Metalloproteinases in idiopathic pulmonary fibrosis.

An invited review of the literature for MMP series.

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Abstract:

In this review, we outline the current state of knowledge about the balance between collagen production and degradation in idiopathic pulmonary fibrosis (IPF). The dysregulated action of metalloproteinases implicated in IPF may play a central role in the disease pathogenesis. Inhibiting metalloproteinases in IPF may therefore have therapeutic potential but our knowledge of their pathophysiological role is held back by limited animal models and the lack of specific inhibitors.
Pulmonary fibrosis – varying mechanisms with a fibrotic endpoint:

Diffuse interstitial lung disease is characterised by varying degrees of inflammation and fibrosis resulting in derangement of the gas-exchange units of the lung. A hallmark of these diseases is the abnormal deposition of collagen. Many interstitial lung diseases are of known aetiology, i.e. exposure to organic (Farmer's lung) or inorganic particles (e.g. asbestosis), induced by drugs (e.g. amiodarone), or associated to rheumatological disease, such as systemic sclerosis and rheumatoid arthritis. Around half of ILD are of unknown aetiology and are classified as idiopathic interstitial pneumonias(1). By far the most common is idiopathic pulmonary fibrosis (IPF), which has a prognosis worse than many cancers.

Current evidence suggests that IPF results from an abnormal response to a currently unidentified alveolar epithelial injury. Theories speculate that IPF results from abnormal wound healing in response to multiple microscopic sites of alveolar epithelial cell (AEC) injury and activation (see figure 1). This is thought to result in a persistently abnormal epithelial repair which promotes fibroblast proliferation generating a “reticulum of activated fibroblasts and collagen which progressively restructures the lung architecture” (2). In addition to this there is increased epithelial cell apoptosis and cell loss especially adjacent to the fibroblastic foci. In IPF, aberrantly activated alveolar epithelial cells synthesize almost all if not all the mediators that provoke and sustain the fibrotic reaction probably through a bi-directional aberrant communication between epithelial and mesenchymal cells(3). Fibroblast type cells arise also by recruitment of fibrocytes from the circulation and possibly by the process of epithelial mesenchymal transformation(4).

In addition to myofibroblast foci formation and epithelial cell injury, there is variable evidence of inflammation as evidenced by increased macrophage and neutrophil counts (5), intra-alveolar coagulapathy (6) and the formation of new blood vessels in the IPF lung(7). Abnormal angiogenesis has further been linked to the development of fibrotic disorders of the lung (7). Together, these changes result in an increase in the permeability of the alveolar capillary barrier, which can be detected clinically by increased DTPA clearance (8). Increased alveolar capillary barrier permeability may also be associated with early mortality in IPF(9).

Therefore, at least two different cellular routes exist, an inflammatory pathway represented by most ILD, and an epithelial pathway (as seen IPF) that lead to the development of lung
fibrosis(10). In this review we will focus upon the importance of metalloproteinases in the pathophysiology of IPF.

Collagen and the pathophysiology of IPF:

There are many different species of collagen but types I and III predominate within both healthy and fibrotic lungs(11). Fibrillar collagens are secreted as soluble precursors (bearing large extension propeptides at both amino and carboxyl termini) that self associate to form an insoluble triple helix fibril. The triple helical conformation of collagen fibrils renders the molecule resistant to proteolytic attack by most enzymes except the metalloproteinases – the biology of which has been outlined in the first article of this series.

Considerable evidence exists that both type I and type III collagen production is increased in IPF. Most studies looking at type I or type III production have looked at either the procollagen carboxy-terminal propeptide (PICP / PIIICP). These have been used as surrogate markers of increased collagen production since collagen itself is insoluble and cannot therefore be sampled directly without invasive biopsy.

In idiopathic pulmonary fibrosis both PICP and PIIICP have been found to be elevated in bronchoalveolar lavage fluid but not serum of patients. PICP levels in the BALF and epithelial lining fluid had a significant negative correlation for diffusion capacity (DLco/Va)(12). In immunohistochemical or in situ mRNA studies on lung tissue, type III collagen is predominant in the thickened alveolar septa and interstitium, whereas type I collagen appeared to be the principal collagen at later stages in the disease course(13). Type I procollagen was mostly present as intracellular spots in newly formed fibrosis in UIP while type III α1-collagen was expressed extracellularly underneath metaplastic alveolar epithelium(14). Increases in other constituents of the extracellular matrix including type V, VI, and VII collagens, fibronectin, elastin and proteoglycans are also present(10).

The degradatory environment in interstitial lung disease.

Elevated levels of procollagen production do not necessarily equate with increased collagen deposition since collagen degradation is a dynamic process regulated by the matrix metalloproteinases and their inhibitors. In order to assess whether net collagen is deposited in the lung some assessment of collagen degradation is also needed.

Several lines of early study have pointed to the abnormalities of collagen degradation within the fibrotic lung. Using zymographic methods from lung homogenates Gadek et al in 1979 demonstrated elevated collagenase activity in fifteen of 21 IPF patients but none in normal
controls or sarcoidosis patients. Conversely there is evidence that collagenolysis is reduced in hypersensitivity pneumonitis, experimental silicosis, and bleomycin induced pulmonary fibrosis in animals. Further, immunohistochemical studies demonstrated high levels of expression of the tissue inhibitors of metalloproteinases (TIMPs) within the IPF lung. TIMP-1 was found in interstitial macrophages and TIMP-2 in fibroblast foci. TIMP-3 revealed an intense staining mainly decorating the elastic lamina in vessels. TIMP-4 was expressed in IPF lungs by epithelial and plasma cells. Additionally Montano et al. found that collagenase inhibitory activity was much higher in biopsy samples from patients with IPF and hypersensitivity pneumonitis than in control subjects. Given that IPF lung tissue derived fibroblasts express a profibrotic secretory phenotype (reduced collagenase and elevated TIMP expression) these early studies suggested that a non-degrading fibrillar collagen microenvironment might prevail in interstitial lung disease.

Whilst initially a defect in collagenolysis was suspected to lead to an excess of extracellular matrix deposition in pulmonary fibrosis, this view now seems overly simplistic. Several studies have suggested that there is an increase in MMPs, rather than a loss of MMPs in IPF (see figure 2). Elevated levels of MMP-1, MMP-2, MMP-3, MMP-7, 8 and MMP-9 have been reported. MMP-12 and MMP-13 have also been implicated in experimental fibrosis. Their roles and potential significance are discussed below:

**MMP-1**

MMP-1 has been shown to be elevated in some but not all bronchoalveolar lavage fluid (BALF) studies of patients with IPF with one study suggesting increased plasma levels as well. Microarray data suggests that MMP-1 mRNA is significantly upregulated in whole lung tissue from IPF patients compared to hypersensitivity pneumonitis as well as normal control lung. MMP-1 expression is also higher in patients with familial IPF compared to sporadic IPF. Interestingly a polymorphism in the MMP-1 gene promoter is more common in smokers with IPF revealing a putative gene-environment interaction in this disease.

The observation that MMP-1 is upregulated in IPF, a condition associated with accumulation of both type I and type III collagen is at first glance a paradox, especially as it has also been implicated in the pathogenesis of COPD where loss of elastic tissue is a feature. One potential explanation is that the expression of MMP-1 is primarily in the reactive epithelium not in the interstitial compartment where collagen is accumulating. Alternatively, the activity of MMP-1 may be counterbalanced by tissue inhibitors resulting in only weak activity.
However, the biological roles for MMP1, in addition to collagen degradation, include processing cytokines such as pro-TNFα, regulation of cell migration, as well as potentially cell growth.(25) These multiple biological functions of MMP-1 along with the clinical data suggest an important role in IPF pathogenesis.

**MMP-2**

MMP-2 (Gelatinase A) has been reported to be widely expressed in fibrotic lungs especially areas of hyperplastic epithelial cells covering intra-alveolar fibrosis as well as by mesenchymal cells in the fibroblastic foci(17). In BALF MMP-2 has been reported to be elevated but western blots looking for active MMP-2 from another study suggest only weak activity (especially compared to BOOP)(9, 26). MMP-2 degrades a wide range of matrix and non-matrix substrates in particular type IV collagen and other basement membrane proteins. In addition, MMP2 is usually up-regulated in experimental models of lung fibrosis and its over-expression as well as that of MMP-9 has been suspected to be implicated in basement membrane disruption (27). This may be important because the structural integrity of the alveolar wall depends on the basement membrane and it is recognised that destruction of the sub-epithelial basement membrane may precede the development of alveolar fibrosis. A discontinuity of the basement membrane potentially allows greater access for exudative factors and interstitial cells to the alveolar space, promoting further tissue destruction and progressive fibrosis (9, 28).

**MMP-3**

MMP-3 (Stromolysin 1) levels are elevated in the BALF of patients with IPF and are higher in those who died within 3 years of diagnosis(9). MMP-3 may be important as a driver of fibrosis since MMP-3 expression in epithelial cells of transgenic mice stimulates development of fibrosis and subsequent tumour formation. Further exposure of mammary epithelial cells to MMP-3 induces epithelial–mesenchymal transition in which the cells acquire myofibroblast-like characteristics and that this process is dependent upon the generation of cellular reactive oxygen species. Data from culture models in which MMPs are inducibly expressed in human lung cell lines, and transgenic mouse models in which MMPs are inducibly expressed in lung alveolar epithelial cells, suggest that similar processes likely occur in the lung (29, 30). MMP-3 has also been implicated in the release of anti-angiogenic collagen degradation products such as endostatin that can promote alveolar epithelial apoptosis, which is believed to be an important driver of ongoing fibrosis (31, 32).
**MMP-7**

MMP-7, also known as matrilysin, has been reported to be one of the genes most consistently elevated in fibrotic lungs. MMP-7 expression does not differ between familial and sporadic IPF (33, 34). In IPF lungs, the increased immunoreactive protein is expressed primarily by the abnormal alveolar epithelium and active protein has demonstrated by tissue zymography in IPF lungs (33). In BALF, MMP-7 levels relate to the severity of lung function impairment in IPF (9). Recently it has also been shown that elevated levels of MMP-7 can also be found in nonspecific interstitial pneumonia and sarcoidosis (35) suggesting that increased MMP-7 expression is not specific to IPF.

MMP-7 has been described as a pro-fibrotic metalloproteinase(10). Several lines of research suggest that MMP-7 may promote a fibrotic response via regulatory effects on epithelial repair and release of latent TGF-β. MMP-7 null mice are relatively protected from bleomycin-induced fibrosis suggesting that this MMP is a central driver of the tissue response in fibrosis. MMP-7 has a broad substrate affinity for extracellular matrix components including collagen type IV, laminin, fibronectin, gelatin, elastin and osteopontin. MMP-7 also has the ability to process numerous bioactive substrates such as FasL, β4 integrin, E-cadherin, pro-TNF-α, pro-α-defensin, endostatin, syndecan, and α2-macroglobulin. MMP-7 can also activate proteases including itself and pro-MMPs -1, -2, and -9. Release of preformed TGF-β from the ECM by MMP-7 is the main regulator of TGF bioactivity which could promote fibroblast growth, survival and collagen synthesis. Thus MMP-7’s role in pulmonary fibrosis is likely to be pleiotropic due to its diverse biological roles being implicated in apoptosis, inflammation, fibroproliferation, and innate immunity(10).

**MMP-8**

MMP-8 (collagenase-2 or neutrophil collagenase), is derived from neutrophils and to a lesser extent from fibroblasts, endothelial, epithelial and plasma cells. MMP-8 levels are elevated in BALF from IPF patients and correlate with the collagenolytic capacity of the BALF(36). MMP-8 levels in the BALF of IPF patients are highest in those with rapidly progressive disease, poor survival and alveolar levels do not reduce with combination therapy (prednisolone, azathioprine and NAC)(9). Thus elevated MMP-8 levels appear to be associated with adverse outcome in IPF.

Neutrophilic alveolitis is a feature of patients with IPF and the degree of neutrophilia in BALF has been related to mortality in one large series(5) so it is interesting to speculate whether the neutrophilia drives matrix turnover via MMP-8, atleast in some patients. Alternatively
MMP-8 has been implicated in the migration and homing of fibrocytes. Fibrocytes are unique bone marrow-derived mesenchymal progenitor cells that are defined by their growth characteristics and surface phenotype, as they express markers compatible with leukocytes, hematopoietic progenitor cells and fibroblasts. Fibrocytes have been found within the lungs of patients with IPF and circulating fibrocyte levels are a marker of poor prognosis(37, 38) in IPF. Attenuation of fibrocyte trafficking in mouse models also directly correlates with a reduction in pulmonary fibrosis. Clearly the potentially important role that MMP-8 may have in this apparently important process needs further study.

MMP-9

MMP-9 (gelatinase B) has been widely studied in patients with IPF where it is predominantly expressed by alveolar macrophages, neutrophils and epithelial cells. In the normal lung, MMP-9 is not produced by resident cells, but under various forms of stimulation, bronchial epithelial cells, Clara cells, alveolar type II cells, fibroblasts, smooth muscle cells, and endothelial cells can produce MMP-9 (39). MMP-9 gene expression and protein have been found to be elevated in both human and experimental lung fibrosis(40, 41). Fibroblasts and alveolar macrophages extracted from IPF patient lung produced elevated MMP-9 compared to normal cells(19, 40).

Levels of MMP-9 in BALF and activity of MMP-9 are greatest in samples from rapidly progressive IPF cases(9, 42). Whether elevations of MMP-9 are a marker of activated neutrophils or involved in the alveolar damage in this subset of patients is unknown. The elevations of MMP-9 in the BALF of patients with bronchiolitis obliterans organizing pneumonia (BOOP) exceed those seen in IPF (43) however, suggesting perhaps an association with neutrophils rather than the lung histology.

Animal studies using knockout mice for MMP-9 display some conflicting results about the role of this metalloproteinase. After bleomycin installation, MMP-9 null mice develop fibrosis that is similar to that developed by wild-type animals, although the lungs of MMP-9 deficient mice showed minimal alveolar bronchiolisation suggesting that that MMP-9 facilitates migration of Clara cells and other bronchiolar cells into the regions of alveolar injury(44). Alternatively over expression of MMP-9 in macrophages has been shown to attenuate bleomycin induced fibrosis (45). The reduction of profibrotic mediators such as TIMP1 and IGFBP-3 in the MMP-9 transgenic mice was identified as potential mechanisms of the diminished fibrotic response. It is difficult to reconcile the above findings but they suggest that MMP-9 may promote or reduce the fibrotic response. It seems likely that the overall response depends upon which cell produces the MMP-9, the local tissue inhibitor levels, and the available target molecules / substrates.
MMP-12

MMP-12 (macrophage elastase) has been implicated in the fibrotic response in animal studies of fibrosis using FasL. Mice treated with a Fas activating antibody had increased caspase-3 activation in alveolar wall cells and increased total lung collagen on Day 21 after exposure. Gene expression profiling showed sequential activation of co-regulated profibrotic genes, including marked up-regulation of matrix metalloproteinase 12 (MMP-12). Targeted deletion of MMP-12 protected mice from Fas-induced pulmonary fibrosis, even though the inflammatory responses in the lungs were similar to those of wild-type mice(46).

There is little data about MMP-12 levels or expression in patients with IPF. In one small study MMP-12 was only detectable in the BALF 3 out of 18 patients with IPF, and represented only 0.022% of total IPF BALF MMP levels as measured by luminex array(47). It is important to recognize that the tissue levels of MMP-12 do not necessarily clearly reflect BALF levels due to the complex regulation of MMPs by local inhibitors. Thus current evidence does not support a clear role for MMP-12 in human lung fibrosis associated with IPF.

MMP-13

MMP-13 has been implicated in the severity of inflammation and fibrosis in experimental asbestos induced lung injury along with MMPs 2, 9 and 12. Use of a general MMP inhibitor GM6001 attenuated both the inflammation and the degree of fibrotic reaction(48). MMP-13 knockout mice also have reduced acute inflammation and fibrosis when exposed to radiation(49).

In contrast in pulmonary fibrosis induced in rats with paraquat and hyperoxia, Ruiz et al. demonstrated reduced levels of collagenases MMP-8 and MMP-13 with an increase in TIMP-1 and TGF-β.(27) Thus similar to the results of the MMP-9 animal models described above, results about MMP-13 are conflicting. Little is known about the role of MMP-13 in human IPF although MMP-13 is undetectable in IPF BALF (D Thickett unpublished observations). This data is backed up by immunohistochemistry and RT-PCR of IPF lungs(17).
Membrane associated and other metalloproteinases in IPF:

A subset of MMPs, the membrane bound MMPs (MT-MMPs) participate in activation of pro-MMP-2 to form MMP-2. These MT-MMPs have been shown to be present in IPF lung tissue. MT1- and MT2-MMPs were found in alveolar epithelial cells, MT3-MMP in fibroblasts from fibroblastic foci and alveolar epithelial cells and MT5-MMP in basal bronchiolar epithelial cells and in areas of squamous metaplasia. In lung fibroblasts, TGF-beta1 induced a strong up regulation of MT3-MMP, both at the gene and protein level(50).

The increasing diversity of known MMP biology means that many potentially important MMPs remain poorly studied in IPF. For example microarray studies suggest elevated MMP-10 and MMP-28 in IPF tissue but their cellular sources, substrates and function are poorly characterised. MMP-28 may be of particular interest since it has been proposed to have a role in epithelial mesenchymal transformation as well as proteolytic cleavage of TGF-beta(51). Further work to clarify the importance of these novel MMPs is ongoing.

Effect of current treatment for IPF on MMP expression in the lung:

Current treatment for IPF remains unsatisfactory and there is little evidence that the fibrosis seen in usual interstitial pneumonia (UIP) pattern on lung biopsy ever regresses with treatment. A recent trial has, however, suggested that treatment with prednisolone, azathioprine and N-acetylcysteine slows progression [24] but whether this treatment reduces aberrant collagen turnover is unclear. The only study to address MMP levels pre and post treatment in the same individual, demonstrated that combination drug therapy does not have any suppressive activity upon BALF MMP immunoreactivity(9). This is despite the fact that a previous study suggests that steroid treatment reduces MMP-9 production from IPF patients [25]; however, in that study, patients were not individually studied consecutively pre- and post-treatment.

Pirfenidone is a novel anti-fibrotic agent, which has been shown to decrease collagen deposition in a variety of animal models in vivo. Trials in IPF patients (currently unpublished) show that it reduces the rate of decline of lung function which has led to the recent recommendation by the European Medicines Agency that pirfenidone be licensed for use in IPF in Europe. Although the mechanism of action of pirfenidone remains unclear, a few non-clinical studies currently published suggest that modulation of MMP activity may be involved. In a hepatic fibrosis model, the anti-fibrotic effect of pirfenidone was mainly due to the reduced expression of procollagen and TIMP-1, most likely through the down-regulation of transforming growth factor beta1 mRNA, and of matrix metalloproteinase-2(52). In mice given intratracheal LPS, pirfenidone reduces MMP-9 due to reduced neutrophil recruitment.
Whereas low dose pirfenidone suppressed transforming growth factor beta-1 and tissue inhibitor of metalloproteinase-1, and protects rats from lung fibrosis induced by bleomycin but had no effect on the expression of MMP-13 (54).

**Metalloproteinases as biomarkers in IPF:**

The findings of increased levels of metalloproteinases in the lungs of IPF and reported relationships to severity of lung function decline or progressive disease on BALF has led to interest in whether there is potential for a biomarker panel for use in IPF. A BALF biomarker would have limited use given the logistics of BAL, variable dilution effects and wide biological variability in individual MMP levels.

Recently a panel of 49 plasma proteins was tested in plasma of IPF patients to define a five protein signature that distinguished patients from controls. MMP-7 and MMP-1, the two plasma proteins whose levels were most increased in patients with IPF compared to controls, were key components of this signature (MMP-7, MMP-1, MMP-8, IGFBP1, and TNFRSF1A). The panel was sufficient to distinguish patients from controls with a sensitivity of 98.6%. These results were further verified in an independent validation cohort of patients with IPF, familial pulmonary fibrosis, subclinical interstitial lung disease, as well as control individuals (55). Given that these plasma proteins can be measured in a single luminex assay this panel of markers has some potential especially as levels were elevated in patients with subclinical disease identified by HRCT. However, the relationship between this panel and disease progression was not reported. What we need in clinical practice is more a biomarker of disease activity than a diagnostic panel.

**Metalloproteinases as therapeutic targets in IPF.**

The up-regulation of MMPs in cancer and inflammatory diseases has made them attractive targets for drug development over the last 20 years. Can we expect MMP inhibitors to be effective in patients with IPF where excessive collagen deposition is a characteristic pathological finding?

The increasing recognition of the complexity of biological functions of metalloproteinases in terms of wound repair, angiogenesis as well as their effects on cytokine, chemokines and growth factor release suggest that there is potential for inhibition to modulate the aberrant alveolar remodelling seen in IPF. Bleomycin induced pulmonary fibrosis is attenuated by the
non-specific MMP inhibitor actions of the antibiotic doxycycline(56). This action is associated with reduced pulmonary inflammation and decreased MMP activity within BALF(57). Further a small open label study of doxycycline therapy in 7 IPF patients did not document any fall in FVC despite over 17 months of daily doxycycline usage. Such a lack of progression would be unusual in a typical IPF patient cohort but the quality of the study does not allow any conclusions to be drawn about the efficacy of doxycycline therapy in IPF patients(58).

Enthusiasm for metalloproteinase inhibition must also be tempered by the failure of early trials using broad spectrum inhibitors in cancer, as well as concerns over the potential adverse effects of therapeutic reduction of collagen degradation in a fibrosing disease may have. Ideally MMP inhibitors for use in IPF would have specificity for individual MMPs. The challenge for researchers in this area is therefore to identify if any individual MMP is a key mechanistic driver of IPF. Such work is hindered by reliance on models such as bleomycin induced injury which fails to properly model human disease(59).

Concluding Remarks:

IPF is a devastating disease and current / emerging therapy is unsatisfactory due to toxicity and limited efficacy. Current theories of the pathogenesis of IPF suggest that alveolar epithelial injury provokes the migration and proliferation of mesenchymal cells with fibroblast foci formation. Pathologically this results in areas of exaggerated collagen deposition in some parts of the lung with the loss of epithelial structures and honeycomb formation. Despite the progressive scarring that is seen, evidence has emerged that there is augmented production of metalloproteinases. The roles of these enzymes are currently unclear as they have pleiotropic effects upon both the extracellular matrix and in the processing of chemokines, cytokines and growth factors. It is possible that up-regulated matrix degradation is therefore a mechanistic driver of progressive fibrosis in IPF. Research in this area is hindered by the lack of good animal models of IPF but a better understanding of the pathophysiology of IPF and collagen turnover should identify novel therapeutic targets for this devastating disease.
Figure 1 Mechanisms of IPF. The aetiology of IPF is undetermined. It is postulated that whatever triggers IPF results in epithelial damage and that consequent epithelial activation that leads to the core features of UIP, namely chaotic epithelial repair, fibroblast proliferation and collagen deposition, which become self perpetuating.

Fig. 2 Bronchoalveolar lavage fluid (BALF) matrix metalloproteinase (MMP) levels expressed as a scatter plot. BALF data were combined from baseline and follow-up bronchoscopy (n=28). The scatter plot demonstrates that the majority of BALF MMP protein is MMP-7, -8 and -9. BALF levels of MMP-2, 3, 7, 8 and 9 were significantly elevated compared to normal controls. Reproduced from (9).


