkä H, *et al.* Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells: regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997;272:31504–31509.
37. Herman MP, Sukhova GK, Libby P, Gerdes N, Tang N, Horton DB, Kilbride M, Breitbart RE, Chun M, Schönbeck U. Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. *Circulation* 2001;104:1899–1904.
38. Craig VJ, Polverino F, Lauchó-Contreras ME, Shi Y, Liu Y, Osorio JC, Tsefaigzi Y, Pinto-Plata V, Gochuico BR, Rosas IO, *et al.* Mononuclear phagocytes and airway epithelial cells: novel sources of matrix metalloproteinase-8 (MMP-8) in patients with idiopathic pulmonary fibrosis. *PLoS One* 2014;9:e97485.
39. Henry MT, McMahon K, Mackarel AJ, Prikk K, Sorsa T, Maisi P, Sepper R, Fitzgerald MX, O'Connor CM. Matrix metalloproteinases and tissue inhibitor of metalloproteinase-1 in sarcoidosis and IPF. *Eur Respir J* 2002;20:1220–1227.
40. Willems S, Verleden SE, Vanaudenaerde BM, Wynants M, Doms C, Yserbyt J, Somers J, Verbeken EK, Verleden GM, Wuyts WA. Multiplex protein profiling of bronchoalveolar lavage in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. *Ann Thorac Med* 2013;8:38–45. [Published erratum appears in *Ann Thorac Med* 8:85.]
41. García-Prieto E, González-López A, Cabrera S, Astudillo A, Gutiérrez-Fernández A, Fanjul-Fernández M, Batalla-Solis E, Puente XS, Fueyo A, López-Otín C, *et al.* Resistance to bleomycin-induced lung fibrosis in MMP-8 deficient mice is mediated by interleukin-10. *PLoS One* 2010;5:e13242.
42. Jiang D, Liang J, Hodge J, Lu B, Zhu Z, Yu S, Fan J, Gao Y, Yin Z, Homer R, *et al.* Regulation of pulmonary fibrosis by chemokine receptor CXCR3. *J Clin Invest* 2004;114:291–299.
43. Tager AM, Kradin RL, LaCamera P, Bercury SD, Campanella GS, Leary CP, Polosukhin V, Zhao LH, Sakamoto H, Blackwell TS, *et al.* Inhibition of pulmonary fibrosis by the chemokine IP-10/CXCL10. *Am J Respir Cell Mol Biol* 2004;31:395–404.
44. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest* 2004;113:243–252.
45. Owen CA, Campbell EJ. The cell biology of leukocyte-mediated proteolysis. *J Leukoc Biol* 1999;65:137–150.
46. Lemjabbar H, Gosset P, Lechapt-Zalcman E, Franco-Montoya ML, Wallaert B, Harf A, Lafuma B. Overexpression of alveolar macrophage gelatinase B (MMP-9) in patients with idiopathic pulmonary fibrosis: effects of steroid and immunosuppressive treatment. *Am J Respir Cell Mol Biol* 1999;20:903–913.
47. Suga M, Iyonaga K, Okamoto T, Gushima Y, Miyakawa H, Akaike T, Ando M. Characteristic elevation of matrix metalloproteinase activity in idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2000;162:1949–1956.
48. Yu WH, Woessner JF Jr, McNeish JD, Stamenkovic I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev* 2002;16:307–323.
49. Betsuyaku T, Fukuda Y, Parks WC, Shipley JM, Senior RM. Gelatinase B is required for alveolar bronchiolization after intratracheal bleomycin. *Am J Pathol* 2000;157:525–535.
50. Odajima N, Betsuyaku T, Nasuhara Y, Nishimura M. Loss of caveolin-1 in bronchiolization in lung fibrosis. *J Histochem Cytochem* 2007;55:899–909.
51. Kawamoto M, Fukuda Y. Cell proliferation during the process of bleomycin-induced pulmonary fibrosis in rats. *Acta Pathol Jpn* 1990;40:227–238.
52. Collins JF, Orozco CR, McCullough B, Coalson JJ, Johanson WG Jr. Pulmonary fibrosis with small-airway disease: a model in nonhuman primates. *Exp Lung Res* 1982;3:91–108.
53. Fukuda Y, Takemura T, Ferrans VJ. Evolution of metaplastic squamous cells of alveolar walls in pulmonary fibrosis produced by paraquat. An ultrastructural and immunohistochemical study. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1989;58:27–43.

54. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L, Piccoli P, Cangi G, Semenzato G, Doglioni C. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. *Lab Invest* 2002;82:1335–1345.
55. Kawanami O, Ferrans VJ, Crystal RG. Structure of alveolar epithelial cells in patients with fibrotic lung disorders. *Lab Invest* 1982;46:39–53.
56. Lanone S, Zheng T, Zhu Z, Liu W, Lee CG, Ma B, Chen Q, Homer RJ, Wang J, Rabach LA, et al. Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest* 2002;110:463–474.
57. Cabrera S, Gaxiola M, Arreola JL, Ramírez R, Jara P, D'Armiento J, Richards T, Selman M, Pardo A. Overexpression of MMP9 in macrophages attenuates pulmonary fibrosis induced by bleomycin. *Int J Biochem Cell Biol* 2007;39:2324–2338.
58. Pilewski JM, Liu L, Henry AC, Knauer AV, Feghali-Bostwick CA. Insulin-like growth factor binding proteins 3 and 5 are overexpressed in idiopathic pulmonary fibrosis and contribute to extracellular matrix deposition. *Am J Pathol* 2005;166:399–407.
59. Ruiz XD, Mlakar LR, Yamaguchi Y, Su Y, Larregina AT, Pilewski JM, Feghali-Bostwick CA. Syndecan-2 is a novel target of insulin-like growth factor binding protein-3 and is over-expressed in fibrosis. *PLoS One* 2012;7:e43049.
60. Klass CM, Couchman JR, Woods A. Control of extracellular matrix assembly by syndecan-2 proteoglycan. *J Cell Sci* 2000;113:493–506.
61. Chen L, Klass C, Woods A. Syndecan-2 regulates transforming growth factor-beta signaling. *J Biol Chem* 2004;279:15715–15718.
62. Park H, Kim Y, Lim Y, Han I, Oh ES. Syndecan-2 mediates adhesion and proliferation of colon carcinoma cells. *J Biol Chem* 2002;277:29730–29736.
63. Belaouaj A, Shipley JM, Kobayashi DK, Zimonjic DB, Popescu N, Silverman GA, Shapiro SD. Human macrophage metalloelastase: genomic organization, chromosomal location, gene linkage, and tissue-specific expression. *J Biol Chem* 1995;270:14568–14575.
64. Huang WC, Sala-Newby GB, Susana A, Johnson JL, Newby AC. Classical macrophage activation up-regulates several matrix metalloproteinases through mitogen activated protein kinases and nuclear factor- κ B. *PLoS One* 2012;7:e42507.
65. Manetti M, Guiducci S, Romano E, Bellando-Randone S, Conforti ML, Ibba-Manneschi L, Matucci-Cerinic M. Increased serum levels and tissue expression of matrix metalloproteinase-12 in patients with systemic sclerosis: correlation with severity of skin and pulmonary fibrosis and vascular damage. *Ann Rheum Dis* 2012;71:1064–1072.
66. Manetti M, Ibba-Manneschi L, Fatini C, Guiducci S, Cuomo G, Bonino C, Bazzichi L, Liakouli V, Giacomelli R, Abbate R, et al. Association of a functional polymorphism in the matrix metalloproteinase-12 promoter region with systemic sclerosis in an Italian population. *J Rheumatol* 2010;37:1852–1857.
67. Kang HR, Cho SJ, Lee CG, Homer RJ, Elias JA. Transforming growth factor (TGF)-beta1 stimulates pulmonary fibrosis and inflammation via a Bax-dependent, bid-activated pathway that involves matrix metalloproteinase-12. *J Biol Chem* 2007;282:7723–7732.
68. Shukla A, Barrett TF, Nakayama KI, Nakayama K, Mossman BT, Lounsbury KM. Transcriptional up-regulation of MMP12 and MMP13 by asbestos occurs via a PKCdelta-dependent pathway in murine lung. *FASEB J* 2006;20:997–999.
69. Manoury B, Nenau S, Guenon I, Boichot E, Planquois JM, Bertrand CP, Lagente V. Macrophage metalloelastase (MMP-12) deficiency does not alter bleomycin-induced pulmonary fibrosis in mice. *J Inflamm (Lond)* 2006;3:2.
70. Matute-Bello G, Wurfel MM, Lee JS, Park DR, Frevert CW, Madtes DK, Shapiro SD, Martin TR. Essential role of MMP-12 in Fas-induced lung fibrosis. *Am J Respir Cell Mol Biol* 2007;37:210–221.
71. Madala SK, Pesce JT, Ramalingam TR, Wilson MS, Minnicozzi S, Cheever AW, Thompson RW, Mentink-Kane MM, Wynn TA. Matrix metalloproteinase 12-deficiency augments extracellular matrix degrading metalloproteinases and attenuates IL-13-dependent fibrosis. *J Immunol* 2010;184:3955–3963.
72. Sand JM, Larsen L, Hogaboam C, Martinez F, Han M, Rossel Larsen M, Nawrocki A, Zheng Q, Karsdal MA, Leeming DJ. MMP mediated degradation of type IV collagen alpha 1 and alpha 3 chains reflects basement membrane remodeling in experimental and clinical fibrosis—validation of two novel biomarker assays. *PLoS One* 2013;8:e84934.
73. Ravanti L, Heino J, López-Otín C, Kähäri VM. Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. *J Biol Chem* 1999;274:2446–2455.
74. Knäuper V, Will H, López-Otín C, Smith B, Atkinson SJ, Stanton H, Hembry RM, Murphy G. Cellular mechanisms for human procollagenase-3 (MMP-13) activation: evidence that MT1-MMP (MMP-14) and gelatinase a (MMP-2) are able to generate active enzyme. *J Biol Chem* 1996;271:17124–17131.
75. Nkyimbeng T, Ruppert C, Shiomi T, Dahal B, Lang G, Seeger W, Okada Y, D'Armiento J, Günther A. Pivotal role of matrix metalloproteinase 13 in extracellular matrix turnover in idiopathic pulmonary fibrosis. *PLoS One* 2013;8:e73279.
76. Flechsig P, Hartenstein B, Teurich S, Dadrich M, Hauser K, Abdollahi A, Gröckl HJ, Angel P, Huber PE. Loss of matrix metalloproteinase-13 attenuates murine radiation-induced pulmonary fibrosis. *Int J Radiat Oncol Biol Phys* 2010;77:582–590.
77. Sen AI, Shiomi T, Okada Y, D'Armiento JM. Deficiency of matrix metalloproteinase-13 increases inflammation after acute lung injury. *Exp Lung Res* 2010;36:615–624.
78. Uchinami H, Seki E, Brenner DA, D'Armiento J. Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. *Hepatology* 2006;44:420–429.
79. Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, Iredale JP. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol* 2007;178:5288–5295.
80. Stracke JO, Hutton M, Stewart M, Pendás AM, Smith B, López-Otín C, Murphy G, Knäuper V. Biochemical characterization of the catalytic domain of human matrix metalloproteinase 19: evidence for a role as a potent basement membrane degrading enzyme. *J Biol Chem* 2000;275:14809–14816.
81. Mauch S, Kolb C, Kolb B, Sadowski T, Sedlacek R. Matrix metalloproteinase-19 is expressed in myeloid cells in an adhesion-dependent manner and associates with the cell surface. *J Immunol* 2002;168:1244–1251.
82. Hieta N, Impola U, López-Otín C, Saarialho-Kere U, Kähäri VM. Matrix metalloproteinase-19 expression in dermal wounds and by fibroblasts in culture. *J Invest Dermatol* 2003;121:997–1004.
83. Yu G, Kovkarova-Naumovski E, Jara P, Parwani A, Kass D, Ruiz V, Lopez-Otín C, Rosas IO, Gibson KF, Cabrera S, et al. Matrix metalloproteinase-19 is a key regulator of lung fibrosis in mice and humans. *Am J Respir Crit Care Med* 2012;186:752–762.
84. Huang SK, White ES, Wettlaufer SH, Griffka H, Hogaboam CM, Thannickal VJ, Horowitz JC, Peters-Golden M. Prostaglandin E(2) induces fibroblast apoptosis by modulating multiple survival pathways. *FASEB J* 2009;23:4317–4326.
85. Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* 2010;190:693–706.
86. White ES, Atrasz RG, Dickie EG, Aronoff DM, Stambolic V, Mak TW, Moore BB, Peters-Golden M. Prostaglandin E(2) inhibits fibroblast migration by E-prostanoid 2 receptor-mediated increase in PTEN activity. *Am J Respir Cell Mol Biol* 2005;32:135–141.
87. Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M. Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. *J Clin Invest* 1995;95:1861–1868.
88. Beck IM, Rückert R, Brandt K, Mueller MS, Sadowski T, Brauer R, Schirmacher P, Mentlein R, Sedlacek R. MMP19 is essential for T cell development and T cell-mediated cutaneous immune responses. *PLoS One* 2008;3:e2343.

89. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, Feingold E, Juan-Guardela BM, Richards TJ, Lussier Y, *et al.* Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. *Sci Transl Med* 2013;5:205ra136.
90. Jirouskova M, Zbodakova O, Gregor M, Chalupsky K, Sarnova L, Hajduch M, Ehrmann J, Jirkovska M, Sedlacek R. Hepatoprotective effect of MMP-19 deficiency in a mouse model of chronic liver fibrosis. *PLoS One* 2012;7:e46271.
91. Illman SA, Keski-Oja J, Parks WC, Lohi J. The mouse matrix metalloproteinase, epilysin (MMP-28), is alternatively spliced and processed by a furin-like proprotein convertase. *Biochem J* 2003;375:191–197.
92. Lohi J, Wilson CL, Roby JD, Parks WC. Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. *J Biol Chem* 2001;276:10134–10144.
93. Pardo A, Selman M, Kaminski N. Approaching the degradome in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008;40:1141–1155.
94. Gharib SA, Johnston LK, Huizar I, Birkland TP, Hanson J, Wang Y, Parks WC, Manicone AM. MMP28 promotes macrophage polarization toward M2 cells and augments pulmonary fibrosis. *J Leukoc Biol* 2014;95:9–18.
95. Song E, Ouyang N, Hörbelt M, Antus B, Wang M, Exton MS. Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. *Cell Immunol* 2000;204:19–28.
96. Gibbons MA, MacKinnon AC, Ramachandran P, Dhaliwal K, Duffin R, Phytian-Adams AT, van Rooijen N, Haslett C, Howie SE, Simpson AJ, *et al.* Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. *Am J Respir Crit Care Med* 2011;184:569–581.
97. Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, Gulati M, Homer RJ, Russell T, van Rooijen N, *et al.* TGF- β driven lung fibrosis is macrophage dependent and blocked by serum amyloid P. *Int J Biochem Cell Biol* 2011;43:154–162.
98. Illman SA, Lehti K, Keski-Oja J, Lohi J. Epilysin (MMP-28) induces TGF- β mediated epithelial to mesenchymal transition in lung carcinoma cells. *J Cell Sci* 2006;119:3856–3865.
99. Balbín M, Fueyo A, Knäuper V, López JM, Alvarez J, Sánchez LM, Quesada V, Bordallo J, Murphy G, López-Otin C. Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation. *J Biol Chem* 2001;276:10253–10262.
100. Dumin JA, Dickson SK, Stricker TP, Bhattacharyya-Pakrasi M, Roby JD, Santoro SA, Parks WC. Pro-collagenase-1 (matrix metalloproteinase-1) binds the $\alpha(2)\beta(1)$ integrin upon release from keratinocytes migrating on type I collagen. *J Biol Chem* 2001;276:29368–29374.
101. Riikonen T, Westermarck J, Koivisto L, Broberg A, Kähäri VM, Heino J. Integrin $\alpha 2 \beta 1$ is a positive regulator of collagenase (MMP-1) and collagen $\alpha 1(I)$ gene expression. *J Biol Chem* 1995;270:13548–13552.
102. Suzuki K, Enghild JJ, Morodomi T, Salvesen G, Nagase H. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry* 1990;29:10261–10270.
103. Checa M, Ruiz V, Montañó M, Velázquez-Cruz R, Selman M, Pardo A. MMP-1 polymorphisms and the risk of idiopathic pulmonary fibrosis. *Hum Genet* 2008;124:465–472.
104. Vij R, Noth I. Peripheral blood biomarkers in idiopathic pulmonary fibrosis. *Transl Res* 2012;159:218–227.
105. Selman M, Ruiz V, Cabrera S, Segura L, Ramírez R, Barrios R, Pardo A. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis: a prevailing nondegradative lung microenvironment? *Am J Physiol Lung Cell Mol Physiol* 2000;279:L562–L574.
106. Fanjul-Fernández M, Folgueras AR, Fueyo A, Balbín M, Suárez MF, Fernández-García MS, Shapiro SD, Freije JM, López-Otin C. Matrix metalloproteinase Mmp-1a is dispensable for normal growth and fertility in mice and promotes lung cancer progression by modulating inflammatory responses. *J Biol Chem* 2013;288:14647–14656.
107. Herrera I, Cisneros J, Maldonado M, Ramírez R, Ortiz-Quintero B, Anso E, Chandel NS, Selman M, Pardo A. Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. *J Biol Chem* 2013;288:25964–25975.
108. Vaalamo M, Mattila L, Johansson N, Kariniemi AL, Karjalainen-Lindsberg ML, Kähäri VM, Saarialho-Kere U. Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. *J Invest Dermatol* 1997;109:96–101.
109. Yang C, Hu G, Tan D. Effects of MMP-1 expressing plasmid on rat liver fibrosis [in Chinese]. *Zhonghua Gan Zang Bing Za Zhi* 1999;7:230–232.
110. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 1994;370:61–65.
111. Owen CA. Leukocyte cell surface proteinases: regulation of expression, functions, and mechanisms of surface localization. *Int J Biochem Cell Biol* 2008;40:1246–1272.
112. Fukuda Y, Ishizaki M, Kudoh S, Kitaichi M, Yamanaka N. Localization of matrix metalloproteinases-1, -2, and -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. *Lab Invest* 1998;78:687–698.
113. Hayashi T, Stetler-Stevenson WG, Fleming MV, Fishback N, Koss MN, Liotta LA, Ferrans VJ, Travis WD. Immunohistochemical study of metalloproteinases and their tissue inhibitors in the lungs of patients with diffuse alveolar damage and idiopathic pulmonary fibrosis. *Am J Pathol* 1996;149:1241–1256.
114. Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S. Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *J Biol Chem* 1997;272:22389–22392.
115. Ruiz V, Ordóñez RM, Berumen J, Ramírez R, Uhal B, Becerril C, Pardo A, Selman M. Unbalanced collagenases/TIMP-1 expression and epithelial apoptosis in experimental lung fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L1026–L1036.
116. Bergers K, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, *et al.* Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2000;2:737–744.
117. Nguyen M, Arkell J, Jackson CJ. Human endothelial gelatinases and angiogenesis. *Int J Biochem Cell Biol* 2001;33:960–970.
118. Keane MP, Arenberg DA, Lynch JP III, Whyte RI, Iannettoni MD, Burdick MD, Wilke CA, Morris SB, Glass MC, DiGiovine B, *et al.* The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. *J Immunol* 1997;159:1437–1443.
119. Keane MP, Belperio JA, Burdick MD, Lynch JP, Fishbein MC, Strieter RM. ENA-78 is an important angiogenic factor in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001;164:2239–2242.
120. Hamada N, Kuwano K, Yamada M, Hagimoto N, Hiasa K, Egashira K, Nakashima N, Maeyama T, Yoshimi M, Nakanishi Y. Anti-vascular endothelial growth factor gene therapy attenuates lung injury and fibrosis in mice. *J Immunol* 2005;175:1224–1231.
121. Narumiya H, Zhang Y, Fernandez-Patron C, Guilbert LJ, Davidge ST. Matrix metalloproteinase-2 is elevated in the plasma of women with preeclampsia. *Hypertens Pregnancy* 2001;20:185–194.
122. Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 2002;160:673–680.
123. Aresu L, Benali S, Garbisa S, Gallo E, Castagnaro M. Matrix metalloproteinases and their role in the renal epithelial mesenchymal transition. *Histol Histopathol* 2011;26:307–313.
124. Cheng S, Pollock AS, Mahimkar R, Olson JL, Lovett DH. Matrix metalloproteinase 2 and basement membrane integrity: A unifying mechanism for progressive renal injury. *FASEB J* 2006;20:1898–1900.
125. Seomun Y, Kim J, Lee EH, Joo CK. Overexpression of matrix metalloproteinase-2 mediates phenotypic transformation of lens epithelial cells. *Biochem J* 2001;358:41–48.

126. Song W, Jackson K, McGuire PG. Degradation of type IV collagen by matrix metalloproteinases is an important step in the epithelial-mesenchymal transformation of the endocardial cushions. *Dev Biol* 2000;227:606–617.
127. Wu B, Crampton SP, Hughes CC. Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. *Immunity* 2007;26:227–239.
128. Lam AP, Herazo-Maya JD, Sennello JA, Flozak AS, Russell S, Mutlu GM, Budinger GR, DasGupta R, Varga J, Kaminski N, et al. Wnt coreceptor Lrp5 is a driver of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014;190:185–195.
129. Meuten T, Hickey A, Franklin K, Grossi B, Tobias J, Newman DR, Jennings SH, Correa M, Sannes PL. WNT7B in fibroblastic foci of idiopathic pulmonary fibrosis. *Respir Res* 2012;13:62.
130. Chilosi M, Poletti V, Zamò A, Lestani M, Montagna L, Piccoli P, Pedron S, Bertaso M, Scarpa A, Murer B, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. *Am J Pathol* 2003;162:1495–1502.
131. Kim TH, Kim SH, Seo JY, Chung H, Kwak HJ, Lee SK, Yoon HJ, Shin DH, Park SS, Sohn JW. Blockade of the Wnt/ β -catenin pathway attenuates bleomycin-induced pulmonary fibrosis. *Tohoku J Exp Med* 2011;223:45–54.
132. Sueblinvong V, Neujahr DC, Mills ST, Roser-Page S, Ritzenthaler JD, Guidot D, Rojas M, Roman J. Predisposition for disrepair in the aged lung. *Am J Med Sci* 2012;344:41–51.
133. Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R, Trounson A. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol* 2009;175:303–313.
134. Dancer RC, Wood AM, Thickett DR. Metalloproteinases in idiopathic pulmonary fibrosis. *Eur Respir J* 2011;38:1461–1467.
135. Ruiz PA, Jarai G. Discoidin domain receptors regulate the migration of primary human lung fibroblasts through collagen matrices. *Fibrogenesis Tissue Repair* 2012;5:3.
136. Martínez de Lizarondo S, Roncal C, Calvayrac O, Rodríguez C, Varo N, Purroy A, Lorente L, Rodríguez JA, Doeuvre L, Hervás-Stubbs S, et al. Synergistic effect of thrombin and CD40 ligand on endothelial matrix metalloproteinase-10 expression and microparticle generation *in vitro* and *in vivo*. *Arterioscler Thromb Vasc Biol* 2012;32:1477–1487.
137. Steele MM, Schieler AM, Kelley PM, Tempero RM. Beta1 integrin regulates mmp-10 dependant tubulogenesis in human lymphatic endothelial cells. *Matrix Biol* 2011;30:218–224.
138. Kassim SY, Gharib SA, Mecham BH, Birkland TP, Parks WC, McGuire JK. Individual matrix metalloproteinases control distinct transcriptional responses in airway epithelial cells infected with *Pseudomonas aeruginosa*. *Infect Immun* 2007;75:5640–5650.
139. Ma JY, Mercer RR, Barger M, Schwegler-Berry D, Scabilloni J, Ma JK, Castranova V. Induction of pulmonary fibrosis by cerium oxide nanoparticles. *Toxicol Appl Pharmacol* 2012;262:255–264.
140. Murray MY, Birkland TP, Howe JD, Rowan AD, Fidock M, Parks WC, Gavrilovic J. Macrophage migration and invasion is regulated by MMP10 expression. *PLoS One* 2013;8:e63555.
141. Rohani MG, McMahan RS, Razumova MV, Hertz AL, Cieslewicz M, Pun SH, Regnier M, Wang Y, Birkland TP, Parks WC. MMP-10 regulates collagenolytic activity of alternatively activated resident macrophages. *J Invest Dermatol* 2015;135:2377–2384.
142. Lijnen HR, Van Hoef B, Vanlinthout I, Verstreken M, Rio MC, Collen D. Accelerated neointima formation after vascular injury in mice with stromelysin-3 (MMP-11) gene inactivation. *Arterioscler Thromb Vasc Biol* 1999;19:2863–2870.
143. Mukhi S, Brown DD. Transdifferentiation of tadpole pancreatic acinar cells to duct cells mediated by Notch and stromelysin-3. *Dev Biol* 2011;351:311–317.
144. Aoyagi-Ikeda K, Maeno T, Matsui H, Ueno M, Hara K, Aoki Y, Aoki F, Shimizu T, Doi H, Kawai-Kowase K, et al. Notch induces myofibroblast differentiation of alveolar epithelial cells via transforming growth factor-beta-Smad3 pathway. *Am J Respir Cell Mol Biol* 2011;45:136–144.
145. Rowe RG, Keena D, Sabeh F, Willis AL, Weiss SJ. Pulmonary fibroblasts mobilize the membrane-tethered matrix metalloprotease, MT1-MMP, to destructively remodel and invade interstitial type I collagen barriers. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L683–L692.
146. Garcia-Alvarez J, Ramirez R, Sampieri CL, Nuttall RK, Edwards DR, Selman M, Pardo A. Membrane type-matrix metalloproteinases in idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006;23:13–21.
147. Atkinson JJ, Holmbeck K, Yamada S, Birkedal-Hansen H, Parks WC, Senior RM. Membrane-type 1 matrix metalloproteinase is required for normal alveolar development. *Dev Dyn* 2005;232:1079–1090.
148. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, et al. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* 1999;99:81–92.
149. Koenig GC, Rowe RG, Day SM, Sabeh F, Atkinson JJ, Cooke KR, Weiss SJ. MT1-MMP-dependent remodeling of cardiac extracellular matrix structure and function following myocardial infarction. *Am J Pathol* 2012;180:1863–1878.
150. Karow M, Popp T, Egea V, Ries C, Jochum M, Neth P. Wnt signalling in mouse mesenchymal stem cells: impact on proliferation, invasion and MMP expression. *J Cell Mol Med* 2009;13:2506–2520.
151. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broadus VC, Nishimura SL. The integrin α (v) β 8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF- β 1. *J Cell Biol* 2002;157:493–507.
152. Folgueras AR, Valdés-Sánchez T, Llano E, Menéndez L, Baamonde A, Denlinger BL, Belmonte C, Juárez L, Lastra A, García-Suárez O, et al. Metalloproteinase MT5-MMP is an essential modulator of neuro-immune interactions in thermal pain stimulation. *Proc Natl Acad Sci USA* 2009;106:16451–16456.
153. Goldberg GL, Strongin A, Collier IE, Genrich LT, Marmer BL. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. *J Biol Chem* 1992;267:4583–4591.
154. Kim KH, Burkhardt K, Chen P, Frevert CW, Randolph-Habecker J, Hackman RC, Soloway PD, Madtes DK. Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. *Am J Respir Cell Mol Biol* 2005;33:271–279.
155. Koskivirta I, Kassiri Z, Rahkonen O, Kiviranta R, Oudit GY, McKee TD, Kytö V, Saraste A, Jokinen E, Liu PP, et al. Mice with tissue inhibitor of metalloproteinases 4 (Timp4) deletion succumb to induced myocardial infarction but not to cardiac pressure overload. *J Biol Chem* 2010;285:24487–24493.
156. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L152–L160.
157. Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol Rev* 2007;87:69–98.
158. Tan RJ, Fattman CL, Niehouse LM, Tobolewski JM, Hanford LE, Li Q, Monzon FA, Parks WC, Oury TD. Matrix metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. *Am J Respir Cell Mol Biol* 2006;35:289–297.
159. Corbel M, Caulet-Maugendre S, Germain N, Molet S, Lagente V, Boichot E. Inhibition of bleomycin-induced pulmonary fibrosis in mice by the matrix metalloproteinase inhibitor batimastat. *J Pathol* 2001;193:538–545.
160. Drummond AH, Beckett P, Brown PD, Bone EA, Davidson AH, Galloway WA, Gearing AJ, Huxley P, Laber D, McCourt M, et al. Preclinical and clinical studies of MMP inhibitors in cancer. *Ann N Y Acad Sci* 1999;878:228–235.
161. Baxter AD, Bhogal R, Bird J, Keily JF, Manallack DT, Montana JG, Owen DA, Pitt WR, Watson RJ, Wills RE. Arylsulphonyl hydroxamic acids: potent and selective matrix metalloproteinase inhibitors. *Bioorg Med Chem Lett* 2001;11:1465–1468.
162. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 2002;295:2387–2392.
163. Wilson CL, Ouellette AJ, Satchell DP, Ayabe T, López-Boado YS, Stratman JL, Hultgren SJ, Matrisian LM, Parks WC. Regulation of intestinal α -defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999;286:113–117.

164. Balbín M, Fueyo A, Tester AM, Pendás AM, Pitiot AS, Astudillo A, Overall CM, Shapiro SD, López-Otín C. Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. *Nat Genet* 2003;35:252–257.
165. Sela-Passwell N, Kikkeri R, Dym O, Rozenberg H, Margalit R, Arad-Yellin R, Eisenstein M, Brenner O, Shoham T, Danon T, et al. Antibodies targeting the catalytic zinc complex of activated matrix metalloproteinases show therapeutic potential. *Nat Med* 2012;18:143–147.
166. Marshall DC, Lyman SK, McCauley S, Kovalenko M, Spangler R, Liu C, Lee M, O'Sullivan C, Barry-Hamilton V, Ghormazien H, et al. Selective allosteric inhibition of MMP9 is efficacious in preclinical models of ulcerative colitis and colorectal cancer. *PLoS One* 2015;10:e0127063.
167. Lim NH, Meinjohanns E, Bou-Gharios G, Gompels LL, Nuti E, Rossello A, Devel L, Dive V, Meldal M, Nagase H. In vivo imaging of matrix metalloproteinase 12 and matrix metalloproteinase 13 activities in the mouse model of collagen-induced arthritis. *Arthritis Rheumatol* 2014;66:589–598.
168. Purcell BP, Lobb D, Charati MB, Dorsey SM, Wade RJ, Zellars KN, Doviak H, Pettaway S, Logsdon CB, Shuman JA, et al. Injectable and bioresponsive hydrogels for on-demand matrix metalloproteinase inhibition. *Nat Mater* 2014;13:653–661.
169. Hirata M, Kugimiya F, Fukai A, Saito T, Yano F, Ikeda T, Mabuchi A, Sapkota BR, Akune T, Nishida N, et al. C/EBP β and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2 α as the inducer in chondrocytes. *Hum Mol Genet* 2012;21:1111–1123.
170. Mueller MS, Mauch S, Sedlacek R. Structure of the human MMP-19 gene. *Gene* 2000;252:27–37.
171. Cisneros J, Hagood J, Checa M, Ortiz-Quintero B, Negreros M, Herrera I, Ramos C, Pardo A, Selman M. Hypermethylation-mediated silencing of p14(ARF) in fibroblasts from idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L295–L303.
172. Coward WR, Feghali-Bostwick CA, Jenkins G, Knox AJ, Pang L. A central role for G9a and EZH2 in the epigenetic silencing of cyclooxygenase-2 in idiopathic pulmonary fibrosis. *FASEB J* 2014;28:3183–3196.
173. Coward WR, Watts K, Feghali-Bostwick CA, Jenkins G, Pang L. Repression of IP-10 by interactions between histone deacetylation and hypermethylation in idiopathic pulmonary fibrosis. *Mol Cell Biol* 2010;30:2874–2886.
174. Dakhallah D, Batte K, Wang Y, Cantemir-Stone CZ, Yan P, Nuovo G, Mikhail A, Hitchcock CL, Wright VP, Nana-Sinkam SP, et al. Epigenetic regulation of miR-17~92 contributes to the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med* 2013;187:397–405.
175. Rabinovich EI, Kapetanaki MG, Steinfeld I, Gibson KF, Pandit KV, Yu G, Yakhini Z, Kaminski N. Global methylation patterns in idiopathic pulmonary fibrosis. *PLoS One* 2012;7:e33770.
176. Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, Bray M, Zhang K, Thannickal VJ, Hagood JS. Altered DNA methylation profile in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:525–535.
177. Yang IV, Pedersen BS, Rabinovich E, Hennessy CE, Davidson EJ, Murphy E, Guardela BJ, Tedrow JR, Zhang Y, Singh MK, et al. Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014;190:1263–1272.
178. Yan C, Wang H, Toh Y, Boyd DD. Repression of 92-kDa type IV collagenase expression by MTA1 is mediated through direct interactions with the promoter via a mechanism, which is both dependent on and independent of histone deacetylation. *J Biol Chem* 2003;278:2309–2316.
179. Qin L, Han YP. Epigenetic repression of matrix metalloproteinases in myofibroblastic hepatic stellate cells through histone deacetylases 4: implication in tissue fibrosis. *Am J Pathol* 2010;177:1915–1928.
180. Chen KC, Wang YS, Hu CY, Chang WC, Liao YC, Dai CY, Juo SH. Oxidative up-regulates microRNA-29b, leading to epigenetic modifications of mmp-2/mmp-9 genes: a novel mechanism for cardiovascular diseases. *FASEB J* 2011;25:1718–1728.
181. Tam EM, Wu YI, Butler GS, Stack MS, Overall CM. Collagen binding properties of the membrane type-1 matrix metalloproteinase (MT1-MMP) hemopexin C domain: the ectodomain of the 44-kDa autocatalytic product of MT1-MMP inhibits cell invasion by disrupting native type I collagen cleavage. *J Biol Chem* 2002;277:39005–39014.
182. Gao W, Zhu J, Westfield LA, Tuley EA, Anderson PJ, Sadler JE. Rearranging exosites in noncatalytic domains can redirect the substrate specificity of ADAMTS proteases. *J Biol Chem* 2012;287:26944–26952.
183. Huhtala P, Tuuttila A, Chow LT, Lohi J, Keski-Oja J, Tryggvason K. Complete structure of the human gene for 92-kDa type IV collagenase. Divergent regulation of expression for the 92- and 72-kilodalton enzyme genes in HT-1080 cells. *J Biol Chem* 1991;266:16485–16490.
184. Kumar B, Koul S, Petersen J, Khandrika L, Hwa JS, Meacham RB, Wilson S, Koul HK. p38 mitogen-activated protein kinase-driven MAPKAPK2 regulates invasion of bladder cancer by modulation of MMP-2 and MMP-9 activity. *Cancer Res* 2010;70:832–841.
185. Murthy S, Ryan A, He C, Mallampalli RK, Carter AB. Rac1-mediated mitochondrial H₂O₂ generation regulates MMP-9 gene expression in macrophages via inhibition of SP-1 and AP-1. *J Biol Chem* 2010;285:25062–25073.
186. Murthy S, Ryan AJ, Carter AB. SP-1 regulation of MMP-9 expression requires Ser586 in the PEST domain. *Biochem J* 2012;445:229–236.
187. Chubinskaya S, Huch K, Mikecz K, Cs-Szabo G, Hasty KA, Kuettner KE, Cole AA. Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1 beta in human cartilage from knee and ankle joints. *Lab Invest* 1996;74:232–240.
188. Wang X, Manner PA, Horner A, Shum L, Tuan RS, Nuckolls GH. Regulation of MMP-13 expression by RUNX2 and FGF2 in osteoarthritic cartilage. *Osteoarthritis Cartilage* 2004;12:963–973.
189. Yang CM, Chien CS, Yao CC, Hsiao LD, Huang YC, Wu CB. Mechanical strain induces collagenase-3 (MMP-13) expression in MC3T3-E1 osteoblastic cells. *J Biol Chem* 2004;279:22158–22165.
190. Anglard P, Melot T, Guérin E, Thomas G, Basset P. Structure and promoter characterization of the human stromelysin-3 gene. *J Biol Chem* 1995;270:20337–20344.
191. Hess J, Porte D, Munz C, Angel P. AP-1 and Cbfa/runt physically interact and regulate parathyroid hormone-dependent MMP13 expression in osteoblasts through a new osteoblast-specific element 2/AP-1 composite element. *J Biol Chem* 2001;276:20029–20038.
192. Reunanen N, Li SP, Ahonen M, Foschi M, Han J, Kähäri VM. Activation of p38 alpha MAPK enhances collagenase-1 (matrix metalloproteinase (MMP)-1) and stromelysin-1 (MMP-3) expression by mRNA stabilization. *J Biol Chem* 2002;277:32360–32368.
193. Guo H, Zucker S, Gordon MK, Toole BP, Biswas C. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. *J Biol Chem* 1997;272:24–27.
194. Brabletz T, Jung A, Dag S, Hlubek F, Kirchner T. beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. *Am J Pathol* 1999;155:1033–1038.
195. Crawford HC, Fingleton B, Gustavson MD, Kurpios N, Wagenaar RA, Hassell JA, Matrisian LM. The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrilysin transcription in intestinal tumors. *Mol Cell Biol* 2001;21:1370–1383.
196. Rivat C, Le Floch N, Sabbah M, Teyrol I, Redeuilh G, Bruyneel E, Mareel M, Matrisian LM, Crawford HC, Gespach C, et al. Synergistic cooperation between the ap-1 and lef-1 transcription factors in activation of the matrilysin promoter by the src oncogene: implications in cellular invasion. *FASEB J* 2003;17:1721–1723.
197. Raza SL, Nehring LC, Shapiro SD, Cornelius LA. Proteinase-activated receptor-1 regulation of macrophage elastase (MMP-12) secretion by serine proteinases. *J Biol Chem* 2000;275:41243–41250.
198. Wu L, Fan J, Matsumoto Si, Watanabe T. Induction and regulation of matrix metalloproteinase-12 by cytokines and CD40 signaling in monocyte/macrophages. *Biochem Biophys Res Commun* 2000;269:808–815.

199. Illman SA, Keski-Oja J, Lohi J. Promoter characterization of the human and mouse epilysin (MMP-28) genes. *Gene* 2001;275:185–194.
200. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* 2003;253:269–285.
201. Rajasekaran S, Vaz M, Reddy SP. Fra-1/AP-1 transcription factor negatively regulates pulmonary fibrosis *in vivo*. *PLoS One* 2012;7:e41611.
202. Becerril C, Pardo A, Montaña M, Ramos C, Ramírez R, Selman M. Acidic fibroblast growth factor induces an antifibrogenic phenotype in human lung fibroblasts. *Am J Respir Cell Mol Biol* 1999;20:1020–1027.
203. Kerkelä E, Ala-aho R, Lohi J, Grénman R, M-Kähäri V, Saarialho-Kere U. Differential patterns of stromelysin-2 (MMP-10) and MT1-MMP (MMP-14) expression in epithelial skin cancers. *Br J Cancer* 2001;84:659–669.
204. Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 1999;13:781–792.
205. Esparza J, Vilardell C, Calvo J, Juan M, Vives J, Urbano-Márquez A, Yagüe J, Cid MC. Fibronectin upregulates gelatinase B (MMP-9) and induces coordinated expression of gelatinase A (MMP-2) and its activator MT1-MMP (MMP-14) by human T lymphocyte cell lines: a process repressed through RAS/MAP kinase signaling pathways. *Blood* 1999;94:2754–2766.
206. Nawrocki-Raby B, Gilles C, Polette M, Bruyneel E, Laronze JY, Bonnet N, Foidart JM, Mareel M, Birembaut P. Upregulation of MMPs by soluble E-cadherin in human lung tumor cells. *Int J Cancer* 2003;105:790–795.