

EDITORIAL

Clinical and Experimental Allergy

Molecular portfolios: cells interacting with matrix in repairing airway epithelium

This editorial discusses the findings of the paper in this issue by Stevens et al. [3].

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In both developing and adult tissues, local cell–cell and cell–matrix interactions largely determine how tissues are organized and how cells work together. It is easy to forget that extracellular matrix is not a static ‘gap filler’ but a dynamic structure degraded and replenished by proteases and synthases. The molecular players in these processes are many and their interactions complex. Once the machinery has evolved to organize molecules or groups of molecules into the right place, there is no reason these molecules cannot fulfil more than one role, indeed it makes sense that they do. This is the case for the plasminogen activator inhibitor (PAI)-1. PAI-1 is part of the plasminogen activating system (PAS, Fig. 1) involved in regulating the activity of the protease plasmin, but also with protease-independent roles in cell adhesion and migration [1, 2]. Increased expression of PAI-1 in epithelial cells from asthmatic children, described in this issue [3], highlights the importance of understanding such ‘portfolio molecules’ and how they may be involved in disease.

The plasminogen activating system

Because of their destructive power, proteases are typically found in the body as inactive pro-enzymes and are only cleaved to produce active proteases at exactly the time and place they are required. Plasmin, a key protease in the cellular toolkit used to regulate and repair matrix is produced by cleavage of the inactive precursor plasminogen, found in the blood and many tissues [4]. Once activated, plasmin is a broad spectrum matrix protease, breaking down fibrin, fibronectin, thrombospondin and

laminin while also able to activate a range of collagenases by cleaving pro-matrix metalloproteases (pro-MMPs) to form MMPs. Plasmin is activated by either tissue plasminogen activator (tPA), predominantly involved in vascular fibrinolysis or by urokinase-type plasminogen activator (uPA), more important in migration and matrix remodeling [4]. uPA is bound to cell surfaces by the uPA receptor (uPAR). This GPI-linked uPA binding protein can thus focus protease activity in the immediate pericellular region and augment activity by increased local concentration and proximity to pericellular matrix components. Under conditions where matrix degradation is needed, protease activity can thus be localized to the cell surface providing appropriate and coordinate expression of plasminogen, uPAR, uPA and active MMPs.

Switching off proteases

So much for activating proteolysis, but how is it switched off? Another key regulatory mechanism in the matrix protease toolkit is the production of inhibitors. These are often produced at the same time as, or even before, their target protease providing for tight regulation and rapid reduction in activity when required. In the PAS, there are two specific PAIs, PAI-1 and PAI-2. PAI-1 binds to vitronectin, either in the extracellular matrix or in solution. Binding of PAI-1 to the uPA/uPAR complex inhibits plasminogen cleavage, so that plasmin is not formed, pro-MMPs are not activated and matrix degradation is consequently reduced. PAI-1 binding to the uPA/uPAR complex also leads to internalization, with degradation of uPA and recycling of unoccupied uPAR to the cell surface [5] leading to more sustained reduction in uPA activity.

Thus, the PAI can inhibit plasmin formation by direct short-term or long-term interactions by internalization of uPA complexes from the cells surface. But cells – especially normally sedentary cells such as epithelial cells – typically express relatively low levels of active matrix

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proteases under resting conditions. That does not mean there is no activity – this is a dynamic system and there is always some turnover, but significantly increased protease activity tends to reflect tissue remodelling. Involution of the mammary gland after weaning provides a physiological example, where proteases are required to breakdown the extra matrix formed to support the additional epithelial structures needed during lactation. In this system, PAI-1 and the MMP inhibitory TIMPs are expressed before the relevant proteases are up-regulated [6]. In the respiratory tract, expression of proteases is likely to reflect ongoing repair of damage or response to environmental stress. Simple mechanical stimulation of bronchial epithelial cells, for example, causes increased expression of PAS components [7]. In cell culture, the epithelium is disrupted then re-forms so it is consequently also likely to show increases in proteases, mirrored by coordinate expression of inhibitors, as reported by Stevens et al. [3] and seen in other studies [8].

Epithelial repair and the plasminogen activating system

Airway epithelial damage increases the risk of infection and further damage, so repair needs to be rapid, largely autonomous and primarily driven by local responses commensurate with the degree of local damage [9–11]. In alveolar epithelial repair, uPA is expressed by cells at the leading edge of wounds and at the extremities of actin filaments [12]. In addition to binding and localizing uPA, uPAR has been shown to bind to both matrix proteins and integrins [13] (Fig. 1). This binding is independent of protease activity, but can bring together matrix molecules, the matrix signalling activities of the integrins and the potential proteolytic activities of uPA. These local matrix interactions are essential in the migratory phase of epithelial repair, where cells are constantly making matrix contacts at the leading edge and breaking them at the trailing edge while sensing and modulating cell–matrix interactions through integrins [14]. This further complements the protease-independent roles of PAI-1 reported in cell migration, with PAI-1 up-regulation in wounding and associated with some invasive tumours [2, 15]. Binding of PAI-1 is thought to reduce cell adhesion by reduced uPAR, integrin and matrix interactions [16, 17]. This reduced adhesion will influence both cell migration and the metastatic ability of tumour cells.

Asthma, matrix and epithelial repair

Changes in extracellular matrix are linked to two characteristic pathophysiological features of asthma. First, sub-epithelial fibrosis can have progressive effects on airway patency and responsiveness due to physical changes in the airway wall. Second, airway epithelial damage has long been associated with samples from

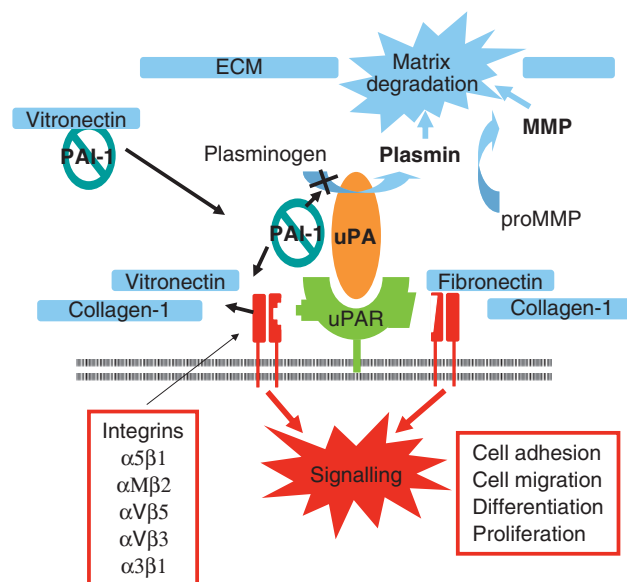


Fig. 1. Plasminogen activating system (PAS). Simplified diagrammatic illustration of the interactions between members of the PAS system in plasminogen activation and their potential involvement in non-proteolytic activities. uPA, urokinase-like plasminogen activator; uPAR, uPA receptor; PAI-1, plasminogen activator inhibitor-1; MMP, matrix metalloproteinase; pro-MMP, pro-matrix metalloproteinase.

asthmatic subjects [18], reflecting changes in epithelial repair processes. It is perhaps no surprise therefore to find links between the PAS and asthma. At a genetic level, polymorphisms in the genes encoding PAI-1 [19, 20] and uPA [21] have been associated with asthma. Asthma is a complex disease with chronic immunological and structural cell interactions. To understand the significance of altered protease pathway molecules in asthma, it is therefore critical to understand both proteolytic and non-proteolytic roles of these molecules and how they might also contribute to its pathogenesis.

Looking first at non-asthmatic subjects, the recent paper by Stevens et al. [3] shows that PAI-1 is present in epithelial cells and lysates collected from paediatric samples by bronchial brushing. The specific localization and context of the PAS molecules is critical, but in studies of tissues we often know only the overall level of expression – and occasionally activity – in a particular tissue or cell. This is where experimental models using controlled stimuli such as the wounding model used by Stevens and colleagues are invaluable, and the ability to manipulate molecules in such systems doubly so. When the primary epithelial cells, obtained by bronchial brushing, were cultured, PAI-1 production was increased and more so if those cultures were wounded. Using siRNA to silence PAI-1 gene expression reduced cell proliferation in culture and also reduced repair. This suggests that PAI-1 is indeed needed for repair but it may also be involved in the signalling to promote cell proliferation following the

immediate migratory response in epithelial repair. Going on to look at epithelial cells from asthmatic children showed that epithelia grown from these cells took longer to repair. They also contained more PAI-1 mRNA and protein compared with non-asthmatics, although plasma PAI-1 protein levels in the subjects were similar. PAI-1 gene silencing reduced proliferation but did not show significant effects on the low level of repair. The failure to rescue repair by reducing PAI-1 levels in cells from asthmatics may be explained in several ways. There may be other molecules required for repair that are also affected in the asthmatics, but not involved in the proliferative response. There could be specific problems with the siRNA in samples from asthmatic subjects, or the optimum PAI-1 concentration required for repair in these samples is different and is not achieved by the siRNA treatment. Chronically high levels of PAI-1 could cause alternative pathways of repair to be activated as uPAR-matrix interactions are reduced [16, 17] or conversely, chronic repair processes may affect the regulation of the PAS in asthmatics. Other studies in an alveolar epithelial repair system showed increased repair by adding plasmin but decreased repair on treatment with anti-PAI-1 antibodies indicating that the proteolytic and non-proteolytic effects may differ [2].

Conclusion

The portfolios of PAI-1 and uPAR are not limited to regulating proteolytic activity but also include important contributions to non-proteolytic processes such as migration occurring at their site of expression. Considering PAI-1 as a matricellular protein family member as has recently been suggested would better reflect this portfolio [2]. The matricellular family of proteins include thrombospondin-1, tenascin-C and an increasing number of other multi-functional molecules characterized by their involvement in cell-matrix interactions, although not themselves being structural matrix proteins [22]. The cytokine-like activities reported for tPA would also be consistent with functions extending beyond simple proteolytic processes [23].

In multicellular organisms context defines function. Extracellular matrix components, and there are many hundreds of them, provide the building materials to organize cells into tissues. Few signals get to or from cells without interacting extensively with the extracellular milieu. In so doing, information about location in the tissue, tissue architecture, previous cellular activity and the state of neighbouring cells is 'encrypted'. Embodying more than one activity in the same molecule could streamline functional links between migration-related signals and local proteolysis. No additional signalling mechanisms are needed and the activities are necessarily co-located, thus simultaneously strengthening and

simplifying regulation. This clearly increases the complexity of the system for the experimental scientist, emphasizing the need to consider multiple interactions rather than single processes. In complex diseases such as asthma, dysfunction in one system may have important and otherwise unpredictable consequences in other physiological systems. As we start to understand the molecular activities held in such portfolios, it may become possible to devise therapies focussed on the molecules with 'keystone' portfolios, allowing us to simultaneously intervene in several interrelated pathways.

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