

Matrix Metalloproteinases in Acute Lung Injury: mediators of injury and drivers of repair

A Davey¹, DF McAuley², CM O'Kane^{2*}

¹ Anaesthetics department, Raigmore hospital, Inverness.

² Centre for Infection and Immunity, Queen's University Belfast.

*Corresponding author: CM O'Kane Centre for Infection and Immunity, Queen's University Belfast, Room 1.14 HSB, 97 Lisburn Road, Belfast BT9 7BL. Tel 0044 2890 972039 email c.okane@qub.ac.uk

Running head: MMPs and ARDS

Word count 7920

Key words: Acute Respiratory Distress Syndrome, Acute Lung Injury, Matrix Metalloproteinases, Inflammation, Repair, Extracellular Matrix

Abstract – 200 words

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) comprise a spectrum of acute inflammatory pulmonary oedema resulting in refractory hypoxaemia in the absence of an underlying cardiogenic cause. There are multiple pulmonary and extra-pulmonary causes and ALI/ARDS patients are a clinically heterogeneous group with associated high morbidity and mortality.

Inflammatory injury to the alveolar epithelial and endothelial capillary membrane is a central event in the pathogenesis of ALI/ARDS, and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs) have been implicated in a wide variety of pulmonary pathologies and are capable of degrading all components of the extracellular matrix including the basement membrane and key non-matrix mediators of lung injury such as chemokines and cell surface receptors.

While many studies implicate MMPs in the injurious process there are significant gaps in our knowledge of the role of specific proteases at different phases of injury and repair. This article examines the role of MMPs in injury and repair of the alveolar epithelial-endothelial capillary barrier and discusses the potential for MMP modulation in the prevention and treatment of ALI. The need for further mechanistic and *in vivo* studies to inform appropriate subsequent clinical trials of MMP modulation will be highlighted.

Introduction

Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) are part of a disease spectrum associated with high mortality and considerable morbidity. The most recent diagnostic criteria for ALI/ARDS developed by the 1994 American European Consensus Conference Committee are based on the gross clinico-radiological findings; acute onset, bilateral infiltrates on chest x-ray, absence of left ventricular failure and $\text{PaO}_2(\text{mmHg}):\text{FiO}_2$ ratio of <200 (ARDS) or <300 (ALI) (1-3). However, ALI/ARDS patients are clinically heterogeneous and this definition does not take into account the underlying aetiology or severity of illness reflected by failure of other organ systems (1). Irrespective of aetiology, the pathogenesis of ALI/ARDS consists of an excessive and inappropriate inflammatory response to a range of pulmonary or extrapulmonary insults resulting in damage to the alveolar epithelial-endothelial capillary barrier (2,3). Clinically, this manifests as refractory hypoxaemia and decreased pulmonary compliance. Pathologically, three phases are recognised: an initial acute inflammatory or exudative phase characterised by proteinaceous oedema and neutrophil invasion of alveoli, subsequent hyaline membrane formation and interstitial fibroproliferation, followed by a variable degree of resolution (1-4). These events are co-ordinated by a cascade of inflammatory mediators that ultimately results in the generation of reactive oxygen and nitrogen species along with proteolytic enzyme release with resultant tissue destruction and respiratory failure. Histopathologically, there is disruption of the alveolar epithelial -endothelial capillary barrier (3,4). Also known as the blood air barrier, it consists of alveolar epithelium, capillary endothelium, extracellular matrix and other cells such as alveolar macrophages and fibroblasts.

These elements are configured to optimise both surface area and thickness of the blood air barrier for efficient gas exchange while maintaining an extracellular matrix (ECM) framework with favourable mechanical properties to facilitate ventilation. Structurally, the blood air barrier has either a thick or thin configuration in relation to the type I alveolar epithelium, underlying capillary endothelium and ECM (5,6). Gas exchange occurs across the thinnest part of the blood air barrier where the type I alveolar cell is closely applied to the endothelium by fused basal lamina. For the remaining portion of the blood air barrier, a layer of ECM is located between the epithelium and endothelium; this thick portion of the blood air barrier is responsible for liquid and solute exchange (6). ECM is a generic term that encompasses both the basement membrane and interstitial connective tissue and is an important functional component of all tissues (7). In the lung, ECM provides a mechanical framework that permits cyclical volume change in the terminal respiratory units and consists of type I collagen, type III collagen, elastin and proteoglycans. In the thin segment of the blood air barrier, alveolar epithelium overlies a basement membrane composed of type IV collagen, laminin, type V collagen, and proteoglycans (7), of which type IV collagen is the principal component. Recently, however, it has become evident that ECM is more than just an inert scaffold with dynamic mechanical properties. A range of processes, such as cell survival, proliferation and migration are influenced by cell-matrix interactions and matrix turnover, making matrix biology in disease an expanding field of interest (7).

Function and regulation of MMP activity

Matrix metalloproteinases (MMPs) are a diverse family of extracellular proteinases of which twenty four, loosely classified according to numerical sequence of discovery, have been identified in humans (8). They are also commonly classified using combination of domain organisation, sequence homology and substrate specificity as “collagenases” (MMP1, -8, -13), “gelatinases” (MMP-2, -9), “stromelysins” (MMP-3, -10), “matrilysins” (MMP-7, -26), “membrane bound” (MMP-14, -15, -16, -17, -24, -25) and others (MMP-11, -12, -19, -20, -21, -22, -23, -27 -28) as outlined in table 1 (8-10). MMPs are produced by a variety of connective tissue, epithelial and inflammatory cells and together are capable of degrading all known components of the ECM with significant substrate overlap, suggesting some degree of functional redundancy between MMPs. It has also been demonstrated that MMPs act on several non-ECM substrates such as pro-TNF α , pro-IL1 β , pro-TGF β 2, chemokines, antiproteases and pro-proteases with effects on processes such as cell growth, proliferation, survival and migration (see table 1) (8). Regulation of activity occurs at both pre and post translational levels. With the exception of neutrophils and to a lesser extent macrophages, cells do not store MMPs, and expression is induced by a variety of transcription factors, growth factors, cytokines/chemokines, reactive oxygen species and other exogenous environmental or pathogen derived agents (8). Secretion of a latent proenzyme form that requires activation by cleavage of the prodomain and the presence of natural inhibitors of MMPs such as tissue inhibitors of metalloproteinases (TIMPs) are important mechanisms that constrain the action of secreted MMPs.

Furthermore, cellular and tissue localisation or compartmentalisation is also important in control of MMP activity (11).

Matrix metalloproteinases are known to be involved in a variety of physiological and pathological processes and MMP involvement in embryological development (12), inflammation (13), innate immunity (14), tissue remodelling (15) and ECM biology (16) has been reviewed elsewhere. Reports implicating MMPs in ALI/ARDS are found in the literature from the early 1990s; however, research efforts have failed to translate into effective pharmacotherapies. Furthermore, recently published reviews tend to focus on the role of MMPs under the broad spectrum of destructive pulmonary pathologies (17,18), without recognising their role in mediating tissue repair as demonstrated in more recent investigation (19). This article will review the evidence implicating MMPs in aspects of ALI/ARDS most relevant to translational research. First, the potential for discovery of novel therapeutic targets by investigating the role of MMPs in mediating alveolar epithelial-endothelial barrier injury during the acute phase of ALI/ARDS will be discussed, followed by similar consideration of MMPs during lung regeneration and repair following ALI/ARDS.

MMPs in alveolar injury

Degradation of protein components in the alveolar epithelial-endothelial unit, including intercellular junction proteins, basement membrane itself and proteins anchoring cells to the basement membrane, is considered a central process in the pathogenesis of ALI/ARDS.

***In vivo* models**

Several studies have specifically investigated the activity of MMPs in experimental lung injury. In hyperoxia treated pigs, animals developed features of lung injury such as increased wet-to-dry ratio, decreased PaO₂/FiO₂ and increased bronchoalveolar lavage (BAL) fluid cellularity after 72 hours of exposure to 80% oxygen (20). This was associated with elevated MMP-9 and -2 in BAL after 72 hours; parameters of lung injury correlated dose-dependently with BAL MMP-9 concentrations. Investigators noted these results were not reflected in the analysis of lung homogenates from representative animals. However, no mention is made of an attempt to standardise the assay by measuring total protein content in each of the different samples, which limits any conclusion regarding the difference between MMP activities in BAL or lung tissue in this study. It was also noted that MMP-2 levels were detected earlier than MMP-9 with a peak at 72 hours in contrast to 96 hours for MMP-9. Immunohistochemistry localised MMP-9 to neutrophils, macrophages and epithelial cells whereas MMP-2 mainly localised to alveolar macrophages (20). Of note, the dominant form of both MMP-2 and -9 detected was the high molecular weight latent form. Investigators also report detection of additional but less abundant molecular weight bands corresponding to the active forms of MMP-2 and -9.

Another model of hyperoxia in rats investigated MMP-2, -8, and -9 in BAL and lung parenchyma by zymography and immunohistochemistry (21). Lung injury was assessed by protein levels in BAL and myeloperoxidase (MPO) activity in lung samples to reflect capillary permeability and neutrophil accumulation, respectively. Investigators demonstrated increased latent forms of MMP-2 & -9 in BAL by zymography after 48 hours of hyperoxia but also identified different molecular weight (MW) forms of MMP-8

in BAL by western blotting (21). Authors reported these different forms corresponded to neutrophil and mesenchymal derived MMP-8. Mesenchymal-cell derived MMP-8 was identified as the dominant form by western blotting and immunohistochemistry revealed the mesenchymal MMP-8 localised to cells with large cytoplasm, considered on this basis by the authors to be recruited macrophages (21).

Bleomycin injury in rats has also been used to investigate the profile of MMP-2 and -9 in early and late phases of lung injury (22). Histology and BAL analysis demonstrated an acute injury pattern on day four following intratracheal instillation 1.5unit/kg Bleomycin. Neutrophils were the dominant source of MMP-9 detected in BAL and macrophages were the main source of MMP-2 (22).

A swine model of lung injury after cardiopulmonary bypass (CPB) also demonstrated a rise of both MMP-2 and -9 in BAL that correlated with the alveolar-arterial oxygen gradient ($AaDO_2$) (23). However, no other markers of lung injury were used in this study to rule out increased $AaDO_2$ caused by a high V/Q ratio or physiological dead space secondary to suboptimal perfusion on CPB. Neutropenic septic lung injury induced by cyclophosphamide and caecal ligation and puncture (CLP) in mice is also associated with increased MMP-9 in lung tissue four to seven days post CLP (24). In this study, investigators measured MMP activity by zymography in lung homogenates of equal protein content in immunosuppressed or normal mice following CLP or sham operation.

Human data

Both adult and paediatric clinical studies provide useful information on the activity of MMPs in ALI/ARDS in patient cohorts with a spectrum of underlying aetiologies.

Although neonatal RDS with progression to bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD) is distinct from ALI/ARDS in adults, similar inflammatory mechanisms may be involved in pathogenesis of both conditions. Therefore, both studies on neonatal RDS and ALI/ARDS in the paediatric age group are included in the following section.

Adult studies

An early but small prospective case control study investigated the presence of MMP-2 and -9 in BAL fluid from patients admitted to ICU with ARDS compared to patients ventilated for other indications such as stroke, encephalitis and sepsis or pancreatitis without ARDS (25). Zymography demonstrated the presence of activated and latent forms of MMP-2 and -9 but only MMP-2 levels were significantly elevated in the ARDS patients. In contrast to this, another small clinical study demonstrated a significant elevation in MMP-9 but not MMP-2 in BAL from patients with permeability pulmonary oedema compared to ventilated patients diagnosed with hydrostatic pulmonary oedema (26). However, this study has some limitations in that patients were recruited over an 8 year period and the criteria for selection/recruitment are unclear. Furthermore the control group was much smaller than the study group (8 vs. 23) In contrast to the above studies where the control groups comprised patients with hydrostatic oedema or patients ventilated with systemic illness but no pulmonary involvement a two year prospective clinical study investigated concentration and activity of MMP-2, -9, TIMP-1 and TIMP-2 in BAL from ALI/ARDS patients and compared these with levels and activity in BAL from patients with hospital acquired pneumonia (27). Levels of MMP-2 and -9 were elevated in ALI/ARDS but less than the hospital acquired

pneumonia group. There were two groups of ALI/ARDS patients in this study, group 1 with illness less than or equal to four days and group 2 with illness lasting more than eight days. Authors found high levels of MMP-9 and a MMP-9:TIMP1 ratio >1 at day zero in group 1 and a marked reduction in MMP-9 and the MMP-9/TIMP-1 ratio at day four in a group of ARDS patients with duration of illness greater than 8 days(27). This suggests that MMP activity may also play a role in progression or persistence of the disease although it is not clear from this study which MMP-9 and TIMP1 profile reflects the true causal relationship. Assessment of both MMP and TIMP levels in a more recent prospective case control study demonstrated elevated levels of MMP-2, -8, -9 and TIMP-1 compared to 12 healthy volunteer controls (28). This study recruited 28 patients with ALI/ARDS from medical ICU and controls from the local medical centre and BAL MMP-2 & -9 levels were assessed by zymography with MMP-1, -3, -8 and TIMP-1 & -2 levels in BAL assessed by ELISA. Increased levels of MMP-2, -8, -9 were not significantly correlated with prolonged ICU admission, duration of ventilation, APACHE III score or $\text{PaO}_2/\text{FiO}_2$ ratio at BAL. Although MMP-1 or -3 levels were not elevated in most patients, investigators noted that a subset of patients with high mortality and more severe APACHE III scores also had detectable MMP-1 and -3 in BAL (28). However, timing of BAL was not reported and a wide time frame of 48 hours from ARDS diagnosis was permitted for sampling. It is therefore possible this association could simply be temporal bias in the MMP profile from delayed BAL in patients with more severe illness involving other organ failure.

A very recent clinical study utilised a multianalyte array to profile MMP-1, -2, -3, -7, -8, -9, -12 and -13 in BAL from ALI/ARDS patients in the control arm of a phase II

randomised control trial of intravenous beta 2 agonists in acute lung injury (the BALTI trial) (19). Compared to BAL from seven healthy controls, patients with ALI/ARDS had increased levels of all eight MMPs analysed within 48 hours of fulfilling the criteria for ALI. This study was the first to describe the temporal changes in MMP profile over the course of lung injury. MMP-1, -3, both present at baseline fell by approximately one third by day 4, while MMP-2 showed a non-significant trend to falling over the same time course. MMP-7, -8, -12 and -13 were all significantly elevated in ARDS BAL compared with BAL from normal volunteers, but the concentrations did not change appreciably between baseline and day 4. In contrast MMP-9 was the only MMP measured that showed a trend to elevation (2 fold increase in median concentration) over the first 4 days, although this was not statistically significant. Further analysis of MMP-9 concentration and activity in the treatment arm of this study will be considered along with lung repair in ALI/ARDS below. Inhalation of low dose lipopolysaccharide (LPS) by healthy volunteers was used in another recent clinical study to mimic the neutrophilic alveolar inflammation that occurs in ALI (29). BAL was performed 6 hours after LPS inhalation, and significantly increased MMP-2, -7, -8 and -9 were detectable (but not MMP-1, -3, -12 and -13).

Paediatric studies

An early study on neonates with respiratory distress, investigated activity of type I collagenases (MMP-1 and -8) in babies requiring mechanical ventilation within the first six days of life, born before 33 weeks (30). Measurement of MMP-1 and -8 in 100 BAL samples from 45 children by ELISA did not detect MMP-1. MMP-8 was detected in 68 samples and tended to rise over the first 6 days. Higher MMP-8 concentrations were found in BAL during this period from patients who proceeded to develop CLD than those who did not. In another study in preterm infants with RDS, investigators measured MMP-2, -8, -9 and TIMP-2 by western blot analysis in tracheal aspirates of 16 babies intubated at birth. Both latent and active forms of MMP-2 and -9 and two MW forms of latent and active MMP-8 were detected (31). There was no association between MMP-2 or -9 and the development of BPD. Authors reported the higher MW form of MMP-8 to be polymorphonuclear (PMN) in origin and the lower MW form to be mesenchymal cell in origin, although there are no data in this study to confirm the source of either form detected. When MMP-8 was compared between babies that developed BPD an association between BPD and significantly higher PMN MMP-8 was found. Analysis of TIMP-2 demonstrated elevated levels in 15 babies and a significant correlation between TIMP-2 and both surfactant treatment and prolonged mechanical ventilation (31). In contrast to this, a larger prospective study found MMP-2 and -9 in 73% and 79% of BAL samples from premature babies requiring ventilation within the first 48 hours of life (32). Analysis by degree of prematurity and subsequent development of CLD demonstrated significantly elevated MMP-9:TIMP1 ratio in very premature babies who developed CLD.

More recently, MMP and TIMP activity in children with ALI/ARDS were investigated in a case control study comparing serial lung secretions obtained by endotracheal suctioning within 24 hours of diagnosis to samples from otherwise healthy patients ventilated for minor elective surgery (33). Authors demonstrated detectable MMP-8, -9 and TIMP-1 in the majority of ALI/ARDS samples by western blotting. ELISA based activity assays demonstrated significantly increased activity of MMP-8 and -9 in children with ALI/ARDS. When authors looked at MMP profile and disease progression they used ALI/ARDS lasting 10 or more days to define a prolonged course. Analysis of the MMP-8 profile demonstrated the predominance of a lower MW form after day ten that was presumed, but not confirmed, to be of mesenchymal cell origin. Also, in cases of ALI/ARDS lasting less than ten days the MMP-9:TIMP-1 ratio was significantly elevated compared to patients with ALI/ARDS lasting less than ten days (33)

From the studies discussed so far, MMP-1, -2, -3, -7, -8, -9, -12 and -13 are implicated in the pathogenesis of ALI/ARDS with most attention focused on collagenolytic (MMP-1 and -8), gelatinolytic (MMP -2 and -9) MMPs and stromelysin (MMP-3). However, significant detail is still lacking on the basic profile of *in vivo* MMP activity and potential substrates in ALI/ARDS. This is important, as differences in MMP form, source, site and presence of inhibitors would have significant implications for both net MMP activity and potential substrates *in vivo*, which are obviously important for both determination of an underlying causal role, and understanding the potential mechanisms of action for MMPs in the pathogenesis of ALI/ARDS.

In most studies reported, the dominant form of MMPs detected was a higher molecular weight latent form with the inhibitory domain still attached. However, net

MMP activity depends on the balance between active protease concentration and presence of endogenous inhibitors. Therefore, if MMPs have a pathogenetic role in ALI/ARDS it must be demonstrated that net MMP activity is increased. Attempts to explain detection of MMP in the latent form were limited, but some authors did postulate that activation of the latent form *in vivo* by reactive oxygen or nitrate species without proteolytic cleavage could account for the large proportion of latent form detected. Other studies, but not all, also analysed the presence of TIMP in samples collected, which gives a better indication of the likely net MMP activity. Therefore, further studies investigating a possible role for a given MMP should consider both its form and the presence of any endogenous inhibitors in addition to a simple semiquantitative description of a particular MMP.

Accepting that MMP activity is elevated in lung injury, the next step is to consider how these proteinases may be involved in the pathogenesis of ALI/ARDS. The early structural degradation concept outlined above is likely to be oversimplified as the ECM mediates a wide variety of cellular functions important to acute inflammation and tissue injury, in addition to providing structural integrity to the alveolar epithelial-endothelial unit. General mechanisms by which proteolysis of ECM/Basement membrane (BM) and non-ECM substrates by MMPs may affect inflammatory injury have been extensively reviewed elsewhere and were briefly outlined above (12-16). However, it is not possible to determine the exact role of MMPs in ALI/ARDS from the above data and several possible conclusions can be drawn from observations that a range of MMPs are elevated in ALI/ARDS and they are produced by a variety of cells. Implication of so many MMPs may reflect MMP interaction or amplification in a proteolytic cascade.

Alternatively, each MMP may have a specific and separate role mediating degradation of ECM / BM and non-ECM substrates with various subsequent biological effects, at discrete time points in the evolution of the illness. Other explanations include functional redundancy, based on the wide range of overlapping substrates, between MMPs currently implicated in ALI/ARDS; or that simple variation between species and insults in experimental models used accounts for the range of MMPs implicated. In terms of overlapping substrate specificity and potential functional redundancy, it is important to understand that *in vivo* substrate specificity is determined by many factors, as outlined above, in addition to *in vitro* biochemical properties of stereospecificity and reaction constants for a given enzyme-substrate interaction. Obviously use of a wide range of animal species and various injurious insults does limit comparison between studies, but such studies are useful for hypothesis generation and should not be ignored.

Taken together, the information above suggests that MMPs may have multiple pathophysiologic roles as both the mediators and effectors of tissue injury in ALI/ARDS. Although significant and specific details are still lacking on MMP profiles, detection and profiling of MMPs in fluids and tissues of animals with experimental ALI/ARDS cannot establish details of a causal role and specific mechanisms of action. More sophisticated techniques and models are required and manipulation of MMP activity either pharmacologically or genetically, in experimental lung injury, offers important information on both the role and mechanisms of MMP involvement in alveolar injury in ALI/ARDS.

Use of chemical inhibitors to study the role of MMPs in ALI

Regulation of MMP activity *in vivo* occurs at multiple levels and there are several agents available that act at both pre and post-transcriptional levels to reduce MMP activity. Doxycycline and other chemically modified tetracyclines such as COL-3 and CMT-3 are the most commonly used non-specific MMP inhibitors. Tetracyclines both directly inhibit MMP activity and transcription (34). The hydroxamates, Prinomastat (AG-3340) and Batimastat (BB-94), are chemicals that chelate metal ions including zinc which is found in the MMP active site (35). Although these agents are reported to be relatively specific, the hydroxamates are not zinc specific chelators and therefore have the potential to inhibit other closely related protease families (35). Both Prinomastat and Batimastat have been used in clinical trials in cancer and rheumatological diseases to inhibit MMPs, and have been used in animal models to study their role in ALI.

Tetracyclines

Rats ventilated with high tidal volumes (30ml/kg) for two hours developed ventilator induced lung injury as determined by wet to dry ratio, histopathological scoring and neutrophil MPO activity and count. Investigators demonstrated that injury was associated with increased MMP-9 expression and activity but when pre-treated with CMT-3, histological indices of injury were reduced (36). More recently the effect of tetracycline-based MMP inhibition on the pulmonary proteome was investigated in rat model of lung injury (37). Oral pre-treatment with doxycycline improved oxygenation and compliance in rats undergoing high volume ventilation compared to placebo treatment and low volume ventilation. Proteomic analysis of lung homogenates identified elevated levels of nine proteins in the treatment group, including soluble receptor for advanced glycation endproduct (sRAGE), ApolipoproteinA-1(ApoA-1),

Peroxiredoxin II, four molecular forms of albumin and two unnamed proteins (37). Soluble RAGE has been proposed to act as a decoy receptor that might attenuate inflammation induced by RAGE ligands in lung injury but the pathogenic significance of the other proteins in ALI/ARDS is uncertain. However, this does highlight some potentially novel non-ECM MMP substrates that may modulate alveolar injury in ALI/ARDS. Pigs pre-treated with COL-3 twelve hours prior to IV LPS (100microg/kg)-induced lung injury demonstrated less histological injury, less hypoxia and were easier to ventilate than control groups (38). In an indirect model of ALI in rats subjected to caecal ligation and puncture (CLP), which were treated with COL-3 at time of injury, showed reduced lung water, improved histological grading for lung injury and increased survival (39). Importantly, the effect on survival was noted to be more pronounced with further dosing post lung injury (39). This has significant clinical importance as the first evidence in animals that treatment post lung injury with a non-specific MMP inhibitor can reduce both severity of lung injury and improve survival. However, intervention groups were small and there were no physiological measures of hypoxia or compliance that would have made this model potentially more comparable with the human disease.

In a CPB swine model of lung injury, CMT-3 was used to assess the response to MMP inhibition (40). Thirty minutes after CPB was initiated either low dose LPS (1microg/kg), a control or simultaneous LPS and CMT-3 were given. Animals receiving CPB and LPS developed ARDS-like pathology with 60% survival. Clinical features of ARDS were prevented by CMT-3 treatment with a 100% survival compared to 60% during the 270min of experimentation (40). It is also worth noting that these all animals

were subject to tidal volumes of 12ml/kg during this experiment, which essentially provides another injurious stimulus to the lungs in all groups.

Subcutaneous administration of doxycycline in a pancreatitis model of lung injury reduces the histological grade of ALI and amount of MMP-9 in lung as detected by an enzyme activity fluorescence assay (41). In a separate *in vitro* experiment transmigration of neutrophils across matrigel and MMP-9 levels in chamber supernatants were reduced by addition of doxycycline in a concentration to match that achieved in the plasma after subcutaneous administration in the animal experiment (41).

Hydroxamates

Prinomastat (AG-3340) has been shown to reduce lung injury in rat model of VILI (42). Animals in this study were exposed to high volume ventilation for four hours and severity of lung injury assessed by wet to dry weight ratio and BAL protein content. Increased latent and active MMP-2 and -9 in untreated groups were detected by zymography and immunoblotting of BAL after high tidal volume ventilation (42). Levels of TNF α and extracellular matrix metalloproteinase inducer (EMMPRIN) were also elevated in VILI as determined by *in situ* hybridisation and ELISA, respectively. Administration of AG-3340 intraperitoneally (IP) for two days and two hours before ventilation reduced MMP-2 and -9 expression in lung tissues and activity in BAL. Wet to dry weight ratio and BAL protein content were also reduced. Investigators noted that prinomastat reduced levels of TNF α , likely due to the inhibitory effect of AG-3340 on TNF α converting enzyme (TACE) which cleaves and activates TNF α .

Use of Batimastat (BB-94) has also been shown to reduce lung injury following acute pancreatitis in rats. Investigators demonstrated reduced proteinaceous exudate and neutrophil accumulation in alveoli during acute pancreatitis with intraperitoneal injection of BB-94 48 hours prior to initiation of injury (43). Histological grading of lung injury also improved with pre-administration of BB-94. In another study, investigators assessed *in vivo* and *in vitro* role of MMP-9 in pancreatitis - associated lung injury using BB-94. Investigators demonstrated pancreatitis was associated with lung injury as determined by albumin leak in BAL and increased levels of MMP-9 in lung homogenates (44). Neutrophils isolated from animals with pancreatitis - associated lung injury demonstrated increased levels of MMP-9 in the cell culture supernatants. Stimulation of human neutrophils by IL-1 β and TNF α in this study also demonstrated increased migration across a matrigel chamber that was abrogated by the administration of BB-94 (44).

Anti-proteases

Manipulation of MMP activity has also been demonstrated by the administration of recombinant tissue inhibitors of matrix metalloproteinases (TIMPs) or a specific anti-TIMP immunoglobulin. Such studies help demonstrate that endogenous protease inhibitors are important contributors to the overall activity of a given protease *in vivo*. An early study on the role of neutrophil proteases in pathogenesis of ALI/ARDS demonstrated that activated neutrophil secretions possessed both serine protease and gelatinase activity. Inhibition of these enzymes by addition of recombinant human protease inhibitors, secretory leukocyte protease inhibitor (SLPI) and TIMP-2, suppressed immune complex mediated lung injury in rats (45). Activity of a given

protease can also be augmented by inhibiting its natural inhibitor using specific immunoglobulins. Administration of anti-TIMP-2 and anti-secretory leukocyte protease inhibitor (SLPI) IgG in a rat model of immune complex mediated lung injury demonstrated intensified lung injury with increased neutrophil recruitment to the alveolar space (46).

Other inhibitors

Non-specific anti-inflammatory agents that affect MMP gene transcription such as tyrosine kinase inhibitors or pentoxifyline (PTX) have also been shown to reduce lung injury by mechanisms involving decreased MMP-2 & -9 levels in models of lung injury secondary to sepsis and resuscitation from haemorrhagic shock. In a rat model of i.v. LPS-induced lung injury, administration of 5mg/kg LPS via internal jugular vein caused histologically confirmed lung injury with increased levels of MMP-2 in BAL and MMP-9 in plasma at four hours (47). Co-administration of 25mg/kg pentoxifyline, a non-specific phosphodiesterase inhibitor, reduced severity of lung injury and MMP-2 levels in BAL. There was no reduction of plasma MMP-9 and authors did not comment on reasons for omitting results for plasma MMP-2 levels. Authors analysed NF κ B activation using electrophoretic mobility shift assay and found increased activation in the LPS group, suggesting that these effects were mediated via NF κ B dependent transcription of MMPs (47). The lack of reduction in MMP-9 in this study may represent the release of stored MMP-9 from neutrophils in the treated group. In another study intratracheal (IT) administration of 6mg/kg LPS in rats was used to induce lung injury defined by increased BAL protein and LDH content (48). Investigators measured MMP-9 activity by zymography in BAL and cell cultures of alveolar macrophages retrieved from BAL in

the same animals. In the treatment arm of the study animals were pre-treated with IT administration of the tyrosine kinase inhibitor Genistein two hours prior to injury. Both BAL and supernatant from alveolar macrophage cultures had increased levels of MMP-9 but treatment with Genistein reduced MMP-9 levels in BAL associated with reduced NF κ B activation in lung tissue and alveolar macrophages (48). Use of PTX along with hypertonic saline resuscitation from haemorrhagic shock in rats has also been shown to reduce lung injury and MMP-2 and -9 levels in BALF and lung homogenates compared to animals with standard Ringers Lactate resuscitation strategy (49)

Use of Knockout mice to study the role of MMPs in ALI

Chemical inhibition of MMPs is relatively non-specific and is likely to have multiple effects on other cellular processes and mediators involved in lung injury. Therefore, data from such studies have significant limitations in determining details on the roles of, or interactions between, MMPs as either mediators or terminal effectors of lung injury. Use of knock-out mice lacking expression of MMPs offers a more selective method of investigating the role of an individual MMP in ALI/ARDS. Recent experiments using a range of knockout mice models have provided further information on the potential role of MMP-3, -7 -8 and -9 in the pathogenesis of injury in ALI/ARDS.

Results from studies using MMP-9 knockout mice in several different models of lung injury contradict each other and conclusions from experiments using non-specific chemical inhibition of MMPs. Investigation of hyperoxic injury in the developing lung using MMP-9 knockout mice demonstrated that deficient MMP-9 seems to abrogate the bronchopulmonary dysplastic like features generated by hyperoxia in wild type controls (50). Lung injury induced by intratracheal instillation of IgG in mice was shown to be

reduced in MMP-9 knock outs compared to wild type controls (51). However, a recent investigation using an IL-1 β -induced model of BPD in MMP-9 $-/-$ mice demonstrated worse lung injury in mutants compared to wild type controls (52). In another study using MMP-9 knockout mice, investigators demonstrated that lack of MMP-9 resulted in a more severe lung injury following ventilator induced lung injury (VILI) (53), suggesting a protective role for MMP-9 in this model of injury.

Other MMPs such as MMP-3 and MMP-7 have also been shown to have a pathogenic role in development of experimental lung injury in knockout (KO) models. In mice lacking MMP-3, intratracheal administration of IgG resulted in less lung injury and neutrophil influx (54). In another study investigators reported a significant difference in neutrophil recruitment between MMP-3 KO mice and MMP-9 KO animals with the same injury, suggesting that MMP-3 plays an essential role in migration of neutrophils from the pulmonary circulation to the alveolus (51).

In the Bleomycin model of lung injury MMP-7 contributes to neutrophil migration by shedding of Syndecan-1 (55). Instillation of Bleomycin in MMP-7 null mice resulted in decreased migration of neutrophils into the alveolus. The authors showed that MMP-7 cleaved syndecan-1 from the mucosal surface of the epithelium: absence of shed syndecan-1 prevented the formation of a KC (murine orthologue of CXCL8)-syndecan gradient that directed neutrophils to the alveolar space (55).

Regulation of MMP activity *in vivo* has also been demonstrated by TIMP-1 KO mice. Deficiency of this endogenous inhibitor *in vivo* resulted in significantly greater lung injury as measured by pulmonary neutrophilia, permeability and haemorrhage in TIMP-1 null mice compared to wild type controls (56). Investigators assessed

gelatinase activity by zymography and demonstrated increased activity of MMP-9, emphasising the importance of a balance between protease and antiproteases in determining the overall proteolytic activity in ALI/ARDS. However the absence of TIMP-1 would leave functionally unopposed activity other proteases in addition to MMP-9 and this study did not investigate other MMPs. Therefore concluding that increased lung injury in this study was due to the functionally unopposed MMP-9 activity is not possible.

Investigation of MMP-8 involvement in lung injury using knockout mice in a variety of models has yielded conflicting results. In other animal models and clinical studies MMP-8 has been detected in BAL and was possibly produced by both neutrophils and mesenchymal cells. In these studies involving MMP-8 KO mice investigators report activity of MMP-8 is derived from neutrophils and has been demonstrated to be largely membrane bound and highly resistant to TIMP. Mice lacking MMP-8 had greater lung injury in an intratracheal LPS model, suggesting an anti-inflammatory role for MMP-8 (57). Furthermore MMP-8 deletion was shown to increase severity of injury and inflammation in LPS, Bleomycin and hyperoxia induced lung injury (58). Investigators demonstrated increased neutrophil and macrophage recruitment due to increased macrophage inflammatory protein alpha (MIP-1 α), but not other traditional PMN chemottractants, in BALF of MMP-8^{-/-} mice (58). In contrast to an anti-inflammatory effect, MMP-8 has been shown to play an important role in mediating VILI as mice lacking MMP-8 have reduced lung injury when subjected to injurious ventilatory strategies (59).

From the above evidence it is clear that MMPs are more than just terminal effectors of ECM/BM destruction in an inappropriate and or excessive innate response

to tissue injury or infection. Matrix metalloproteinase activity is elevated in ALI/ARDS but individual roles in pathogenesis are likely determined by the source of secretion, substrates available at the site of activity, and a local balance between latent or active form and any inhibitors present. Figure 1 highlights the key MMPs and cells involved in the pathogenesis of ALI/ARDS. Furthermore MMPs may have a role in repair such that after established injury, a deficiency of one or more proteases may inhibit the epithelial reparative process. This highlights the need for more work on these basic aspects of MMP biology to better characterise the pathogenic role of individual MMPs at any given stage of the ALI process. Non-specific inhibition of MMPs has demonstrated reduced lung injury in pre-treatment models but in one study a treatment effect was noted after lung injury had been caused. Importantly, this suggests a possible therapeutic window exists for the treatment of early ALI/ARDS in humans with agents that are non-specific MMP inhibitors. Gene targeting of individual MMPs offers a potentially selective method of controlling individual MMP activity. Results, however, have been conflicting. MMP-8 and -9 KO mouse models of lung injury demonstrate both exacerbation and reduction of lung injury from a variety of insults. MMP-7 mediated shedding of syndecan-1 seems to be consistently important *in vivo*, but MMP-7 has also been implicated in epithelial repair, while MMP-3 also seems to have an important role in mediating experimental lung injury but no specific substrate has been identified. This gap in knowledge between the lung injury phenotype of KO mice and lung injury in other methods of MMP inhibition highlights the importance of assessing both the molecular and cellular consequences of MMP manipulation *in vivo*.

MMPs in repair of the alveolar epithelial endothelial unit

By the time ALI is clinically recognized an overwhelming inflammatory cascade is already established. MMPs are also known to be involved in physiological processes that may be important to tissue repair, such as development and morphogenesis, and there are some data indicating they are important in alveolar epithelial repair. Further understanding of their role in repair may highlight potential new strategies to aid resolution of ALI.

Epithelial wound repair in the lung

Basic mechanisms of wound repair in the lung have been extensively reviewed and current understanding focuses on cellular and molecular aspects of epithelial repair in the alveolar capillary membrane. Essentially, the alveolar epithelial defect (wound) is filled by spreading and migration of neighbouring type II alveolar epithelial cells along a provisional ECM followed by proliferation and differentiation to type I alveolar epithelial cells with restoration of epithelial integrity (60). However, recent reviews (61-63) highlight that understanding of this process and what influences the time course (onset of fibroproliferative response) and trajectory (balance between repair and fibroproliferation) of healing in the lung is limited as there are obvious difficulties in modelling *in vivo* alveolar epithelial cell repair *in vitro*.

Time course and trajectory of epithelial repair in the lung

Currently, the accepted paradigm of ALI/ARDS evolution is that of a linear progression through exudative, fibroproliferative and resolution phases. However, more recent investigation of repair and remodelling in lung injury demonstrates that fibroproliferation begins early in the development of ALI/ARDS. Using an intraperitoneal injection of paraquat model of lung injury in rats, investigators demonstrated that altered

tissue mechanics associated with markers of ECM remodelling are detected as early as 24 hours after injury (64). In another animal study, pulmonary mechanics were assessed in mice with lung injury induced by administration of LPS either 125µg IP or 10µg IT (65). Collagen fibre deposition was increased and pulmonary compliance decreased at 24hrs post injury. This finding is also supported in humans with an autopsy study of ALI/ARDS patients demonstrating collagen deposition early in the course of the syndrome (66). Clinical studies in patients with ALI/ARDS also demonstrate that fibroproliferation begins early in the course of disease. In one prospective trial, BAL from 77 patients with ALI/ARDS was assessed for mitogenic activity on human fetal lung fibroblasts and collagen synthesis by presence of procollagen peptide. Compared to a small number of controls patients with ALI/ARDS had potent mitogenic activity and high levels of procollagen peptide III (PCP III) in BAL from day one (67). Another study investigated the relationship between chord compliance calculated from the linear part of pressure volume curves in ventilated patients, PCP III and MMP in patients with ALI/ARDS having BAL within four days of diagnosis. Investigators found decreased chord compliance and evidence for increased collagen synthesis from PCP III levels in these early samples (68).

Some investigators believe the primary site or component of the alveolar epithelial endothelial unit injured has important effects on the trajectory of healing and balance between fibroproliferation and repair after lung injury. It is intuitive that injury to either the epithelial or endothelial face of the alveolar unit would dominate in pulmonary or extrapulmonary ALI/ARDS, respectively. Therefore different causes of ALI/ARDS could be important in determination of the fibroproliferative response to injury.

Investigation of the degree of fibroproliferation in two models of extra-pulmonary experimental lung injury in mice demonstrated that a greater degree of endothelial injury is associated with greater degree of fibrosis following lung injury (69). Investigators assessed mechanical properties and content of collagen and elastin by histology after either 125µg IP LPS or CLP. Mice with lung injury had a greater degree of endothelial injury determined by electron microscopy, associated with more collagen and elastin fibre deposition in the lung and reduced compliance (69). However, it seems the primary hypothesis in this investigation was not formed *a priori* and these findings contradict other studies and current understanding that suggest epithelial damage is associated with a greater degree fibroproliferation (61-63). In contrast to this study, the importance of alveolar epithelial damage as a determinant of fibroproliferation is demonstrated in a mouse model of ALI/ARDS using intratracheal or intraperitoneal LPS already mentioned (65). Investigators noted that animals with a dominant pulmonary insult provided by IT LPS yielded fibroelastogenesis but damage to the endothelium caused fibrosis that resolved early in the course of injury. In humans, autopsy studies already discussed also reveal a potential difference in the degree of collagen deposition depending on the aetiology of injury classified as pulmonary or extra-pulmonary (66).

MMPs and alveolar epithelial repair

As already discussed MMPs have various biological effects by proteolytic action on cell – cell and cell – matrix interactions that ultimately manipulate cell and tissue processes likely to be important to repair. However, current understanding of MMP involvement in remodelling and repair following ALI/ARDS is limited. Epithelial repair in the lung has been demonstrated to involve MMP-7 in the regulation of cell to cell

adhesion and cell matrix adhesion by shedding of E-cadherin and Syndecan-1 from the cell surface (70). In experiments using a variety of *in vitro* and *in vivo* methods investigators assessed the rate of wound closure in A459 adenocarcinoma cell cultures and tracheal explants in WT or MMP-7 ^{-/-} mice. Knockout mice were also used to assess shedding of E-cadherin in BAL of MMP-7 ^{-/-} mice treated with intratracheal Bleomycin. A459 cells transfected with an active MMP-7 gene had increased rates of epithelial wound closure and E-cadherin shedding was reduced in culture supernatant of wounded tracheal explants and in BAL fluid from Bleomycin-induced lung injury in mice lacking MMP-7. Furthermore, immunohistochemistry also demonstrated co-localisation of MMP-7 and E-cadherin in wild type mouse trachea with a controlled wound. Modulation of $\alpha_2\beta_1$ integrin affinity for ECM by MMP-7 mediated syndecan-1 shedding may also play a role in epithelial repair. Syndecan-1 shedding by MMP-7 has already been discussed where this process facilitates neutrophil migration across the epithelium in lung injury (55). Investigation using complex *in vitro* methods demonstrates that shedding of syndecan-1 mediated by MMP-7 also negatively influences $\alpha_2\beta_1$ Integrin affinity for collagens in the ECM therefore facilitating migration of epithelial cells at the wound edge (71). Another study investigated the role of MMP-7 and TIMP-1 in airway epithelial wound repair using *in vitro* and KO models of injury (72). Investigators demonstrated increased spreading and migration of air way epithelial cells *in vitro* from wild type cultures compared to MMP-7 KO cultures. Immunofluorescence in wild type cultures demonstrated co-localisation of MMP-7 and TIMP-1 at cells proximal to the wound edge. Furthermore, wound repair in cell culture experiments from the TIMP-1 null mice demonstrated unrestrained *in vitro* re-epithelialisation by cell

spreading and migration compared to wild type controls (72). These findings were also confirmed *in vivo* using a naphthalene lung injury model suggesting that TIMP-1 plays a functionally important role in determining net MMP-7 activity *in vivo*

MMP-9 may also play a role in alveolar epithelial repair as evidence from an early study using cultured alveolar epithelial cells from hyperoxia treated rats also demonstrated increased migratory phenotype, correlating with increased MMP-9 activity, that was reduced by inhibition using Doxycycline (73). Unexpectedly, in humans, a recent study using BAL from ALI/ARDS patients receiving intravenous salbutamol in the BALTI trial demonstrated a possible role for MMP-9 in alveolar epithelial repair (19). This study showed a significant fold change in MMP-9 at day four in the salbutamol group, which was associated with reduced extravascular lung water (EVLW). Salbutamol specifically increased secretion of MMP-9 from distal lung epithelial cells (DLECs), as a model of alveolar epithelial cells, but not macrophages or neutrophils. Inhibition of MMP-9 in wounded alveolar epithelial cell cultures using dihydrolipoic acid (DHLLA) caused a dose dependent reduction in wound repair. This study suggested that MMP-9 was required for lung epithelial function *in vivo* and wound repair *in vitro* and highlights that broad spectrum inhibition of MMPs also has the potential to inhibit the repair phase of ALI/ARDS and therefore potentially worsen outcomes for patients. The potential role of MMPs in repair of the alveolar capillary membrane is outlined in figure 2 below.

Conclusion

The studies discussed in this review demonstrate that MMPs play a central role as both mediators and effectors of alveolar capillary membrane injury and repair in

ALI/ARDS. Recent animal work and human studies demonstrate the role of inflammatory, mesenchymal and possibly epithelial cells that produce MMP-3, -2, -9, -7 and -8, in development of injury to the alveolar capillary membrane. Non structural ECM components important in chemotaxis such as syndecan-1/KC and MIP-1 α have been shown to be important in mediating injury in experimental lung injury. However, significant detail on cellular source, particularly for MMP-3 and -7, and other key substrates remains to be established.

Understanding of the time course and trajectory of alveolar epithelial- endothelial repair in ALI/ARDS suggests this process starts early, but factors determining the development of fibrosis are poorly understood. However, rapid restitution of the epithelial barrier is likely to be important in limiting fibroproliferation. MMP-7 production by airway epithelium and degradation of syndecan-1 and E-Cadherin has been shown to be important in facilitating cell migration in epithelial repair and MMP-9 production by DLECs has, more recently, also been demonstrated to be important epithelial wound repair

From a translational perspective, targeting of MMP activity using broad spectrum inhibitors has been shown to limit injury when used as a pre-injury treatment strategy and may also be of benefit when given early in the course of experimental lung injury. However, involvement of MMPs in both injury and repair has important implications for potential pharmacotherapeutic strategies as indiscriminate inhibition of MMPs may have deleterious effects on repair.

In conclusion, while there is much evidence implicating MMPs in the injury process in ALI/ARDS significant work is required to fully understand the role of MMPs in

the pathogenesis and progression of ALI/ARDS. More consistent detail on MMP profile in clinical studies over the time course of ALI/ARDS and relevant animal models is required. Further work on KO models of lung injury phenotypes to elucidate roles of individual MMPs and associated alterations of other important mediators of lung injury should provide more detail on the mechanism of MMP involvement in alveolar injury and repair. These data will be necessary to inform which MMPs should be inhibited at which specific stages in the course of ALI, to allow well-designed clinical trials of inhibitors, and also to inform studies of when supplemented or unopposed protease activity may be appropriate to enable epithelial repair and resorption of abnormal collagens.

References

- (1) Ware LB, Matthay MA. The acute respiratory distress syndrome. *N.Engl.J.Med.* 2000; 342: 1334-1349.
- (2) Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K. Acute lung injury review. *Intern.Med.* 2009; 48: 621-630.
- (3) Matthay MA, Zemans RL. The Acute Respiratory Distress Syndrome: Pathogenesis and Treatment. *Annu.Rev.Pathol.* 2010; 6: 147-163.
- (4) Matthay MA, Zimmerman GA. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am.J.Respir.Cell Mol.Biol.* 2005; 33: 319-327.
- (5) Empey DW. Diseases of the respiratory system. Introduction: structure and function of the lungs. *Br.Med.J.* 1978; 1: 631-633.

- (6) Murray JF. The structure and function of the lung. *Int.J.Tuberc.Lung Dis.* 2010; 14: 391-396.
- (7) Dunsmore SE, Rannels DE. Extracellular matrix biology in the lung. *Am.J.Physiol.* 1996; 270: L3-27.
- (8) Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol.Rev.* 2007; 87: 69-98.
- (9) Lemaitre V, D'Armiento J. Matrix metalloproteinases in development and disease. *Birth Defects Res.C.Embryo.Today* 2006; 78: 1-10.
- (10) O'Kane CM, Frank J, McAuley DF. Matrix metalloproteinases: A potential role in the pathogenesis of ARDS. In: Vincent JL, editor. Yearbook of Intensive Care and Emergency Medicine. 1st ed. Heidelberg, Germany: Springer; 2004. p. 287-300.
- (11) McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr.Opin.Cell Biol.* 2001; 13: 534-540.
- (12) Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev.* 2000; 14: 2123-2133.
- (13) Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. *Semin.Cell Dev.Biol.* 2008; 19: 34-41.
- (14) Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat.Rev.Immunol.* 2004; 4: 617-629.
- (15) Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat.Rev.Mol.Cell Biol.* 2007; 8: 221-233.
- (16) Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Curr.Opin.Cell Biol.* 2004; 16: 558-564.

- (17) Elkington PT, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. *Thorax* 2006; 61: 259-266.
- (18) Oikonomidi S, Kostikas K, Tsilioni I, Tanou K, Gourgoulialis KI, Kiropoulos TS. Matrix metalloproteinases in respiratory diseases: from pathogenesis to potential clinical implications. *Curr.Med.Chem.* 2009; 16: 1214-1228.
- (19) O'Kane CM, McKeown SW, Perkins GD, Bassford CR, Gao F, Thickett DR, et al. Salbutamol up-regulates matrix metalloproteinase-9 in the alveolar space in the acute respiratory distress syndrome. *Crit.Care Med.* 2009; 37: 2242-2249.
- (20) Gushima Y, Ichikado K, Suga M, Okamoto T, Iyonaga K, Sato K, et al. Expression of matrix metalloproteinases in pigs with hyperoxia-induced acute lung injury. *Eur.Respir.J.* 2001; 18: 827-837.
- (21) Cederqvist K, Janer J, Tervahartiala T, Sorsa T, Haglund C, Salmenkivi K, et al. Up-regulation of trypsin and mesenchymal MMP-8 during development of hyperoxic lung injury in the rat. *Pediatr.Res.* 2006; 60: 395-400.
- (22) Kim JY, Choeng HC, Ahn C, Cho SH. Early and late changes of MMP-2 and MMP-9 in bleomycin-induced pulmonary fibrosis. *Yonsei Med.J.* 2009; 50: 68-77.
- (23) Eichler W, Bechtel JF, Schumacher J, Wermelt JA, Klotz KF, Bartels C. A rise of MMP-2 and MMP-9 in bronchoalveolar lavage fluid is associated with acute lung injury after cardiopulmonary bypass in a swine model. *Perfusion* 2003; 18: 107-113.
- (24) Hirsh M, Carmel J, Kaplan V, Livne E, Krausz MM. Activity of lung neutrophils and matrix metalloproteinases in cyclophosphamide-treated mice with experimental sepsis. *Int.J.Exp.Pathol.* 2004; 85: 147-157.

- (25) Delclaux C, d'Ortho MP, Delacourt C, Lebargy F, Brun-Buisson C, Brochard L, et al. Gelatinases in epithelial lining fluid of patients with adult respiratory distress syndrome. *Am.J.Physiol.* 1997; 272: L442-51.
- (26) Pugin J, Verghese G, Widmer MC, Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit.Care Med.* 1999; 27: 304-312.
- (27) Lanchou J, Corbel M, Tanguy M, Germain N, Boichot E, Theret N, et al. Imbalance between matrix metalloproteinases (MMP-9 and MMP-2) and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) in acute respiratory distress syndrome patients. *Crit.Care Med.* 2003; 31: 536-542.
- (28) Fligiel SE, Standiford T, Fligiel HM, Tashkin D, Strieter RM, Warner RL, et al. Matrix metalloproteinases and matrix metalloproteinase inhibitors in acute lung injury. *Hum.Pathol.* 2006; 37: 422-430.
- (29) Shyamsundar M, McKeown ST, O'Kane CM, Craig TR, Brown V, Thickett DR, et al. Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. *Am.J.Respir.Crit.Care Med.* 2009; 179: 1107-1114.
- (30) Sweet DG, McMahon KJ, Curley AE, O'Connor CM, Halliday HL. Type I collagenases in bronchoalveolar lavage fluid from preterm babies at risk of developing chronic lung disease. *Arch.Dis.Child.Fetal Neonatal Ed.* 2001; 84: F168-71.
- (31) Cederqvist K, Sorsa T, Tervahartiala T, Maisi P, Reunanen K, Lassus P, et al. Matrix metalloproteinases-2, -8, and -9 and TIMP-2 in tracheal aspirates from preterm infants with respiratory distress. *Pediatrics* 2001; 108: 686-692.

- (32) Sweet DG, Curley AE, Chesshyre E, Pizzotti J, Wilbourn MS, Halliday HL, et al. The role of matrix metalloproteinases -9 and -2 in development of neonatal chronic lung disease. *Acta Paediatr.* 2004; 93: 791-796.
- (33) Kong MY, Gaggar A, Li Y, Winkler M, Blalock JE, Clancy JP. Matrix metalloproteinase activity in pediatric acute lung injury. *Int.J.Med.Sci.* 2009; 6: 9-17.
- (34) Griffin MO, Fricovsky E, Ceballos G, Villarreal F. Tetracyclines: a pleiotropic family of compounds with promising therapeutic properties. Review of the literature. *Am.J.Physiol.Cell.Physiol.* 2010; 299: C539-48.
- (35) Fingleton B. Matrix metalloproteinases as valid clinical targets. *Curr.Pharm.Des.* 2007; 13: 333-346.
- (36) Kim JH, Suk MH, Yoon DW, Lee SH, Hur GY, Jung KH, et al. Inhibition of matrix metalloproteinase-9 prevents neutrophilic inflammation in ventilator-induced lung injury. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 2006; 291: L580-7.
- (37) Doroszko A, Hurst TS, Polewicz D, Sawicka J, Fert-Bober J, Johnson DH, et al. Effects of MMP-9 inhibition by doxycycline on proteome of lungs in high tidal volume mechanical ventilation-induced acute lung injury. *Proteome Sci.* 2010; 8: 3.
- (38) Carney DE, McCann UG, Schiller HJ, Gatto LA, Steinberg J, Picone AL, et al. Metalloproteinase inhibition prevents acute respiratory distress syndrome. *J.Surg.Res.* 2001; 99: 245-252.
- (39) Steinberg J, Halter J, Schiller HJ, Dasilva M, Landas S, Gatto LA, et al. Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. *J.Surg.Res.* 2003; 111: 185-195.

- (40) Carney DE, Lutz CJ, Picone AL, Gatto LA, Ramamurthy NS, Golub LM, et al. Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* 1999; 100: 400-406.
- (41) Sochor M, Richter S, Schmidt A, Hempel S, Hopt UT, Keck T. Inhibition of matrix metalloproteinase-9 with doxycycline reduces pancreatitis-associated lung injury. *Digestion* 2009; 80: 65-73.
- (42) Foda HD, Rollo EE, Drews M, Conner C, Appelt K, Shalinsky DR, et al. Ventilator-induced lung injury upregulates and activates gelatinases and EMMPRIN: attenuation by the synthetic matrix metalloproteinase inhibitor, Prinomastat (AG3340). *Am.J.Respir.Cell Mol.Biol.* 2001; 25: 717-724.
- (43) Muhs BE, Patel S, Yee H, Marcus S, Shamamian P. Inhibition of matrix metalloproteinases reduces local and distant organ injury following experimental acute pancreatitis. *J.Surg.Res.* 2003; 109: 110-117.
- (44) Keck T, Balcom JH, 4th, Fernandez-del Castillo C, Antoniu BA, Warshaw AL. Matrix metalloproteinase-9 promotes neutrophil migration and alveolar capillary leakage in pancreatitis-associated lung injury in the rat. *Gastroenterology* 2002; 122: 188-201.
- (45) Mulligan MS, Desrochers PE, Chinnaiyan AM, Gibbs DF, Varani J, Johnson KJ, et al. In vivo suppression of immune complex-induced alveolitis by secretory leukoproteinase inhibitor and tissue inhibitor of metalloproteinases 2. *Proc.Natl.Acad.Sci.U.S.A.* 1993; 90: 11523-11527.
- (46) Gipson TS, Bless NM, Shanley TP, Crouch LD, Bleavins MR, Younkin EM, et al. Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. *J.Immunol.* 1999; 162: 3653-3662.

- (47) Coimbra R, Melbostad H, Loomis W, Porcides RD, Wolf P, Tobar M, et al. LPS-induced acute lung injury is attenuated by phosphodiesterase inhibition: effects on proinflammatory mediators, metalloproteinases, NF-kappaB, and ICAM-1 expression. *J.Trauma* 2006; 60: 115-125.
- (48) Kang JL, Lee HW, Lee HS, Pack IS, Chong Y, Castranova V, et al. Genistein prevents nuclear factor-kappa B activation and acute lung injury induced by lipopolysaccharide. *Am.J.Respir.Crit.Care Med.* 2001; 164: 2206-2212.
- (49) Deree J, Martins JO, Leedom A, Lamon B, Putnam J, de Campos T, et al. Hypertonic saline and pentoxifylline reduces hemorrhagic shock resuscitation-induced pulmonary inflammation through attenuation of neutrophil degranulation and proinflammatory mediator synthesis. *J.Trauma* 2007; 62: 104-111.
- (50) Chetty A, Cao GJ, Severgnini M, Simon A, Warburton R, Nielsen HC. Role of matrix metalloprotease-9 in hyperoxic injury in developing lung. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 2008; 295: L584-92.
- (51) Warner RL, Beltran L, Younkin EM, Lewis CS, Weiss SJ, Varani J, et al. Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am.J.Respir.Cell Mol.Biol.* 2001; 24: 537-544.
- (52) Lukkarinen H, Hogmalm A, Lappalainen U, Bry K. Matrix metalloproteinase-9 deficiency worsens lung injury in a model of bronchopulmonary dysplasia. *Am.J.Respir.Cell Mol.Biol.* 2009; 41: 59-68.
- (53) Albaiceta GM, Gutierrez-Fernandez A, Parra D, Astudillo A, Garcia-Prieto E, Taboada F, et al. Lack of matrix metalloproteinase-9 worsens ventilator-induced lung injury. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 2008; 294: L535-43.

- (54) Nerusu KC, Warner RL, Bhagavathula N, McClintock SD, Johnson KJ, Varani J. Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. *Exp.Mol.Pathol.* 2007; 83: 169-176.
- (55) Li Q, Park PW, Wilson CL, Parks WC. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* 2002; 111: 635-646.
- (56) Kim KH, Burkhardt K, Chen P, Frevert CW, Randolph-Habecker J, Hackman RC, et al. Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. *Am.J.Respir.Cell Mol.Biol.* 2005; 33: 271-279.
- (57) 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.
- (58) Quintero PA, Knolle MD, Cala LF, Zhuang Y, Owen CA. Matrix metalloproteinase-8 inactivates macrophage inflammatory protein-1 alpha to reduce acute lung inflammation and injury in mice. *J.Immunol.* 2010; 184: 1575-1588.
- (59) Albaiceta GM, Gutierrez-Fernandez A, Garcia-Prieto E, Puente XS, Parra D, Astudillo A, et al. Absence or Inhibition of Matrix Metalloproteinase-8 Decreases Ventilator-induced Lung Injury. *Am.J.Respir.Cell Mol.Biol.* 2009;.
- (60) Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 2010; 298: L715-31.
- (61) Rocco PR, Dos Santos C, Pelosi P. Lung parenchyma remodeling in acute respiratory distress syndrome. *Minerva Anesthesiol.* 2009; 75: 730-740.

- (62) Chapman HA. Disorders of lung matrix remodeling. *J.Clin.Invest.* 2004; 113: 148-157.
- (63) Dos Santos CC. Advances in mechanisms of repair and remodelling in acute lung injury. *Intensive Care Med.* 2008; 34: 619-630.
- (64) Rocco PR, Negri EM, Kurtz PM, Vasconcellos FP, Silva GH, Capelozzi VL, et al. Lung tissue mechanics and extracellular matrix remodeling in acute lung injury. *Am.J.Respir.Crit.Care Med.* 2001; 164: 1067-1071.
- (65) Santos FB, Nagato LK, Boechem NM, Negri EM, Guimaraes A, Capelozzi VL, et al. Time course of lung parenchyma remodeling in pulmonary and extrapulmonary acute lung injury. *J.Appl.Physiol.* 2006; 100: 98-106.
- (66) Negri EM, Hoelz C, Barbas CS, Montes GS, Saldiva PH, Capelozzi VL. Acute remodeling of parenchyma in pulmonary and extrapulmonary ARDS. An autopsy study of collagen-elastic system fibers. *Pathol.Res.Pract.* 2002; 198: 355-361.
- (67) Marshall RP, Bellingan G, Webb S, Puddicombe A, Goldsack N, McAnulty RJ, et al. Fibroproliferation occurs early in the acute respiratory distress syndrome and impacts on outcome. *Am.J.Respir.Crit.Care Med.* 2000; 162: 1783-1788.
- (68) Demoule A, Decailliot F, Jonson B, Christov C, Maitre B, Touqui L, et al. Relationship between pressure-volume curve and markers for collagen turn-over in early acute respiratory distress syndrome. *Intensive Care Med.* 2006; 32: 413-420.
- (69) Chao MC, Garcia CS, de Oliveira MB, Santos RS, Lucas IH, Silva PL, et al. Degree Of Endothelium Injury Promotes Fibroelastogenesis In Experimental Acute Lung Injury. *Respir.Physiol.Neurobiol.* 2010;.

- (70) 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.
- (71) Chen P, Abacherli LE, Nadler ST, Wang Y, Li Q, Parks WC. MMP7 shedding of syndecan-1 facilitates re-epithelialization by affecting alpha(2)beta(1) integrin activation. *PLoS One* 2009; 4: e6565.
- (72) Chen P, McGuire JK, Hackman RC, Kim KH, Black RA, Poindexter K, et al. Tissue inhibitor of metalloproteinase-1 moderates airway re-epithelialization by regulating matrilysin activity. *Am.J.Pathol.* 2008; 172: 1256-1270.
- (73) Buckley S, Driscoll B, Shi W, Anderson K, Warburton D. Migration and gelatinases in cultured fetal, adult, and hyperoxic alveolar epithelial cells. *Am.J.Physiol.Lung Cell.Mol.Physiol.* 2001; 281: L427-34.

Abbreviations

APACHE: Acute physiology and chronic health evaluation
AaDO₂: Alveolar arterial oxygen gradient
ALI: Acute lung injury
ARDS: Acute respiratory distress syndrome
BAL: Bronchoalveolar lavage
BALTI: Beta agonist lung injury trial
BM: Basement membrane
BPD: Bronchopulmonary dysplasia
COL-3 / CMT-3: Chemically modified tetracycline
CLP: Caecal ligation puncture
CPB: Cardiopulmonary bypass
CXCL8: Chemokine also known as IL-8
DHHA: Dihydrolipoic acid
DLECs: Distal lung epithelial cells
ECM: Extracellular matrix
ELISA: Enzyme linked immunosorbent assay
EMMPRIN: Extracellular matrix metalloproteinase inducer
EVLW: Extravascular lung water
FiO₂: Fraction inspired O₂
ICU: Intensive care unit
KC: murine orthologue of CXCL8

KO: Knock out
IL1 β : Interleukin 1 β
IT: Intratracheal
LDH: Lactate dehydrogenase
LPS: Lipopolysaccharide
MIP-1 α : Macrophage inhibitory protein 1 α
MMP: Matrix metalloproteinase
MPO: Myeloperoxidase
MW: Molecular weight
NF κ B: Nuclear factor kappa B
PaO₂: Partial pressure arterial O₂
PCP III: Procollagen peptide III
PMN: Polymorphonuclear leukocyte
PTX: Pentoxifylline
RAGE: Receptor for advanced glycation endproduct
RDS: respiratory distress syndrome
SLPI: Secretory leukocyte peptidase inhibitor
TACE: TNF alpha converting enzyme
TGF β 2: Transforming growth factor beta2
TIMP: Tissue inhibitor of matrix metalloproteinase
TNF α : Tumour necrosis factor alpha
VILI: Ventilator induced lung injury
V/Q: ventilation perfusion ratio
WT: Wild type

Figure legends

Figure 1. MMP involvement in pathogenesis of ALI/ARDS. MMP-9 and MMP-8 are produced by neutrophils. Alveolar macrophages and epithelial cells secrete MMP-2 & -9 and MMP-7, respectively, while MMP-3 is secreted by activated fibroblasts. Likely key substrates include ECM proteins and inflammatory mediators such as chemotaxins. MMP-dependent shedding of syndecan-1 seems to be important in maintaining a chemotactic gradient.

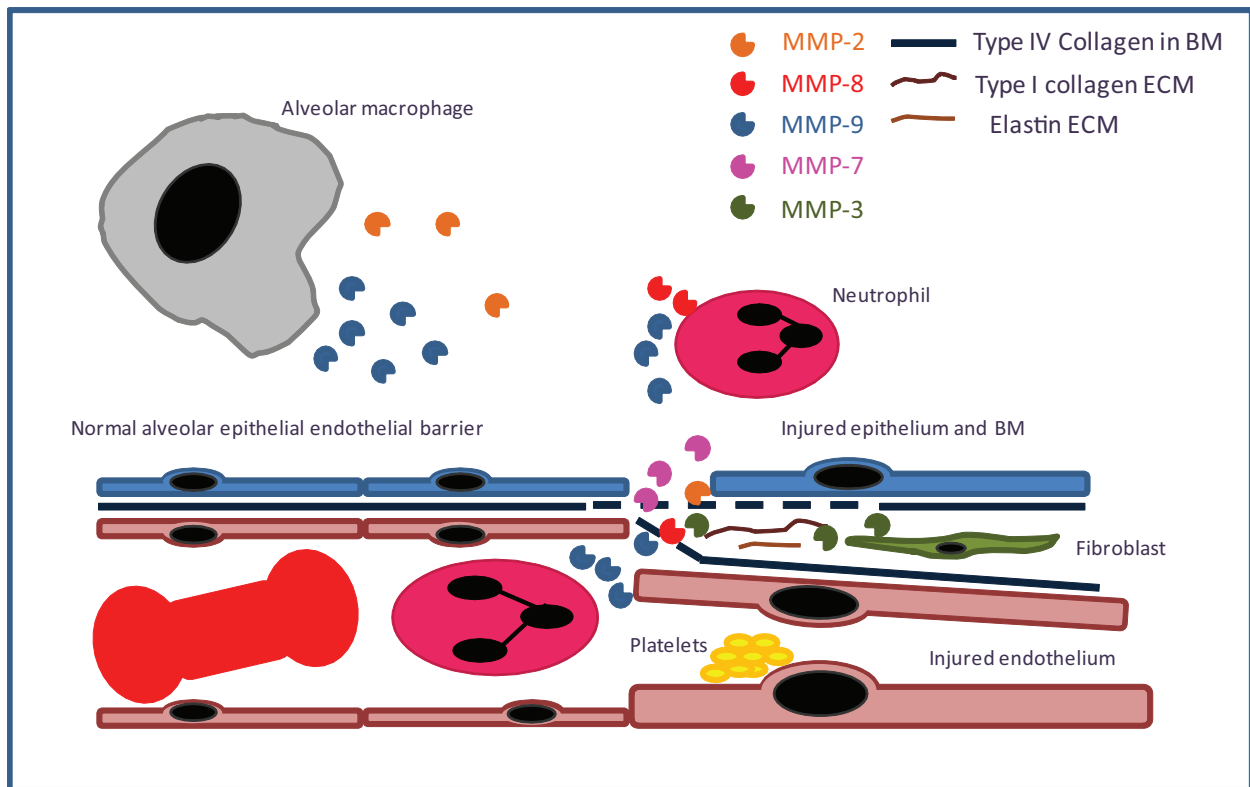


Figure 1

Figure 2. MMP involvement in alveolar epithelial repair. MMP-7 and -9 from distal alveolar epithelial cells are important in epithelial repair. Facilitation of cell migration by shedding of syndecan-1 and probable proteolysis of provisional matrix and abnormal collagens are important in epithelial restitution following ALI/ARDS.

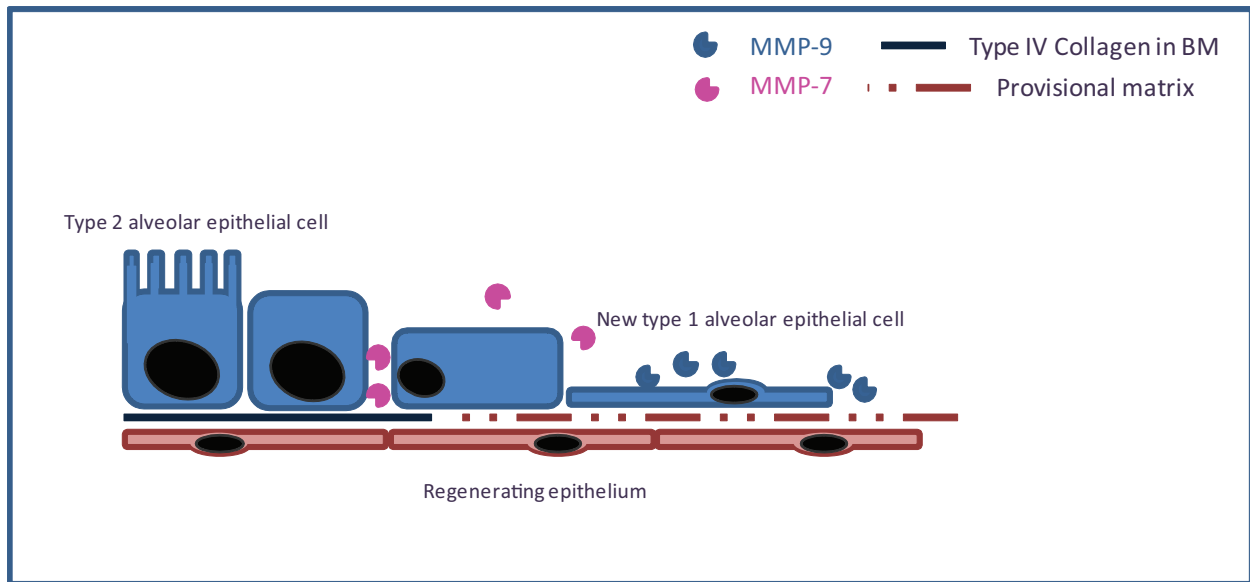


Figure 2

MMP (n=24)	Other Name(s)	<i>In vitro</i> ECM substrates	Other substrate example(s)
MMP-1	Interstitial collagenase	Type I, II, III, VII, VIII, X, XI collagen, Gelatin, Fibronectin, Vitronectin, Laminin	Perclean, IGFBP-2,3, Pro-TNF α , PAR-1
MMP-2	Gelatinase A	Gelatin, type I, II, III, IV, V, VII, X, XI collagen, elastin, fibronectin, vitronectin, laminin	Pro-IL-1B, Pro-TGF- β 2, Pro-TNF α
MMP-3	Stromelysin 1	Laminin, fibronectin, vitronectin, gelatin, elastin, aggrecan, fibrin, type III, IV, V, VII, IX, X, XI collagen	Pro-IL-1 β , Pro MMP-1, Pro-TNF α , α 1-proteinase inhibitor
MMP-7	Matrylsin, Pump-1	Type I collagen, gelatin, elastin, vitronectin. Laminin	Plasminogen, Pro-TNF α
MMP-8	Neutrophil collagenase	Type I, II, III collagen, aggrecan	α 1-proteinase inhibitor
MMP-9	Gelatinase B	Gelatin, Type IV, V, XI, XIV collagen, elastin, vitronectin, laminin	Pro-TGF- β 2, Pro-TNF α , Pro-IL-1 β , α 1-proteinase inhibitor, CXCL8
MMP-10	Stromelysin 2	Gelatin, elastin, fibronectin, aggrecan, type II & IV collagen	Pro-MMP-1, -8, -10
MMP-11	Stromelysin 3	Gelatin, fibronectin, laminin	IGFBP-1, α 1-proteinase inhibitor
MMP-12	Metalloelastase	Elastin, type I & V collagen, fibronectin, vitronectin, laminin	Plasminogen

MMP (n=24)	Other Name(s)	<i>In vitro</i> ECM substrates	Other substrate example(s)
MMP-13	Collagenase 3	type I, II, III, I V, VI, IX, X collagen, gelatin, fibronectin, aggrecan	α 1-antichymotrypsin
MMP-14	MT1-MMP	Types I, II and III collagen, gelatin, fibronectin, vitronectin, laminin, aggrecan	Pro-MMP-2, -13, cell surface CD44
MMP-15	MT2-MMP	Fibronectin, tenascin, entactin, laminin, aggrecan	Cell surface tissue transglutaminase (tTG)
MMP-16	MT3-MMP	Type III collagen, gelatin, fibronectin, vitronectin, laminin	Pro-MMP-2
MMP-17	MT4-MMP	Gelatin	Pro-MMP-2
MMP-19	RASI-1	Gelatin, laminin, entactin, fibronectin, aggrecan	None identified
MMP-20	Enamelysin	Amelogenin	None identified
MMP-21	XMMP	Unknown	None identified
MMP-22	CAMMP	Unknown	None identified
MMP-23	Femalysin	Gelatin	None identified

MMP (n=24)	Other Name(s)	<i>In vitro</i> ECM substrates	Other substrate example(s)
MMP-24	MT5-MMP	Fibronectin, gelatin, chondroitin sulphate proteoglycan, dermatan sulphate proteoglycan	Pro-MMP-2
MMP-25	MT6-MMP	Gelatin, fibronectin, chondroitin sulphate proteoglycan, dermatan sulphate proteoglycan	Pro-MMP-2
MMP-26	Matrilysin-2	Gelatin, fibronectin, vitronectin	α 1-proteinase inhibitor
MMP-27	CMMP	Gelatin, casein	None identified
MMP-28	Epilysin	Casein	None identified

Table 1. List of all 24 identified human MMPs with examples of both matrix and non-matrix substrates. Note non-matrix substrates consist of pro-proteases (including MMPs), inflammatory mediators and other cell surface proteins. Missing MMPs have been removed from the list because further investigation demonstrated duplication or non-existence of MMPs predicted by described gene products.(1-4).