Untangling the protease web in COPD: metalloproteinases in the silent zone

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The idea that unregulated protease activity underlies the pathogenesis of COPD has been prominent since the recognition that genetic loss of α1 antitrypsin led to unregulated neutrophil elastase activity and premature emphysema.[1] Increased proteolysis by other proteases, particularly the matrix metalloproteinases (MMPs), has since been implicated in COPD. The MMPs are a family of over 20 zinc-dependent endopeptidases, initially identified as extracellular matrix (ECM) degrading enzymes and classified according to their ECM substrates. In individual studies, genetic association, overexpression, and in fewer cases, activity of individual proteases have been associated with COPD in humans and smoke-induced emphysema in animals. While a number of MMPs and disintegrin and metalloproteinases have been studied in COPD at some level, most interest has focused on MMP-1, MMP-12 and MMP-9. MMP-1 is overexpressed by alveolar macrophages, type II pneumocytes and airway epithelial cells. The protein is induced by cigarette smoke and is capable of degrading collagens I and III, the most abundant lung proteins.[2 3] MMP-9 expressed by neutrophils and macrophages is overexpressed in COPD although MMP-9 activity, rather than protease expression, is suppressed in stable patients compared with healthy smokers but activated during exacerbations.[4 5] Although MMP-9 has elastase activity in vitro, its action in COPD may be due to generation of prolyl–glycine–proline (PGP), a collagen-derived neutrophil chemotactic matrikine rather than elastolysis.[6] MMP-12 is an elastase associated with the development of COPD by genome-wide association study,[7] whose presence and activity in sputum are associated airflow obstruction[8] and in which single-nucleotide polymorphisms that reduce enzyme activity are associated with protection from emphysema in COPD.[9] While the 'classical'-matrix-degrading actions of these proteases can be easily related to the pathogenesis of COPD, unfortunately the reality is not that straightforward. The rapidly expanding number of non-ECM MMP substrates has highlighted a large number of biological processes modified by MMPs and created potential new opportunities to intervene therapeutically in COPD. For example, proteomic-based interrogation of animal models of inflammation has identified around 150 discrete protein substrates of MMP-12 in vivo. Importantly, proteolytic processing of these substrates was shown to modify inflammation, cell migration, innate and adaptive immunity in addition to ECM remodelling.[10] These far-reaching effects put MMPs and other proteases at the centre of maintenance of normal homeostasis as well as mediators of disease. These important protective functions make the use of MMP protease inhibitors a risky therapeutic strategy. For example, MMP-12 generates angiostatin and suppresses neovascularisation and metastatic lung tumour growth in animals, and inhibiting this protease in those who smoke is likely to be an unacceptable risk in COPD.[11]

In this month's edition of the journal, Ostridge and colleagues address the association of specific aspects of the emphysema phenotype; namely small airway disease and emphysema, with bronchoalveolar lavage MMP and cytokine levels in 24 individuals with COPD and 8 smoking or ex-smoking controls. Emphysema, bronchial wall thickening and small airway obstruction were examined using validated quantitative CT measures. Total MMP protein and cytokine levels were measured by Luminex multiplex microparticle array. Consistent with various previous reports, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-10, although not MMP-12, were elevated in COPD when compared with controls. The interest in this study lies with the associations between protein levels and specific aspects of COPD pathology. Emphysema in COPD was associated with elevation of MMP-3, MMP-7 and MMP-10; interestingly, both MMP-3 and MMP-10 are capable of activating MMP-7 whereas MMP-10 itself is activated by neutrophil elastase. Airflow obstruction, measured using FEV_1, however was associated with MMP-8, MMP-9 and MMP-12, predominantly neutrophil and macrophage-derived proteases capable of generating leucocyte chemotaxins and activating TGFβ.[12] Measures of small airway disease were associated with a different MMP signature again, comprising MMP-3, MMP-7, MMP-8, MMP-9 and MMP-10. The strongest association being with MMP-8. The differing MMP profiles associated with small airway obstruction and emphysema highlight that small airway remodelling is required for airflow obstruction in COPD, independent of loss of elastic recoil due to emphysema.

The challenge is to understand how these tissue-remodelling enzymes contribute to the airway pathology of COPD. This study highlights the need to consider the roles of proteases in other aspects of the COPD other than emphysema, particularly the small airways that are emerging as a critical component of the COPD phenotype. In health, the small airways, defined as those <2 mm contribute <20% of lower airway resistance. The potential for large increases in small airway obstruction to remain relatively asymptomatic has earned this lung compartment the title of the 'silent zone'.[13] A combination of physiological and anatomical studies, recently incorporating micro-CT, suggests that small airway obstruction can progress relatively undetected initially, preceding emphysema, with the development of progressive airway wall thickening and complete loss, due to luminal obliteration, of up to 90% of the small airways. In COPD, increased small airway obstruction may be due to airway wall thickening by ECM deposition and inflammatory cell infiltration, luminal occlusion by mucous and dynamic collapse due to loss of elastic recoil. However, detailed multivariate analyses of physiological and anatomic data point towards airway wall remodelling as the dominant influence on small airway resistance.[14] Taken together, these data suggest that the association between small airway obstruction and MMP-8, a neutrophil-derived collagenase, is likely to be related to MMP-8's profibrotic actions. In vitro, MMP-8 is a potent type I collagen-degrading protease, yet despite its collagenase activity, which might be expected to reduce lung fibrosis in response to injury: in murine airways, MMP-8 inactivates macrophage inflammatory protein-α in allergic airway inflammation and contributes to the generation of the neutrophil chemoattractant PGP.[15] MMP-8 also promotes lung fibrosis in response to bleomycin in the mouse and reduces lung inflammation by cleaving interleukin-10.[16]

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The study by Ostridge and colleagues advances the field by examining multiple proteases in patients with detailed phenotype data. However, the multilevel regulation of proteases in vivo including the presence of endogenous inhibitors dictates that to understand protease activity at a cellular, spatial and temporal level, this type of detailed protein expression and phenotype data need to be analysed together with precise localisation and overall activity data for different protease classes. Furthermore, the redundancy among substrate–protease interactions, of which the MMPs are only a subset, makes the significance of these data harder to interpret. For example, MMP-8 and neutrophil elastase are generally colocalised and have multiple overlapping substrates. The higher potency of neutrophil elastase for most of these substrates makes the expression and contribution of MMP-8 in COPD more difficult to interpret. The next steps will be to describe how these individual changes in protease expression and activity contribute to biological changes at specific anatomical and cellular sites at specific times. Emerging proteomic techniques have begun to identify multiple previously unrecognised MMP substrates in vivo, revealing an increasingly complex 'web' of protease interactions.[10] While MMPs activate or inactivate individual proteins such as cytokines whose actions may be predictable, altering the activity of a single protease also degrades multiple protease inhibitors and activates multiple proproteases affecting their substrates and their targets and so on. For example, proteomic analysis of pharmacological inhibition of MMP-14 in a cell line affected 30 other proteases and protease inhibitors all of which may have had downstream effects.[17] This complexity makes interpreting the biological consequences of changes in individual enzymes difficult. Combining the detailed protein level data reported by Ostridge *et al* with techniques such as in situ zymography to localise changes in overall proteolytic activity at specific anatomical locations should be the next phase of protease research to understand tissue remodelling in disease. Large clinical cohorts are allowing detailed phenotyping of those with COPD and the correlation of genetic and protein expression data: the need now is to incorporate protein activity and localisation into these studies. Complementary approaches to examine the turnover of protease targets can also provide data on overall protease activity. Measurement of ECM protein fragments produced by specific proteases reflect changes in ECM degradation and deposition. Interestingly, these markers can predict disease progression in lung fibrosis and in COPD exacerbations, show both increases in collagen and elastin turnover and simultaneous de novo synthesis of other ECM proteins.[18 19]

The eventual aim of such work is to improve targeting of injurious tissue remodelling and other emerging protease-dependent processes in COPD. Broadspectrum MMP inhibitors in cancer were not efficacious and led to unforeseen side effects. The complexity of protease and inhibitor networks, redundancy among protease functions and the multiple beneficial effects of MMPs at different sites at different times currently make the safe targeting proteases unworkable. Targeting specific processes downstream of these proteases in the future is likely to be safer and more achievable.

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