ERJ Express. **Published on June 23, 2011 as doi: 10.1183/09031936.00024711**

Metalloproteinases in idiopathic pulmonary fibrosis.

An invited review of the literature for MMP series.

Dancer RCA[1]

Wood AM[1]

Thickett DR[1]

[1] Respiratory Medicine, School of Clinical and Experimental Medicine, University of Birmingham, UK, B15 2TT

Correspondence to DR Thickett, address as above

Email: d.thickett@bham.ac.uk

Fax: +44 121 627 2012

Telephone: +44 7976248963

Article word count: 3655 excluding references.

Key words: idiopathic pulmonary fibrosis matrix metalloproteinase.

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 pN-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. (15) Conversely there is evidence that collagenolysis is reduced in hypersensitivity pneumonitis, experimental silicosis, and bleomycin induced pulmonary fibrosis in animals(16). 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(17). 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(18). 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(17-19).

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)(9). 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(9, 20). Microarray data suggests that MMP-1 mRNA is significantly upregulated in whole lung tissue from IPF patients compared to hypersensitivity pneumonitis (21) as well as normal control lung (22). MMP-1 expression is also higher in patients with familial IPF compared to sporadic IPF (23). 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 (24).

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(10). 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-acetylcystein 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

(53). 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).

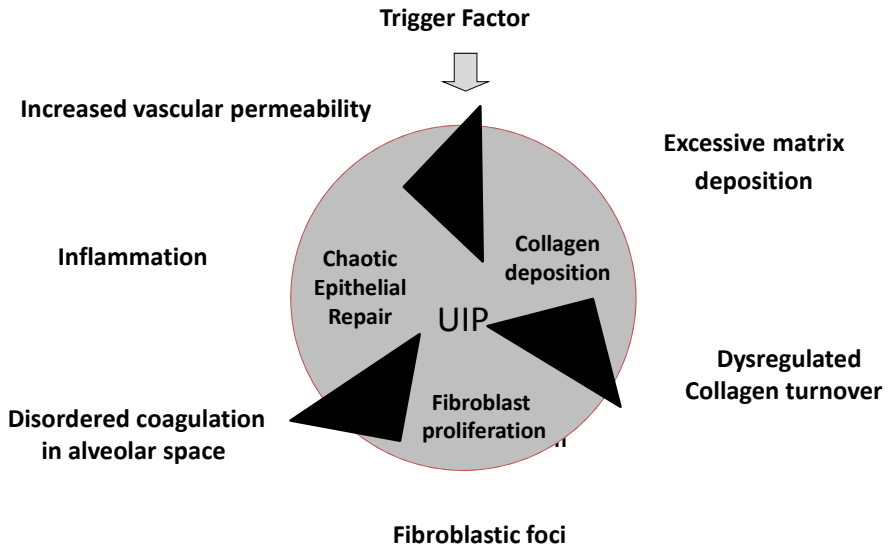
Enthusiasm for metalloproteinase inhibition must also be tempered by the failure of early trials using broad spectrum inhibitors in cancer, as well as concerns over the potential adverse effects of therapeutic reduction of collagen degradation in a fibrosing disease may have. Ideally MMP inhibitors for 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).

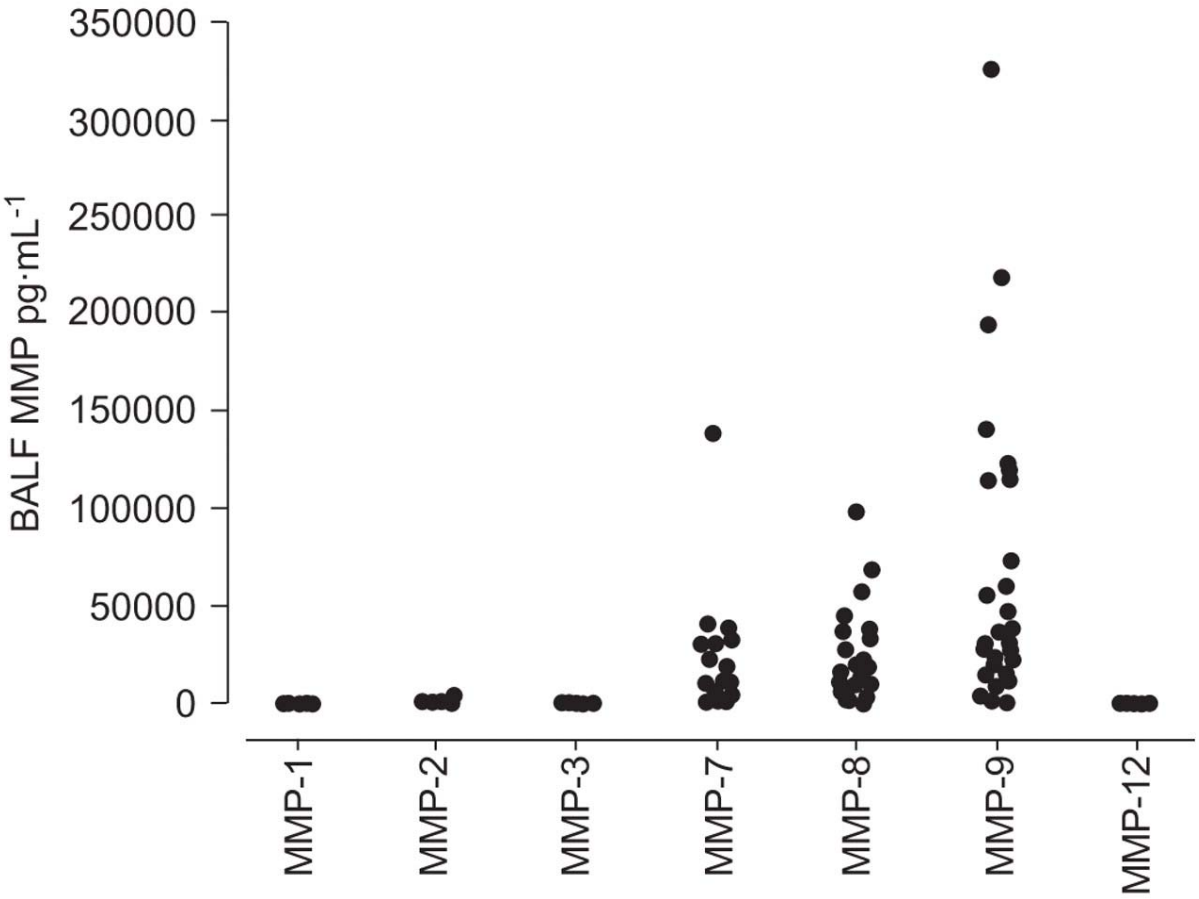
Concluding Remarks:

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).




350000
300000
250000
200000
150000
100000
50000
0
BALF MMP pg·mL-1
MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12

1. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: Clinical relevance of pathologic classification. *Am J Respir Crit Care Med* 1998;157:1301-1315.
2. Cool CD, Groshong SD, Rai PR, Henson PM, Stewart JS, Brown KK. Fibroblast foci are not discrete sites of lung injury or repair: The fibroblast reticulum. *Am J Respir Crit Care Med* 2006;174:654-658.
3. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: From innocent targets to serial killers. *Proc Am Thorac Soc* 2006;3:364-372.
4. Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, Westergren-Thorsson G, Selman M. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008;40:2129-2140.
5. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE, Jr. Baseline bal neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 2008;133:226-232.
6. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld B, Velcovsky HG, Morr H, Grimminger F, Walmrath D, Seeger W. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. *Thromb Haemost* 2000;83:853-860.
7. Cosgrove GP, Brown KK, Schiemann WP, Serls AE, Parr JE, Geraci MW, Schwarz MI, Cool CD, Worthen GS. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: A role in aberrant angiogenesis. *Am J Respir Crit Care Med* 2004;170:242-251.
8. Goh NS, Desai SR, Anagnostopoulos C, Hansell DM, Hoyles RK, Sato H, Denton CP, Black CM, du Bois RM, Wells AU. Increased epithelial permeability in pulmonary fibrosis in relation to disease progression. *Eur Respir J* 2010.
9. 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.
10. Pardo A, Selman M, Kaminski N. Approaching the degradome in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008;40:1141-1155.
11. Madri JA, Furthmayr H. Collagen polymorphism in the lung. An immunochemical study of pulmonary fibrosis. *Hum Pathol* 1980;11:353-366.
12. Lammi L, Ryhanen L, Lakari E, Risteli J, Paakko P, Kahlos K, Lahde S, Kinnula V. Type iii and type i procollagen markers in fibrosing alveolitis. *Am J Respir Crit Care Med* 1999;159:818-823.
13. Raghu G, Striker LJ, Hudson LD, Striker GE. Extracellular matrix in normal and fibrotic human lungs. *Am Rev Respir Dis* 1985;131:281-289.
14. Kaarteenaho-Wiik R, Lammi L, Lakari E, Kinnula VL, Risteli J, Ryhanen L, Paakko P. Localization of precursor proteins and mrna of type i and iii collagens in usual interstitial pneumonia and sarcoidosis. *J Mol Histol* 2005;36:437-446.
15. Gadek JE, Kelman JA, Fells G, Weinberger SE, Horwitz AL, Reynolds HY, Fulmer JD, Crystal RG. Collagenase in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1979;301:737-742.
16. Thickett DR, Poole AR, Millar AB. The balance between collagen synthesis and degradation in diffuse lung disease. *Sarcoidosis Vasc Diffuse Lung Dis* 2001;18:27-33.
17. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez 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:L562-574.
18. Montano M, Ramos C, Gonzalez G, Vadillo F, Pardo A, Selman M. Lung collagenase inhibitors and spontaneous and latent collagenase activity in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. *Chest* 1989;96:1115-1119.
19. Ramos C, Montano M, Garcia-Alvarez J, Ruiz V, Uhal BD, Selman M, Pardo A. Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and tissue inhibitor of metalloproteinases expression. *Am J Respir Cell Mol Biol* 2001;24:591-598.

20. Zhou LF, Jiang L, Li ZH, Kang J. [change of matrix metalloproteinase-1 and matrix metalloproteinase-7 in serum and bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and sarcoidosis]. *Zhonghua Jie He He Hu Xi Za Zhi* 2010;33:441-444.
21. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, Aziz N, Kaminski N, Zlotnik A. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 2006;173:188-198.
22. Konishi K, Gibson KF, Lindell KO, Richards TJ, Zhang Y, Dhir R, Bisceglia M, Gilbert S, Yousem SA, Song JW, Kim DS, Kaminski N. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;180:167-175.
23. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, Berman KG, Speer MC, Sporn TA, Brown KK, Schwarz MI, Schwartz DA. Gene expression profiling of familial and sporadic interstitial pneumonia. *Am J Respir Crit Care Med* 2007;175:45-54.
24. Checa M, Ruiz V, Montano M, Velazquez-Cruz R, Selman M, Pardo A. Mmp-1 polymorphisms and the risk of idiopathic pulmonary fibrosis. *Hum Genet* 2008;124:465-472.
25. Limb GA, Matter K, Murphy G, Cambrey AD, Bishop PN, Morris GE, Khaw PT. Matrix metalloproteinase-1 associates with intracellular organelles and confers resistance to lamin a/c degradation during apoptosis. *Am J Pathol* 2005;166:1555-1563.
26. 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.
27. Ruiz V, Ordonez RM, Berumen J, Ramirez 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:L1026-1036.
28. O'Kane CM, McKeown SW, Perkins GD, Bassford CR, Gao F, Thickett DR, McAuley DF. Salbutamol up-regulates matrix metalloproteinase-9 in the alveolar space in the acute respiratory distress syndrome. *Crit Care Med* 2009;37:2242-2249.
29. Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. Rac1b and reactive oxygen species mediate mmp-3-induced emt and genomic instability. *Nature* 2005;436:123-127.
30. Radisky ES, Radisky DC. Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. *J Mammary Gland Biol Neoplasia* 2010;15:201-212.
31. Uhal BD, Joshi I, Hughes WF, Ramos C, Pardo A, Selman M. Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung. *Am J Physiol* 1998;275:L1192-1199.
32. 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.
33. Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, Sheppard D, Pardo A, Selman M, Heller RA. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci U S A* 2002;99:6292-6297.
34. Kaminski N, Zuo F, Cojocaro G, Yakhini Z, Ben-Dor A, Morris D, Sheppard D, Pardo A, Selman M, Heller RA. Use of oligonucleotide microarrays to analyze gene expression patterns in pulmonary fibrosis reveals distinct patterns of gene expression in mice and humans. *Chest* 2002;121:31S-32S.
35. Vuorinen K, Myllarniemi M, Lammi L, Piirila P, Rytila P, Salmenkivi K, Kinnula VL. Elevated matrilysin levels in bronchoalveolar lavage fluid do not distinguish idiopathic pulmonary fibrosis from other interstitial lung diseases. *APMIS* 2007;115:969-975.
36. 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.

37. Strieter RM, Keeley EC, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells, fibrocytes, in promoting pulmonary fibrosis. *Trans Am Clin Climatol Assoc* 2009;120:49-59.
38. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, Margetts PJ, Farkas L, Dobranowski J, Boylan C, O'Byrne PM, Strieter RM, Kolb M. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:588-594.
39. Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003;28:12-24.
40. Lemjabbar H, Gosset P, Lechapt-Zalcman E, Franco-Montoya ML, Wallaert B, Harf A, Lafuma C. 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.
41. Perez-Ramos J, de Lourdes Segura-Valdez M, Vanda B, Selman M, Pardo A. Matrix metalloproteinases 2, 9, and 13, and tissue inhibitors of metalloproteinases 1 and 2 in experimental lung silicosis. *Am J Respir Crit Care Med* 1999;160:1274-1282.
42. 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.
43. Choi KH, Lee HB, Jeong MY, Rhee YK, Chung MJ, Kwak YG, Lee YC. The role of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in cryptogenic organizing pneumonia. *Chest* 2002;121:1478-1485.
44. 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.
45. Cabrera S, Gaxiola M, Arreola JL, Ramirez 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.
46. 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.
47. Thickett DR, Perkins GD, O'Kane C, McKeown S, McAuley D. Does mmp-12 play a role in human lung fibrosis? *Am J Respir Cell Mol Biol* 2008;38:247; author reply 247.
48. 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.
49. Flechsig P, Hartenstein B, Teurich S, Dadrich M, Hauser K, Abdollahi A, Grone 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.
50. 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.
51. Illman SA, Lehti K, Keski-Oja J, Lohi J. Epilysin (mmp-28) induces tgf-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. *J Cell Sci* 2006;119:3856-3865.
52. Di Sario A, Bendia E, Macarri G, Candelaresi C, Taffetani S, Marzioni M, Omenetti A, De Minicis S, Trozzi L, Benedetti A. The anti-fibrotic effect of pirfenidone in rat liver fibrosis is mediated by downregulation of procollagen alpha1(i), timp-1 and mmp-2. *Dig Liver Dis* 2004;36:744-751.
53. Corbel M, Lanchou J, Germain N, Malledant Y, Boichot E, Lagente V. Modulation of airway remodeling-associated mediators by the antifibrotic compound, pirfenidone, and the matrix metalloproteinase inhibitor, batimastat, during acute lung injury in mice. *Eur J Pharmacol* 2001;426:113-121.
54. Tian XL, Yao W, Guo ZJ, Gu L, Zhu YJ. Low dose pirfenidone suppresses transforming growth factor beta-1 and tissue inhibitor of metalloproteinase-1, and protects rats from lung fibrosis induced by bleomycina. *Chin Med Sci J* 2006;21:145-151.

55. Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, Lindell KO, Cisneros J, Macdonald SD, Pardo A, Sciurba F, Dauber J, Selman M, Gochuico BR, Kaminski N. Mmp1 and mmp7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. *PLoS Med* 2008;5:e93.
56. Huang YH, Li Y, Shang Y, Ouyang ZG, Zhen YS. [inhibition of pulmonary fibrosis by doxycycline: An experiment with mice]. *Zhonghua Yi Xue Za Zhi* 2006;86:182-186.
57. Fujita M, Ye Q, Ouchi H, Harada E, Inoshima I, Kuwano K, Nakanishi Y. Doxycycline attenuated pulmonary fibrosis induced by bleomycin in mice. *Antimicrob Agents Chemother* 2006;50:739-743.
58. Bhattacharyya P, Nag S, Bardhan S, Acharya D, Paul R, Dey R, Ghosh M, Saha I. The role of long-term doxycycline in patients of idiopathic pulmonaryfibrosis: The results of an open prospective trial. *Lung India* 2009;26:81-85.
59. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L152-160.