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The role of matrix metalloproteinases in cystic fibrosis lung disease

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ABSTRACT: Significant airway remodelling is a major component of the increased morbidity and mortality observed in cystic fibrosis (CF) patients. These airways feature ongoing leukocytic inflammation and unrelenting bacterial infection. In contrast to acute bacterial pneumonia, CF infection is not cleared efficiently and the ensuing inflammatory response causes tissue damage. This structural damage is mainly a result of free proteolytic activity released by infiltrated neutrophils and macrophages. Major proteases in this disease are serine and matrix metalloproteases (MMPs). While the role of serine proteases, such as elastase, has been characterised in detail, there is emerging evidence that MMPs could play a key role in the pathogenesis of CF lung disease. This review summarises studies linking MMPs with CF lung disease and discusses the potential value of MMPs as future therapeutic targets in CF and other chronic lung diseases.

KEYWORDS: Antiproteases, cystic fibrosis, elastase, lung disease, neutrophil, proteases

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PROTEOLYTIC ACTIVITY IN CYSTIC FIBROSIS LUNG DISEASE

Chronic progressive destruction of the lung is the major cause of death in patients with cystic fibrosis (CF) [1]. While gastrointestinal symptoms are treated efficiently by established medications, such as pancreatic enzyme replacement therapy, the progression of pulmonary symptoms with destruction of pulmonary tissue cannot, as yet, be controlled sufficiently. CF airways are characterised by airway surface liquid (ASL) depletion producing mucus obstruction and chronic inflammation with persistent leukocyte accumulation, mainly of neutrophils and macrophages. Despite the presence of millions of activated phagocytes, bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae* flourish in the CF airway microenvironment, resulting in chronic infection. Since recruited leukocytes are impaired in their antibacterial capacity due to decreased mucociliary clearance [2], CXCR1 receptor cleavage [3], excessive but inefficient neutrophil extracellular trap formation [4] and other mechanisms, dehydrated mucus and extracellular DNA obstruct the airways and other

toxic products, such as proteases and oxidants, cause host tissue damage. Major proteases in this disease situation are serine proteases and matrix metalloproteinases (MMPs). While the role of serine proteases, such as neutrophil elastase (NE), cathepsin G and proteinase 3 have been characterised in detail in murine and human studies (including natural and synthetic antagonists), there is emerging evidence that MMPs could play a key role in the pathogenesis of CF lung disease. The amount of free proteolytic activity increases over the lifetime of CF patients [3] and overwhelms the antiprotease shield of the airspaces. These enzymes damage cellular and pulmonary structures, including cilia, elastin, fibronectin, surfactant proteins A and D, immunoglobulins and CXCR1 on neutrophils and CD4 on lymphocytes [5]. This article will introduce the MMP family and will highlight the potential of several members as future targets in CF lung disease.

MMPs: STRUCTURE AND FUNCTION

MMPs are one of four subfamilies within the superfamily of metalloendopeptidases known as the metzincins [6]. More than 20 MMPs have

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been identified and, based on structure and substrate specificity, they are further subdivided into groups. All MMPs exhibit an N-terminal signal sequence, a pro-domain region and an active catalytic domain (with a zinc-binding region). Another major domain observed is a haemopexin-like C-terminal domain (found in all MMPs except MMP-7 and -22), which is thought to mediate substrate specificity of the MMP for components of the extracellular matrix. Additional domains include a transmembrane domain (found in MMP-14, -15, -16 and -24) and glycosylphosphatidylinositol (GPI anchor; MMP-17 and -25), important to cell-bound MMPs. Furin-recognition motifs are seen in multiple MMP isoforms' pro-domain regions, and type II fibronectin repeats are seen in gelatinase (MMP-2 and -9) catalytic domains. These regions seem to have important implications for the interaction of the MMP with its substrate (reviewed in [7]).

MMPs have the combined ability to degrade essentially all connective tissue components. While MMPs are involved in many normal homeostatic mechanisms, their expression and activity is commonly elevated in conditions where inflammation and tissue remodelling/repair are operative [8, 9]. MMPs are regulated at various points. Transcription-level regulation, through induction by various cytokines such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, may involve the activation of a diverse group of intracellular signalling cascades (such as p38 mitogen-activated protein kinase (MAPK) or extracellular signal-regulated kinase 1/2 MAPK), leading to activation of nuclear signalling factors such as activator protein-1, nuclear factor-κB, and signal transducer and activator of transcription to induce MMP transcription [10]. Most MMPs are translated to a zymogen (inactive) form. These enzymes have a pro-domain region that is cleaved through a cysteine switch mechanism and is then activated. Activation can be induced by various triggers, including protease-mediated activation (either by another protease or auto-activation by the active form of that MMP), oxidant-mediated activation or matrix-mediated activation.

In addition to regulation of activation, there are numerous inhibitors of MMPs. Although tissue inhibitors of metallo-proteases (TIMPs) are often thought of as the predominant group of inhibitors for MMPs, they are really the most specific endogenous MMP inhibitors. The majority of MMP-related inhibition *in vivo* occurs through relatively nonspecific MMP inhibitors such as α2 macroglobulin [8, 11]. TIMPs are a group of four small (20–24 kDa) MMP-specific inhibitors that bind to MMPs in a 1:1 stoichiometric relationship [12–15]. Animal studies, in addition to human studies in adults, support a role for MMPs and an imbalance between MMPs and TIMPs in the pathogenesis of several well-recognised pulmonary disorders, such as chronic obstructive pulmonary disease (COPD) [16, 17] and asthma [18–20].

MMPs perform numerous biological functions, including degradation of matrix components and remodelling of tissues, release of cytokines, growth factors and chemokines, and modulation of cell mobility and migration [11]. Data suggest that dysregulated cellular production, secretion and activation of MMPs, and/or dysfunction of their inhibitors, are involved in pathological conditions within the lung parenchyma. Through degradation of extracellular matrix components,

MMPs can destroy the alveolar epithelium, as well as disrupt reorganisation during the repair process [8, 9]. Although previously well-studied in other pulmonary disorders, the role of MMPs in CF lung disease has only recently been emerging and suggests important downstream effects of this proteolytic dysregulation.

MMPs IN CYSTIC FIBROSIS LUNG DISEASE

MMPs were first found to be upregulated in the airways of CF patients in 1995, when DELACOURT *et al.* [21] described the increase of a 95/88 kDa gelatinase isoform (presumably MMP-9) in the sputum of patients with CF lung disease (n=27) compared with asthmatic controls (n=9) with an associated imbalance of TIMP. Since this finding, there has been a burgeoning of MMP literature in CF lung disease, focusing not only on relative protease/antiprotease imbalance but also on the increasing evidence for downstream effects of this proteolytic environment. These potential biological effects with regard to CF lung disease are shown in figure 1.

MMP-2

MMP-2 (EC 3.4.24.24) is a protease with gelatinolytic activity (hence its alternate name, gelatinase A), which is found to be expressed constitutively in various cell types found in the lungs. This enzyme has a broad spectrum of substrates and is involved in modulating diverse cellular functions, including angiogenesis [22], tissue remodelling [9] and potentiation of inflammatory response [23]. MMP-2 is activated in a unique membrane-type MMP-dependent manner, demonstrating a classic example of MMP-to-MMP activation [24]. MMP-2 is thought to contribute to the pathogenesis of a variety of pulmonary disorders, including COPD, asthma, lung cancer and interstitial pulmonary fibrosis [25].

MMP-2 has not been extensively studied in CF lung disease but there are some interesting insights into the potential role of this protease in CF. For example, one study has shown that inhibition of MMP-2 in Calu-3 cells (which constitutively express MMP-2) leads to augmentation of chloride transport in these cells, suggesting autocrine regulation of ion transport *via* this protease [26]. In addition, GERAGHTY *et al.* [27] recently demonstrated that NE may augment MMP-2 expression from epithelial cells, potentially leading to increased remodelling and inflammatory response in CF. Interestingly, although MMP-2 elevations have not been consistently observed in pulmonary specimens from individuals with CF [28, 29], a recent manuscript has found that MMP-2 decreases in serum during CF exacerbation [30].

MMP-7

MMP-7 (EC 3.4.24.23), or matrilysin, is the smallest known MMP. It exhibits broad substrate specificity, with the capacity to cleave multiple components of the matrix [31]. In addition, MMP-7 has been observed playing a role in diverse biological responses, such as airway re-epithelialisation, inflammation [32], host defence [33] and cell growth *via* cleavage of insulin growth factor-binding proteins [34]. MMP-7 has been shown to be involved in idiopathic pulmonary fibrosis (IPF), as knockout mice are protected from bleomycin-induced injury [35] and MMP-7 is overexpressed in lung tissues from patients with IPF [36].
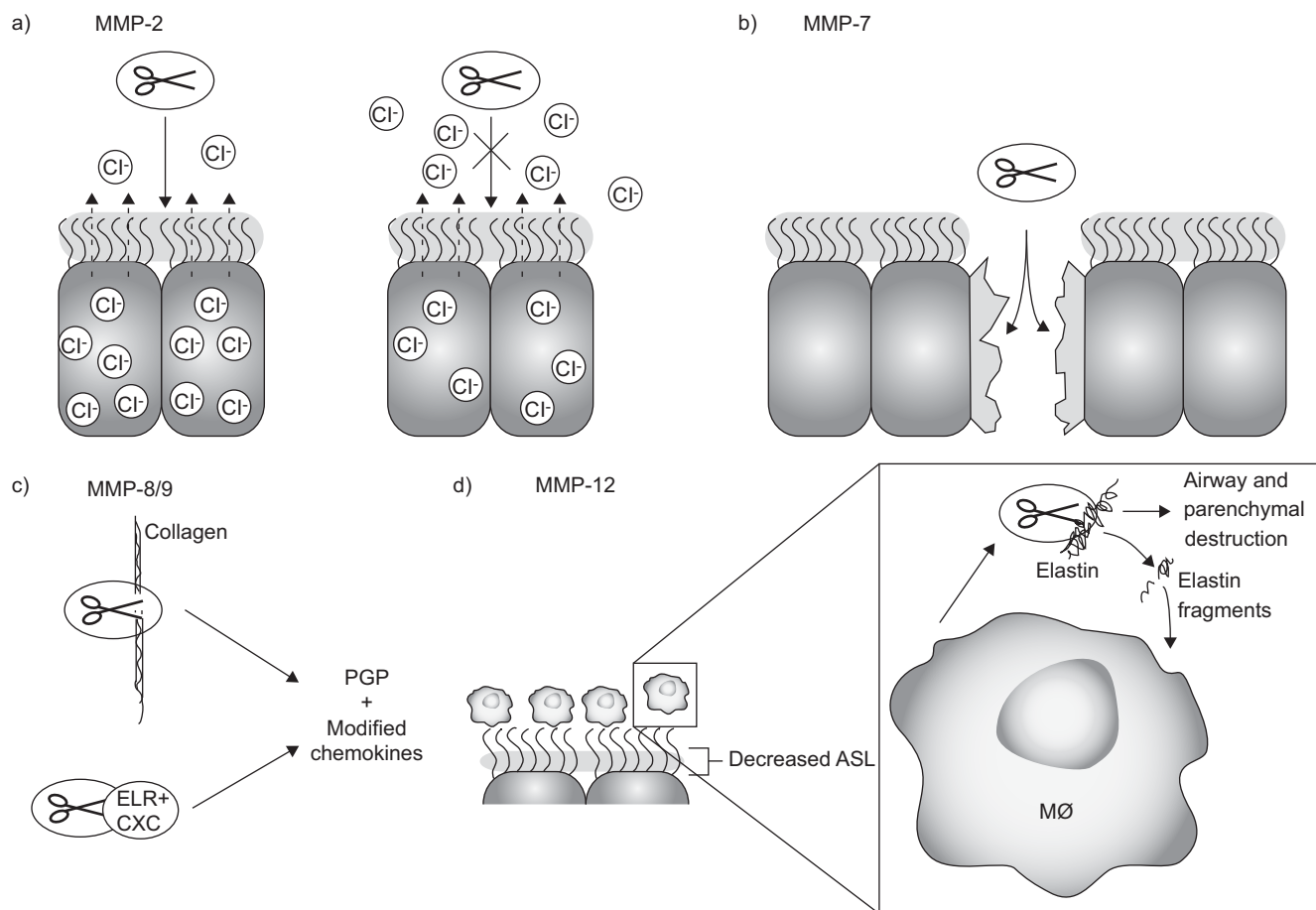
FIGURE 1. Predicted effects of matrix metalloproteinase (MMP) (depicted as ovals with scissors) activity in the context of cystic fibrosis. a) MMP-2 disrupts chloride (Cl^-) current, and inhibition of MMP-2 abrogates this effect. b) MMP-7 functions to repair damage in airway epithelia. c) MMP-8 and -9 are able to cleave collagen and modify ELR+ CXC chemokines, which modulate inflammation in the airways. d) Decreased airway surface liquid (ASL) activates macrophages (MØ) and increases expression of MMP-12, which cleaves elastin, resulting in degradation of the airway and lung parenchyma. These elastin fragments may also increase the recruitment of monocytes and activation of macrophages. PGP: proline-glycine-proline.

MMP-7 may play an important role in the injury/repair response in CF lung disease. DUNSMORE *et al.* [37] have shown increased MMP-7 expression in the airway epithelia of CF patients, and that it is differentially released either apically or basally from these cells. The authors hypothesise that this regulation is important in the injury response and show that human airways treated with an MMP-7 inhibitor fail to re-epithelialise after injury [37]. To the current authors' knowledge, specific studies of the importance of MMP-7 expression in CF transmembrane conductance regulator knockout mice after injury have not yet been published.

MMP-8
MMP-8 (EC 3.4.24.34), or neutrophil collagenase, is one of three collagenase MMPs (MMP-1, -8 and -13) and is highly expressed in neutrophils. These collagenases have common cleavage sites for type I, II and III collagens, producing a three-fourths N-terminal fragment and a one-fourth C-terminal fragment [38–40]. This suggests a potential redundancy for these proteases in disease. MMP-8 has also been shown to cleave aggrecan *in vitro* [41], and may play a role in arthritis [42]. Two specific isoforms of pro-MMP-8 have been described

in humans: one, of ~80 kDa, is highly expressed in polymorphonuclear leukocytes (PMNs); and a 55-kDa isoform is expressed from mesenchymal cells [43]. Beyond the capacity to cleave extracellular matrix, MMP-8 has diverse biological effects, including modulation of chemokines [44], regulation of repair response [45] and innate immunity [46]. Increased MMP-8 expression has been characterised in various lung diseases including COPD [47], IPF [43], bronchiectasis [48] and asthma [49].

MMP-8 expression and activity has also been found to be elevated in the airway secretions of patients with CF lung disease [50]. Furthermore, this enzyme is elevated in the serum of patients with CF, and levels correlate with lung function (MMP-8 level *versus* forced expiratory volume in 1 s (FEV1) % predicted, r= -0.468, p<0.001) [30]. Unfortunately, beyond these characterisations, little else is known regarding the impact of this dysregulation on CF pathogenesis. Recently, a novel neutrophil chemokine (proline-glycine-proline (PGP)) was found in the sputum of CF patients and MMP-8 was found to be an important enzyme involved in its generation [51]. Further examination of the impact of MMP-8 on progression of CF lung disease is certainly warranted.

MMP-9

Perhaps one of the most extensively studied proteases, MMP-9 (EC 3.4.24.35), or gelatinase B, is broadly expressed in a variety of cells in the lung, including inflammatory (PMNs, macrophages), epithelial and endothelial cells. This observed redundancy belies important location-specific functions of MMP-9, some of which seem in opposition to other MMP-9 functions. For example, MMP-9 has observed important proinflammatory effects by generating PGP [51] and increasing the chemokine potency of IL-8 [52], but MMP-9 also plays an important role in the regulation of granuloma formation in tuberculosis [53]. Similarly, MMP-9 had been thought to lead to matrix breakdown, but recently it has been suggested that MMP-9 may have a role in matrix repair [54, 55].

MMP-9 has been extensively examined in the lower airway secretions of CF patients and has been found to be increased in both quantity and activity [28, 50]. One study found a correlation between lung function (FEV1) and MMP-9 ($r=$ -0.78, $p=0.001$) [56]. In addition, MMP-9 expression is elevated in the serum of patients with CF lung disease [30].

MMP-12

MMP-12 (EC 3.4.24.65), also known as macrophage elastase, is a protease secreted by macrophages with the capacity to degrade elastin. MMP-12 was first cloned by SHAPIRO et al. [57]. It seems to be subject to partial regulation by proteases, such as thrombin and plasmin, and can be released from macrophages by a variety of inflammatory cytokines, such as TNF-α and IL-1β [58]. This protease has emerging biological effects in modulating cytokine and chemokine networks, including cleavage of pro-TNF-α [59] and ELR+ CXC chemokines [60]. The physiological role of MMP-12-mediated proteolysis remains poorly understood and may be related to remodelling of connective tissue during growth and development, and migration of macrophages into tissues. Interestingly, recent data have demonstrated that MMP-12 has direct antimicrobial activity and plays an important role in the macrophage-mediated killing of both Gram-negative and Gram-positive bacteria in the lung and other organs [61]. Increased proteolytic activity of MMP-12 has been shown to play an important role in the pathogenesis of emphysema induced by either cigarette smoke [17] or overexpression of the T-helper type 2 cytokine IL-13 in mice [62].

A recent human study identified a single nucleotide polymorphism producing a functional variant in the MMP-12 promoter that is associated with lung function in asthmatic children and the risk of COPD in adult smokers, suggesting that MMP-12 plays an important role in the pathogenesis of asthma and COPD [63]. Recently, expression of MMP-12 in the sputum of individuals with CF has been demonstrated [28]. The role of increased MMP-12 activity in CF lung disease has not been studied. However, recent observations in transgenic mice with airway-specific overexpression of the amiloride-sensitive epithelial sodium ion channel (βENaC-Tg mice), which exhibit ASL depletion and phenocopy CF lung disease [64], suggest that airway surface dehydration and mucostasis cause macrophage activation and MMP-12-dependent emphysema [65]. Further elucidation of the role of MMP-12 in the human CF lung should be facilitated by the recent development of fluorescence resonance energy transfer (FRET) probes

TABLE 1 Evidence supporting the involvement of matrix metalloproteinases (MMPs) in cystic fibrosis (CF) lung disease

	IntEnz number	Alternative name	Molecular weight	Location of expression in airways	Expression in CF	Predicted role in CF	[Ref.]
MMP-2	EC 3.4.24.24	Gelatinase A	72 kDa pro 64 kDa active	Structural cells (epithelia, endothelium) and macrophages	Detected in BAL fluid, not detected in sputum, decreased in serum during exacerbation	Regulation of ion transport, increasing remodelling and inflammatory response	[26–29]
MMP-7	EC 3.4.24.23	Matrilysin	28 kDa pro 19 kDa active	Epithelial cells predominantly	Elevated in airway epithelia	Possible role in injury/repair	[37]
MMP-8	EC 3.4.24.34	Neutrophil collagenase	75 kDa pro 65 kDa active	Neutrophils and epithelia	Elevated in serum and levels negatively correlated with lung function, elevated in BAL fluid	Modulation of inflammatory response, airway remodelling, generation of bioactive collagen fragments	[30, 50, 51]
MMP-9	EC 3.4.24.35	Gelatinase B	92 kDa pro 82 kDa active	Predominately neutrophils but also structural cells (epithelia, endothelium) and macrophages	Elevated in lower airway secretions and negatively correlated with lung function, elevated in serum	Modulation of inflammatory response, airway remodelling, generation of bioactive collagen fragments	[30, 51, 52, 56]
MMP-12	EC 3.4.24.65	Macrophage elastase	54 kDa pro 45 kDa active	Macrophages	Detected in sputum and serum of CF patients	Possible role in airway and parenchymal remodelling, possible role in generating bioactive elastin fragments	[28, 30, 65]

IntEnz: Integrated Enzyme Database; BAL: bronchoalveolar lavage.

designed to assess MMP-12 activity on leukocytes and biological fluids [66]. Based on their high sensitivity (with a detection limit in the sub-nanomolar range for human MMP-12), these novel MMP-12 FRET sensors should be suitable to measure MMP-12 activity in clinical specimens, such as bronchoalveolar lavage fluid or sputum, and determine its role in the pathogenesis and as a potential biomarker of CF and other chronic inflammatory lung diseases.

OUTLOOK: TARGETING MMPs TO TREAT CF LUNG DISEASE

As more information has been uncovered regarding the presence and activity of MMPs in CF lung disease, there has been increased interest in modulating MMP activity to improve disease outcome. Evidence supporting the involvement of MMPs in CF lung disease is summarised in table 1.

MMP inhibition has been extensively studied as a potential therapeutic pathway in cancer, and although MMP-inhibiting drugs have shown significant promise, two phase III trials have shown no clinical benefit [67]. In addition, an MMP inhibitor (PG-116800) has been studied in post-myocardial infarction cardiac remodelling and has shown no benefit. To date, the clinical use of MMP inhibitors in the setting of inflammatory disorders such as osteoarthritis has been largely disappointing [68]. Unfortunately, the usefulness of many synthetic MMP inhibitors has been limited owing to either off-target effects or intolerable side-effect profiles. Despite the difficulties of translating therapeutic approaches into other conditions, interest has turned to the potential of MMP inhibition in lung diseases such as CF. Various approaches have been studied for the inhibition of MMP activities.

TIMPs

TIMPs are naturally occurring inhibitors of MMPs *in vivo*. While TIMP binding blocks MMP activity, TIMPs themselves can be inactivated by proteases such as NE by direct proteolytic degradation [69], or by excessive MMP-12 activity [70]. Though this kind of TIMP/MMP dysregulation has been shown to contribute to the pathology of the disease and to correlate to disease severity in animal and human studies, there have not, to the current authors' knowledge, been attempts to use TIMPs as a treatment for CF. Though TIMPs serve as fairly potent inhibitors of MMP activity, the increased quantities of TIMP required to overcome a pro-proteolytic environment would probably make TIMP therapy cost-prohibitive.

Antibiotics

As previously mentioned, the current standard of long-term clinical care of patients with CF lung disease often involves thrice-weekly dosing of a macrolide antibiotic [71]. While these antibiotics have multiple biological effects, a potent effect is the inhibition of MMP activities. As yet, tetracycline antibiotics have not been systematically examined for similar anti-protease or anti-inflammatory activities in CF. Previous data indicate that tetracyclines have intrinsic anti-inflammatory properties. Doxycycline, a member of the tetracycline family, is able to reduce neutrophil chemotaxis [72] and nitric oxide production from murine lung epithelial cells [73]. More pertinently, doxycycline can inhibit MMP synthesis from human endothelial cells [74]. As MMPs have been positively

regarded as targets for tetracycline antibiotics in disease [75], there is increasing interest in inhibiting MMPs using these agents. Recent *ex vivo* data suggest that this class of antibiotic may effectively reduce MMP activity in CF sputum [28]. A single-centre randomised controlled clinical trial is planned to examine these effects in an adult in-patient CF population in the USA (Clinicaltrials.gov identifier NCT01112059, principal investigator A. Gaggar).

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