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Protean Proteases: at the cutting edge in lung diseases

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Abstract

Proteases were traditionally viewed as mere protein-degrading enzymes with a very restricted spectrum of substrates. A major expansion in protease research has uncovered a variety of novel substrates, and it is now evident that proteases are critical pleiotropic actors orchestrating pathophysiological processes. Recent findings evidenced that the net proteolytic activity also relies upon interconnections between different protease and protease inhibitor families in the protease web. In this review, we provide an overview of these novel concepts with a particular focus on pulmonary pathophysiology. We describe the emerging roles of several protease families including cysteine and serine proteases. The complexity of the protease web is exemplified in the light of multidimensional regulation of serine protease activity by matrix metalloproteases through cognate serine protease inhibitor processing. Finally, we will highlight how deregulated protease activity during pulmonary pathogenesis may be exploited for diagnosis/prognosis purposes, and utilized as a therapeutic tool using nanotechnologies. Considering proteases as part of an integrative biology perspective may pave the way for the development of new therapeutic targets to treat pulmonary diseases related to intrinsic protease deregulation.

Introduction

Proteases represent almost 2% of the human genome comprising 565 members [1, 2]. Based on their different catalytic mechanisms, five major classes of proteases are known in mammals including serine, cysteine, metallo, aspartic, and threonine proteases, the three first families being the most widespread in humans [3]. Protease activity requires tight regulation (Figure 1): elevated protease levels, actively contributing to disease progression, constitute a common feature shared by a broad range of pulmonary pathologies, including idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), emphysema or infection, [4]. The underlying mechanisms of such observations have long remained elusive: indeed, proteases were traditionally viewed as mere protein-degrading enzymes with a restricted spectrum of substrates. During the last decade, a major expansion in protease research from different fields dramatically shifted this paradigm. The study of degradomics (the protease substrate repertoire) revealed that the nature of the dedicated substrates of proteases, and the biological effects triggered by their processing, are exceptionally diverse [5]. Proteases are now considered as key components of regulatory mechanisms, through irreversible cleavage of substrates resulting in their activation, inactivation, or modulation of function. The serine proteases of the coagulation cascade constitute an archetypal example: beyond blood clot formation, these proteases contribute to a variety of pathophysiologies, including IPF, through the irreversible activation of their cellular receptors, the Protease-Activated Receptors[6, 7]. Cellular effects of other protease families, such as cysteine proteases or matrix-metalloproteases (MMPs) began to unfold only recently. Additionally, recent evidences show a regulation of protease activity through interconnection of protease cascades (the protease web).

In this review, these new concepts of integrative protease biology are discussed in the perspective of pulmonary pathophysiology. First, we highlight the striking role in lung pathogenesis of cysteine proteases, which were traditionally circumscribed to lysosomal degradation. We describe the recently discovered involvement of macrophage and neutrophil elastases, which (despite their names) actually belong to different clans, in CF. Defined localization of protease activity is exemplified by the involvement of A Disintegrin And Metalloproteinase (ADAM) proteases in lung inflammation. Next, we focus on the main protease inhibitors, including serpins, to summarize the mechanisms underlying serpinopathies, and review recent evidence on serpin regulation by MMPs, thereby bolstering the concept of the protease web. These data are recapitulated in Table 1. Finally, we highlight

how deregulated protease activity during pulmonary pathogenesis may be exploited for diagnosis/prognosis purposes and utilized as a therapeutic tool using nanotechnologies.

Unusual suspects: the emerging role of cathepsins, neutrophil and macrophage elastases, and ADAMs in lung diseases

Cysteinyl cathepsins: endolysosomal degradation and beyond

It is only very recently that an unexpected role of cathepsins in lung diseases has emerged. Human cysteine cathepsins are a group of 11 papain-like lysosomal proteases including cathepsins B, H, L, C, X, F and O, and are ubiquitously expressed in most tissues whereas cathepsins V, K, S and W have a more limited expression profile. Such diverse tissue-specific expression suggests that their action is not limited solely to the bulk protein degradation, but that they are also involved in the regulation of specific cellular functions. All of this allows cathepsins to regulate a broad variety of important physiological processes, including intracellular protein turnover, immune response, bone remodelling and prohormone processing [8, 9].

With respect to lung pathophysiology, cathepsins have a critical role in MHC II–mediated antigen processing and presentation [3, 10, 11]. In addition, cathepsins, especially cathepsin K, have been found to have also major roles in the processing of TLR receptors, [10, 12, 13]. In CF, levels of cathepsins B, L and S are increased in patient bronchoalveolar lavage fluid (BALF) [14], with bronchial epithelium acting a source of cathepsin S [15]. However, several questions remain unanswered, such as their relative contribution to extracellular proteolysis (as secreted proteases) *vs.* intracellular proteolysis (as lysosomal proteases) remains to be determined, as well as the balance between degradation and/or processing of specific substrates. Over the last decade, the role for cathepsins K and B in IPF has also emerged. In IPF patients, cathepsin K is increased in fibroblasts from fibrotic tissues. Unexpectedly, in the murine model of bleomycin-induced pulmonary fibrosis, cathepsin K deficiency associates with more severe lesions. And indeed, in fact, cathepsin K has a pivotal protective role in pulmonary homeostasis through collagen cleavage thereby contributing to the airway structural integrity [16-18]. Increased expression of cathepsin B was also observed in IPF patient-derived fibroblasts. Cathepsin B contributes to myofibroblast differentiation by triggering activation of the TGF-β1-driven canonical Smad 2-3 pathway [19]. Accordingly,

cathepsin B inhibition and/or genetic silencing diminishes α-smooth muscle actin (α–SMA) expression and fibroblast differentiation.

From the above, the use of cathepsin pharmacological inhibitors seems an obvious therapeutical intervention. However, the off-targets and/or side effects of cathepsin inhibitors remain to be fully elucidated. For instance, cathepsin S inhibitors efficiently reduce lung inflammation but can result in cartilage and bone degradation [20]. Cathepsin K inhibitors, developed for the treatment of osteoporosis, target cathepsin K collagenolytic activity and may impair collagen I turnover thus favoring lung fibrosis [21]. A reversible cathepsin B inhibitor, VBY-376, demonstrated potent activity in a mouse model of CCl_4-induced liver fibrosis [22] (http://www.virobayinc.com/docs/AASLD_Poster_2010.pdf). Promising phase I trials for treatment of hepatic fibrosis were also obtained with VBY-376 (http://adisinsight.springer.com/drugs/800031124). Since cathepsin B-driven responses are common in pulmonary and hepatic fibrosis, the use of VBY-376 may be appropriate for pulmonary fibrosis treatment.

Overall, it has emerged from the last decade that, beyond lysosomal degradation, cathepsins are crucial actors in pulmonary homeostasis and further research is warranted to better delineate their role in pulmonary pathophysiology.

Neutrophil and macrophage elastases in CF: old dogs, new tricks

An imbalance between neutrophil-derived proteases (including neutrophil elastase (NE), cathepsin G (CatG), and proteinase 3 (PR3)) and extracellular inhibitors is considered an important pathogenic mechanism for a number of lung diseases[23-25]. It is assumed that unopposed CatG, NE and PR3 collectively cause severe lung damage and emphysema[26], further underscoring their importance in pathology (for review, see [27]). A fourth elastase-related protease, NSP4, expressed in the lung, has been recently identified. Its proteolytic effects, controlled by natural serine protease inhibitors of the serpin-type, are most likely limited to the intracellular and pericellular microenvironment of activated neutrophils. The natural substrates of NSP4 however yet remain to be determined [28]. Recent studies on mouse NE revealed the existence of a new variant, created by the spontaneous conversion process of the single-chain (sc) form of NE into a specific two-chain (tc) form by autoprocessing. The existence of this tc NE could adversely affect the protease–antiprotease

balance in the lung during NE release, and may lead to the development of more efficient anti-elastase therapies[29].

Strikingly, recent studies in infants and preschool children with CF also identified the increased activity of NE as a key risk factor of the onset and progression of early bronchiectasis [30, 31]. In the context of chronic lung disease, NE is involved in a number of processes including induction of IL-8 expression, increased mucin secretion, inactivation of innate airway proteins and cell surface receptors all of which, combined, identify NE as a pro-inflammatory molecule [32-34, 35 , 36 , 37 , 38 , 39]. Similarly, in other chronic lung diseases, including COPD, A1AT deficiency and bronchiectasis, NE is thought to contribute to disease pathogenesis[40 , 41, 42].

However, it is in the CF airways where the most definitive role regarding NE's deleterious effects has been most clearly demonstrated as shown by the AREST CF group [31]. The development of the βENaC -overexpressing (βENaC-Tg) mouse, featuring CF-like airway surface dehydration, phenocopies key characteristics of CF in patients, including airway inflammation, mucus hypersecretion and obstruction, bacterial infection and structural lung damage [43-45]. Recent studies in this CF model demonstrated that genetic deletion of NE reduces airway neutrophilia, mucin expression, goblet cell metaplasia and distal airspace enlargement without compromising anti-bacterial host defense *in vivo* [46]. In addition, whole-genome expression studies identified macrophage elastase (matrix metalloproteinase (MMP)-12) as a highly up-regulated gene in lungs from βENaC-Tg mice, and demonstrated that MMP12 also contributes to structural lung damage *in vivo* [47]. The clinical relevance of this finding is supported by a genetic study showing that a functional SNP (rs2276109) in the MMP12 promoter, previously associated with reduced lung function in COPD and asthma [48], was also associated with lung function decline in patients with CF [47]. More recent degradomic analysis of MMP12 *in vivo* reveals its broad substrate variety. For instance, MMP12 is potently anti-inflammatory in several *in vivo* models by cleaving and inactivating neutrophil chemokines [49], and terminating and complement factors, C4a and C5a [50,51].

Taken together, these studies support important roles of elastases released from activated neutrophils and macrophages in the *in vivo* pathogenesis of multiple abnormalities in the CF lung including inflammation, mucus hypersecretion and structural lung damage. These studies also demonstrate that the βENaC-Tg mouse will be a useful model for further studies of the roles of other emerging proteases including serine proteases, matrix

metalloproteases and cysteine proteases [15, 24, 50], and their respective inhibitors, in the complex *in vivo* pathogenesis of CF and potentially other chronic inflammatory lung diseases.

Localizing the activity to the membrane: A Disintegrin And Metalloproteinase (ADAM proteases)

The ADAM proteins constitute a major class of membrane-anchored multidomain proteinases that are responsible for the shedding of cell-surface protein ectodomains, including the latent forms of growth factors, cytokines, and receptors. As such, they are described as "signaling scissors". The ADAM family comprises ~34 members, 22 of which have been described in humans. Only 13 human ADAMs carry the zinc-binding motif (HExxHxxGxxH) required for the proteolytic activity. Proteolytically active ADAMs can be regulated by induction of their gene transcription, by posttranslational mechanisms upon stimulation with cytokines or bacterial toxins, or, in some cases, by cleavage of their ectodomain and subsequent release of a soluble form [51, 52]. Several members of proteolytically active ADAMs are involved in lung disease. ADAM17 is critical for generation of proinflammatory mediators, especially TNF-alpha. The shedding of soluble activated TNF-alpha subsequently stimulates a cascade of events causing endothelial permeability and leukocyte recruitment. The overexpression of ADAM10 in lung epithelium causes the development of experimental emphysema [53]. ADAM10 has been identified as potential sheddase for CD23 and the soluble form of this mediator enhances the production of IgE [54]. Polymorphisms in the ADAM33 gene are linked with asthma or COPD [55] and BALF levels of the soluble form of this protease correlate with the severity of the disease [56]. Additionally, ADAM10 and 17 are critically involved in the shedding of several growth factors including TGFα for example [57]. ADAMs are also implicated in host defense against infectious agents. ADAM10 functions as a receptor for *Staphylococcus aureus* toxin, and epithelial ADAM10 deficiency can prevent lethal *S. aureus* infection [58]. Finally, a key role has been proposed for ADAMs in airway remodeling associated with asthma [59]. Based on the above, it has been postulated that ADAM inhibition would decrease edema formation, inflammatory cell recruitment, tissue remodeling, and reduction of *S aureus* toxicity. Accordingly, the use of a selective ADAM17 inhibitor suppressed LPS-induced lung inflammation in mice [51]. From the above it is evident that ADAM proteases are implicated in acute and chronic inflammation processes but also in infection and regeneration. As

inhibition of specific ADAMs might interfere with these processes, temporally and locally restricted and very specific inhibition strategies might be of interest for the treatment of lung disease.

Proteases and natural serine protease inhibitors: the dangerous liaisons

Besides an increase in protease expression and/or activation, deregulation of protease activity can arise from the loss of cognate protease inhibitors. Respiratory anti-proteases comprise serine protease inhibitors (serpins), tissue inhibitors of metalloproteinase (TIMPs), trappin-2/elafin, and secretory leukoprotease inhibitor (SLPI) [60]. These inhibitors not only neutralize protease activity, but also display anti-microbial and immune modulatory actions which are highly relevant in chronic lung pathologies, such as in cystic fibrosis and COPD, where there is a combination of infection and exacerbated host responses [61, 62]. The clinical importance of protease inhibition is supported by studies demonstrating that inhibitor deficiencies result in structural lung damage. For instance, serpinopathies are a relatively newly defined group of disorders, where the underlying cause is the accumulation of misfolded protein, or lack of protein expression, in the affected tissue (Figure 2). A1AT, a prototypical member of the serpin superfamily, is a specific inhibitor of NE, PR3 and catG [63]. Inherited A1AT deficiency (A1ATD), which causes familial emphysema and liver disease [64, 65], is a classic example of serpinopathies. A1ATD is most often associated with the Z (Glu342Lys) mutation. Z-type A1AT forms oligomeric complexe; the intra-cellular retention of such complexes (gain-of-function defect) cause cellular damage [66, 67]. Strikingly, cigarette smoke-mediated oxidation can enhance Z-A1AT polymerization in human alveolar epithelial cells and induce ER-stress and NF-κB-mediated pro-inflammatory cytokine (TNF-α, IL-6 and MCP-1) production [68, 69].

Hence, anti-protease supplements may reduce the deleterious effects of unregulated NE activity. Accordingly, the therapeutic potential of natural endogenous inhibitors of proteases has gained interest over the last decade, and their newly appreciated functions point to extended therapeutic uses. Thus, human plasma-purified A1AT is used in infusion in A1ATD patients to delay the progression of emphysema. Administration of A1AT also triggers the anti-inflammatory pathways necessary for resolution of inflammation and prevention of tissue damage [70]. Inhalation therapy offers another opportunity for easier, more efficient delivery of A1AT directly to the lungs. Inhaled A1AT indeed significantly

ameliorates cigarette smoke-induced emphysema in the mouse [71]. In patients with CF, A1AT inhalation reduces airway inflammation[72]. Similarly, aerosolized recombinant SLPI reduces elastase activity in healthy subjects and CF patients [73]. Altogether, these data indicate the potential value of recombinant natural inhibitors in the treatment of chronic lung disease.

Noteworthy, while cognate inhibitors are essential for protease activity regulation, recent evidence demonstrates that reciprocally, protease inhibitors are also protease substrates. Isotopic labels of cellular and secreted proteins *in vitro* and *in vivo* [74] revealed that serpins are major substrates of MMPs [75]. In other words, serine protease activity is regulated by MMPs through serpin processing. Accordingly, MMP2 cleaves serpin B6 and alpha-1 inhibitor III [76], while MMP14 processes SLPI [77]. Such a regulation of inhibitors through protease processing has significant consequences in pathophysiology. For instance, during lung inflammation, the cleavage of serpinA1 by MMP8 releases elastase activity *in vivo* [78]. In arthritis, a protective role for macrophage MMP12 was demonstrated: among other mechanisms, MMP12 induces antithrombin III degradation and increased thrombin activation [79]. In acute skin inflammation, MMP2 cleaves serpin G1, also known as complement C1 inhibitor. These findings have unveiled a mechanism, referred to as "metallo-serpin switch", by which MMP2 triggers complement cascade activation [74]. Altogether, these data revealed that proteases pervasively influence the activity of other proteases, either directly or by cleaving intermediate proteases or protease inhibitors.

The Protease web: is there a maze in the lung?

As the above-mentioned examples illustrate, proteases can no longer be thought of acting in isolation. Thus, proteases in the same or different families activate protease zymogens, inactivate proteases by autodegradation *in trans*, cleave and release protease substrate binding domains termed exosites (resulting in altered protease specificity and substrates), and, as exemplified above, cleave and inhibit protease inhibitors. By mapping these interactions of all reported biochemical validated cleavages in proteases and inhibitors uploaded to the data base MEROPS and TopFIND [80, 81], graph theory was used to bioinformatically map the protease interactions of proteases termed the protease web [78]. Remarkably, at the highly interconnected core of the protease web lie 255 highly connected proteases and inhibitors representing 50% of known proteases and inhibitors. Reachability analysis was performed,

whereby the connections between proteases and inhibitors (paths) were mapped, and both the connections that are direct ie. 1:1, and indirect ie. further downstream reached by one or more intermediate connections. Thus, within this core, 158 of these proteins reached over 153 (60%) of the protease and inhibitors of the core, spanning all 5 human protease classes. The protease web is highly robust. Removal of one or more nodes did not "break" this highly interconnected core, nor did removal of paths. It was not until 40% of paths were removed did the web connectivity breakdown. Similarly it required removal of more than 6 proteases or inhibitors to destabilise the interconnected core. The connectivity will be shown to further increase as more protease and inhibitor interactions are uploaded to MEROPS and TopFIND by individual investigations upon publication of their data. The important implication of these analyses is that therapy must be designed with great care as blockade of one protease will almost inevitably have knock on effects to other proteases and activity via connections in the protease web and may explain the failure of previous protease inhibitor clinical trials [82-84].

Noteworthy, the above-mentioned findings have also recently emerged from other fields of research in addition to pulmonology. Since, as described earlier, several –if not all- of these actors have been shown to be involved in lung pathophysiology, it is therefore tempting to speculate that these mechanisms are highly relevant in lung disease, and further research in this direction is eagerly awaited.

Protease activity: a tool for diagnosis?

Because elevated protease activity is associated with lung disease, it can be postulated that quantitative measurement of such an activity could serve as a surrogate marker for diagnosis/prognosis in lung diseases. A significant number of fluorescent probes have been developed to directly measure protease activities. Already in the late Seventies, short peptide-based molecules were developed that feature an intrinsic fluorescent entity.

While commercially available and still heavily in use, sophisticated fluorescent reporters, often based on Foerster Resonance Energy Transfer (FRET), have been developed in recent years. Frequently, two fluorophores are employed as FRET donor and acceptor. This provides the possibility to measure FRET with a more sensitive readout in a ratiometric fashion, mostly independent from the amount of reporter present. Recent examples are FRET sensors for MMP12 (LaRee series) and neutrophil elastase (NEmo series) [85, 86], which have revealed elevated MMP-12 and NE activity. While such probes work extremely well with isolated

enzymes in the test tube, their application to biological samples is much more difficult. This is partly due to the fact that most proteases are counteracted by protease inhibitors, also present in mouse or patient samples. Further, the zymogen forms of proteases require cleavage for activation by other proteases and such activation seems to vary locally. For instance, when investigating MMP12 activity from mouse BAL samples by the soluble reporter LaRee1, very little activity was observed. However, the lipidated reporter LaRee1, located to the outer cell membrane of BAL macrophages, reported strong activity. This demonstrates that the enzyme activity resides predominantly on cell surfaces and suggests that the lung lumen is much less exposed [85]. Similarly, NE seems to be mostly blocked in its proteolytic activity in samples from lung lumen, but the lipidated reporter was cleaved readily on the surface of activated neutrophils [86]. FRET-based reporters have now made their way to patient samples and revealed, for instance, elevated strong increase in MMP12 and NE activity on the surface of airway neutrophils and macrophages from βENaC-Tg mice and patients with CF, respectively [46, 47]. Noteworthy, very recently, different cathepsin-targeted imaging probes were developed and successfully used to monitor fibrotic disease progression in the murine model of bleomycin-induced pulmonary fibrosis and in IPF patients [87]. In the future, an extended array of reporters will help to characterize the protease activity landscape on cells isolated from patients and will hopefully assist physicians for the diagnosis of lung diseases, possibly predict disease course and to devise treatment strategies.

Hijacking protease activity for therapeutics

Dysregulation of MMP activity is a characteristic feature of organ fibrosis [88] including in the lung [89] and malignant tumors [1, 90]. Recent progress in the field of nanotechnology has focused on the use of MMP-directed nanoparticles as diagnostic and therapeutic or theranostic devices [91-94]. Compared to commonly used drug and imaging formulations, nano-scaled drug carriers offer additional advantages such as increasing the drug circulation time, minimizing drug degradation, and overcoming first-pass drug metabolism [95]. Mesoporous silica nanoparticles, engineered to release their incorporated chemotherapeutic drug upon gate-opening by MMP2/9 cleavable linkers, have recently been developed [96]. Efficient drug release was shown to be strictly dependent on the presence of MMP9 in human lung tumor cell lines. Moreover, highly effective drug release from our MSN nanoparticles in mouse and human lung tumors. The successful application of therapeutic nanoparticles to *ex vivo* cultures of human lung tumors represents a major

progress in closing the translational gap in the development of novel MMP-targeted nanomedicines for lung tumor treatment. Thus, deregulation in protease activity in other lung diseases could be similarly hijacked for drug delivery and may represent a novel strategy for therapeutic intervention.

Conclusion

It is now clear that the traditional representation of proteases as mere ECM degrading enzymes has shifted. The new paradigm allows a better understanding of how proteases are instrumental in the pathogenesis of a variety of chronic lung diseases. Protease deregulation during pathogenesis may occur through escape to its tight, spatio-temporal, self-limiting control. The wide variety of protease substrates adds a further complexity to this field. Indeed, the examples described in this review emphasize that the close interplay between proteases, substrates and inhibitors, which are interrelated in the protease interactome accounting for the net proteolytic activity in the lung, should be taken into consideration. As a result of the recent emergence of the above-mentioned concepts, the understanding of the interplay and interdependency of these systems remains very scarce in the lung research field. Extrapolations of observations from different systems, together with translational approaches, combining degradomics data, murine models of lung disease and *ex vivo* observations from patients, should allow identification of an increasing number of instrumental proteases and protease inhibitors, in the perspective of integrative protease biology. Understanding in greater detail the complex molecular basis of regulation and function of protease activity will facilitate identification of new therapeutic targets as well as new diagnostic and prognostic tools for personalized medicine, and hopefully result in the development of new drugs to treat pulmonary diseases that result from inherent protease systems deregulation.

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Figure legends

Figure 1: **More-than-normal protease activity in lung pathogenesis.** In the normal lung (left), proteases (represented by scissors) are involved in lung homeostasis and their activity is tightly regulated by (among others) cognate protease inhibitors. (Right) In a diseased lung, elevated protease activity, which can occur through increased protease expression and/or loss in cognate protease inhibitor, results into imbalanced protease activity.

Figure 2: Mechanisms underlying serpinopathies. Point mutations in the gene encoding may result into misfolded protein and misfolded serpin polymerization. Serpin polymers have no inhibitory functions towards serine proteases. Accumulation of serpin polymers has multiple cellular consequences. Other mutations can hinder protein expression. In either case, the loss of serpin results into uncontrolled proteolytic activity, thereby contributing to cell damage and the development of diseases.

Table | 1: Proteases with extracellular function associated with chronic lung diseases: mechanisms of action within the airway microenvironment, reporters and selected (commercially available) pharmacological inhibitors

PROTEASE		MECHANISM OF ACTION	REPORTER	INHIBITORS
CYSTEINE PROTEASES	**Cathepsin B**	Found in fibroblasts of IPF, contributes to myo-fibroblast differentiation via TGF-ß1, Smad 2-3 pathway [19]	FRET reporter (Hu, unpublished observations)	VBY-376 [22] (C) Leupeptin (C) E-64[15] (V)
	Cathepsin K	Cleaves collagen, increased in fibroblasts of fibrotic tissue in IPF [16-18]	FRET reporter (Subramanian, unpublished observations)	CTSK inhibitor II (C) (Calbiochem) Cystatin C (C) [97]
	Cathepsin L	Increased in BAL of CF patients, along with Cathepsin B & Cathepsin K [16]		Pentapeptide amide RKLLW-NH2
	Cathepsin S	Lysosomal protease, unlike other cathepsins remains active at neutral pH [98]	FRET reporter [99]	Leupeptin (C) E-64 [15] (V)
SERINE PROTEASES	**Cathepsin-G**	High affinity for nucleic acids, locates to NETs [27]		Chymostatin (C)
	NSP-4	Recently identified 4th neutrophil-derived protease (along with CTSG, NE & PR-3) substrate unknown [28]		
	Neutrophil elastase	Cleaves elastin, key risk factor for bronchiectasis in CF [29, 30], Induces IL-8, mucin secretion [32-39]	NEmo1, NEmo2[46]	Sivelestat (C) Elastase inhibitor V (V)
	Proteinase-3	Degrades elastin [100]		
METALLOPROTEASES	**ADAM-17** (TACE)	Initiates endothelial permeabilty and leukocyte recruitment via TNF generation [41, 42], involved in shedding TGFα [47]		TAPI-1[101]
	ADAM-10	Causes experimental emphysema[43], possible sheddase for CD23 [44], involved in shedding TGFα [47]		GI254023X [102] (C)
	ADAM-33	Polymorphisms in ADAM-33 gene liked to Asthmas, COPD [45]	FRET probe[103]	Anti-ADAM-33-Antibody
	MMP-2	Cleaves type IV collagen, Serum MMP2 correlates with severity of pulmonary hypertension [104] , cleaves Serpin B6 & alpha-1 inhibitor-3 [76]		GM6001 (C) [105] Actinonin
	MMP-7	Possible marker for IPF (serum, BALF) [106]		GM6001 (C) [105] Actinonin
	MMP-8	Cleaves SerpinA1, releasing elastase activity *in vivo* [78]		Actinonin
	MMP-9 (gelatinase B)	Cleaves type IV & V collagen, recruits leukocytes[107]	FRET reporter (Halls, unpublished observations)	GM6001 (C) [105] Actinonin
	MMP-10	Possible marker for IPF (serum, BALF) [106]		GM6001 (C) [105], Actinonin [106]

	MMP-12 (macrophage elastase)	SNPs associated with lung function decline in COPD, asthma and CF [47, 48] Increased activity in smokers with COPD, asthma [35] and CF [36], associated with lung function decline in CF [47]	LaRee1, LaRee5 [85]	GM6001 (C) [105] MMP408 Actinonin UK 370106
	MMP-14	Processes SLPI [77]		Anti-MMP-14-Antibody
ANTIPROTEASES	**A1AT**	Serpin, inhibits NE, Cathepsin G, and Proteinase-3 [63], deficiency causes emphysema & liver disease [64, 65], therapeutic potential for COPD [71]		
	Elafin (trappin-2)	Inhibits proteases, anti-microbial, modulates immune response [50, 51]		
	SLPI	Inhibits proteases, anti-microbial, modulates immune response [50, 51], therapeutic potential for CF [73]		
				Inhibition type (C) = competitive (V) = covalent

A1AT=alpha-1 antitrypsin, ADAM=a disintegrin and metalloproteinase, BALF=bronchial alveolar lavage fluid, CAP=channel activating protease, CTS= cathepsin, LaRee=lavage reporter, NE=neutrophil elastase, NEmo=neutrophil elastase monitor, NETs=neutrophil extracellular traps, NSP-4=neutrophil serine protease-4, MMP=metalloprotease, SLPI=secretory leukocyte protease inhibitor

Figure 1

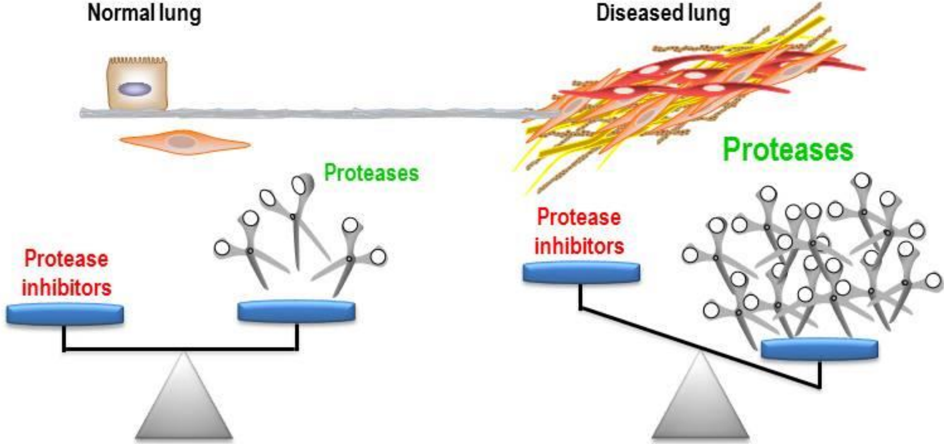

Figure 2