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Urokinase and its receptor uPAR is a critical component of pulmonary renal cascade and is significant for pulmonary function and contributes to the physiological regulation; relationship with Serpin E2

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ABSTRACT:
Many previous studies have shown that induction of uPA, uPA receptor (uPAR), and PAI-1 may occur in lung endothelial cells (ECs) through a uPA-mediated feedback pathway. Soluble urokinase-type plasminogen activator receptor (suPAR) is a soluble form of the urokinase plasminogen activator receptor (uPAR) that is produced upon cleavage of membrane-bound uPAR. It is found in various body fluids, including blood, urine and cerebrospinal fluid. It is now known that Pulmonary function in physiological and patho-physiological condition is regulated by these molecules. On the other hand, Protease nexin-1 or serine protease inhibitor (Serpin E2) is intricately linked in the physiological homeostasis and interacts with uPA system. These are the key elements of the pulmonary renal cascade regulating multiple physiological functions.

Key words: Urokinase, suPAR, lungs, SERPIN, physiological, airways

Introduction

The urokinase plasminogen activator receptor (uPAR) is a three-domain membrane-bound receptor, mainly expressed on immune cells i.e activated T cells, neutrophils and macrophages, but also on smooth muscle and endothelial cells. This receptor is involved in angiogenesis, adhesion, cell migration, proliferation, cell survival, inflammation and proteolysis and respiratory control (1-3). Several pro-inflammatory cytokines (interleukin (IL)-1b, IL-6, tumor necrosis factor (TNF)) and growth factors (epidermal growth factor, basic fibroblast growth factor) regulate the expression of uPAR (4-5). During inflammatory stimulation, shedding of uPAR from the plasma membrane is the main process of soluble urokinase plasminogen activator receptor (suPAR) formation. Association and correlation analyses confirmed coordinated uPAR expression in lung structural and inflammatory cells. These results suggests a complex relationship between different bronchial structural cells and uPAR expression, where it has been suggested that uPAR is involved in a number of airway remodelling linked processes, for example wound repair and epithelial proliferation (6-7). This author first proposed in 2003 that Erythropoietin, urokinase and Serpin E2 mediate a mutually homeostatic pulmonary renal cascade between the lungs and kidneys regulating multiple physiological functions termed the 'Pulmonary renal cascade. Considering the fact that urokinase and erythropoietin and Serpin E2 affect the respiratory system and also play an important role in hemopoiesis, cardiovascular system, muscle contractility and central nervous activity the urokinase enzyme and its two subtypes of receptors contribute effectively and are an important component of the pulmonary renal cascade and are under intensive scientific scrutiny (8).

uPAR and suPAR and its importance in airways disorders: The single chain uPAR (cluster of differentiation 87) is a 55–60 kDa glycoprotein of the Ly-6/uPAR/alpha-neurotoxin family, folded into

three separate domains (DI, DII and DIII). The molecular mass of the truncated DIIDIII form is about 40–45 kDa, whereas the DI domain is 16 kDa. uPAR is bound to the cell membranes by glycosyl phosphatidylinositol (GPI) anchor (Fig.1). The formation of suPAR is fulfilled by several enzymes such as uPA, GPI-specific phospholipase D, matrix metalloproteinases (MMPs) and, cathepsin G, neutrophil elastase and plasmin (9-11). Several growth factors, pro-inflammatory cytokines, bacterial lipopolysaccharide and cell–cell contact are indirect factors that stimulate the suPAR shedding. Full-length suPAR (suPARI-III) consists of all three domains but lacks the GPI anchor and can be cleaved into two soluble forms, suPARI and suPARI-III. Exogenous uPA binds suPAR with a kd in sub-nanomolar ranges comparable to the full-length uPAR, which makes suPAR the ideal candidate to investigate the uPA-uPAR binding interactions *in vitro* (12-14). Both DI and DII–DIII bind uPA, which indicates that each domain recognizes another epitope of uPA. Circulating suPAR levels have been determined in COPD only by a few studies. Two studies analysed suPAR during acute exacerbations. These events are associated with airway and systemic inflammatory responses, and not surprisingly elevated suPAR levels were found during exacerbations . The results in stable disease are contradictory, as both higher and similar levels were found. Thus suPAR is the circulatory form of the uPA receptor which plays critical roles in normal physiology and pathophysiological states (15).

Interaction between uPA and SERPINs regulating physiological homeostasis between pulmonary and hematological system: Serine protease inhibitors (serpins) are protease inhibitors that play central regulatory roles throughout the mammalian body. They serve as important players in normal physiological functions and regulate protease activation in various systems, including coagulation, fibrinolysis, or inflammation. The role of serpins as central regulators of normal physiological functions is well illustrated by the number of diseases caused by serpin dysfunction (16-17). In inflammatory settings, unstable or unbalanced serpin levels can even be lethal, as in severe sepsis with disseminated intravascular coagulation (18). Plasminogen activator inhibitor-1 (PAI-1) is the principal serpin regulating fibrinolysis, whereas antithrombin (AT) and protease nexin-1 (PN-1; serpinE2) are the two principal known physiological inhibitors of thrombin. AT is a plasma serpin and therefore inhibits thrombin generated after vessel injury, whereas PN-1 may be undetectable in the blood and acts essentially at the tissue/cellular level (19-20). Regarding lung injury, elevated expression of PAI-1 has been shown to reduce fibrinolysis in idiopathic pulmonary fibrosis (IPF), but PN-1 (serpinE2) may also contribute. Indeed, PN-1 inhibits thrombin, the plasminogen activators (PAs) urokinase-type PA and tissue PA, and plasmin. PN-1 has been shown to be expressed in the normal adult mouse lung and in normal and pathological human lungs (21-22). Its expression is upregulated in lung tissue, bronchoalveolar lavage fluids (BALFs), and lung fibroblasts from IPF patients, in whom it is able to block thrombin activity. Collectively, this may suggest a potential role for PN-1 in the pathophysiology of IPF. However, because PN-1 exerts both anticoagulant and antifibrinolytic activities, and it plays a protective role in the cellular responses to lung injury has been determined (23).

Does Urokinase and SerpinE2 regulate the physiological homeostasis and also contribute in patho-physiological states: Urokinase and its two receptors alongwith their interaction with SerpinE2 mediate the interaction between the lungs and kidneys in a mutually active cascade.SerpinE 2 may interact with urokinase during stressful conditions for e.g like the acute respiratory distress syndrome and chronic disease states (24). Erythropoietin is another important agent and therefore these three key molecules mediate multiple functions and have a regulatory role on respiration, cardiovascular and central nervous activity apart from their facilitatory role in hemopoiesis. The exacerbation and modulation do occur in pathophysiological states (25). In fact a similar pathway regulating the production of erythropoietin (EPO) has been extensively characterized and shown to involve the interactions of specific transcription factors, such as HIF-1, with discrete enhancer regions in the 38-flanking sequences of the *EPO* gene.Despite restricted cellular production of EPO, hypoxic induction of reporter genes containing the *EPO* enhancer has been observed in a wide variety of cell types, suggesting that HIF-1 may also mediate

other adaptive responses to hypoxia (26-28). Inspection of the 58-flanking region of the uPAR gene shows three potential HIF-1 binding sequences, each of which differs by only one nucleotide from the consensus sequence 58-XACGTGCX, originally identified in the genes encoding EPO and several glycolytic enzymes (29-30). This suggests that erythropoietin and urokinase may be working in tandem and interacting to regulate physiological functions.

Pulmonary protectant effect of SerpinE2: The blood coagulation and fibrinolytic system deregulation has been implicated in the development of idiopathic pulmonary fibrosis, a devastating form of interstitial lung disease. In a recent study use of intratracheal instillation of bleomycin to induce pulmonary fibrosis in mice analyzed the role of serine protease inhibitor E2 (serpinE2)/protease nexin-1 (PN-1), a tissue serpin that exhibits anticoagulant and antifibrinolytic properties (Fig.2). PN-1 deficiency was associated, after bleomycin challenge, with a significant increase in mortality, as well as a marked increase in active thrombin in bronchoalveolar lavage fluids, an overexpression of extracellular matrix proteins, and an accumulation of inflammatory cells in the lungs. Moreover the bone marrow transplantation experiments showed that protective PN-1 was derived from hematopoietic cell compartment. A pharmacological strategy using the direct thrombin inhibitor argatroban reversed the deleterious effects of PN-1 deficiency. Concomitant deficiency of the thrombin receptor protease-activated receptor 4 (PAR4) abolished the deleterious effects of PN-1 deficiency in hematopoietic cells (31). These results demonstrate that prevention of thrombin signaling by PN-1 constitutes an important endogenous mechanism of protection against lung fibrosis and associated mortality. These recent scientific findings suggest that proper doses of thrombin inhibitors or PAR4 antagonists may provide benefit against progressive lung fibrosis with evidence of deregulated thrombin activity. It is believed that increases in SERPINE2 expression in COPD represent a compensatory mechanism, in response to the overwhelmed protease burden and associated antiprotease functional insufficiency. Similar to these findings with Serpine2, recently Baraldo *et al.* (32) demonstrated enhanced T- and B-lymphocyte accumulation and exaggerated lymphoid follicle formation in humans with SERPINA1/α1-antitrypsin deficiency. It should be noted that many, though not all, disease-related effects of SERPINA1 are mediated *via* its ability to regulate elastase activity, whereas SERPINE2 does not inhibit elastase. Alltogether, these results suggest the involvement of SERPINs in complex adaptive immune responses associated with COPD pathology, beyond the regulation of tissue destruction and emphysema. The identification of where these 2 serine protease inhibition pathways converge to regulate COPD-related lymphocyte trafficking is a focus of future investigation with potential for therapeutic implications. Interestingly, it was also found a modest, but significant, congenital airspace enlargement in $SE2^{-/-}$ mice. This difference in respiratory structure is nonprogressive and is not associated with evidence of neutrophil or macrophage inflammation.

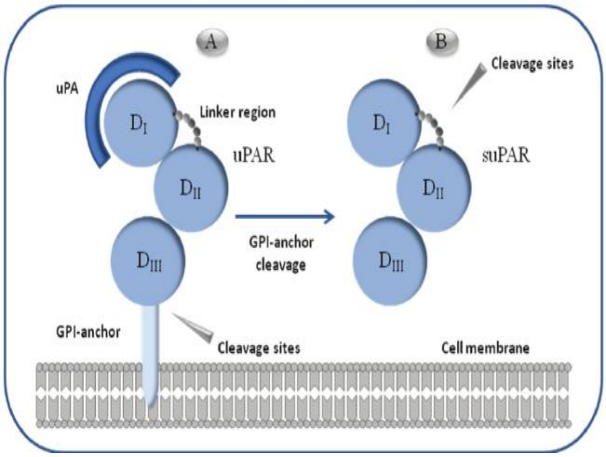

Fig.1 uPA receptor domains

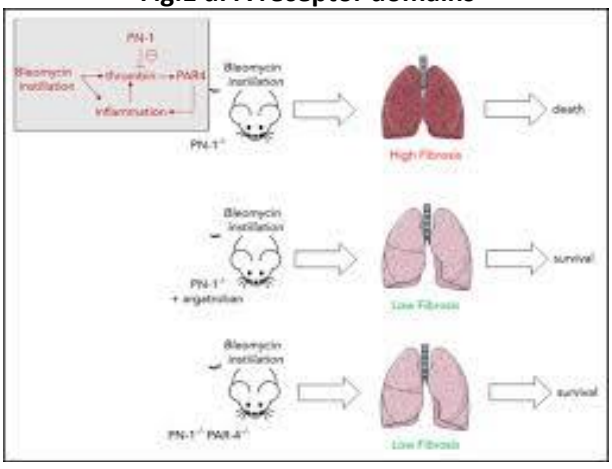

Fig.2 Protease nexin-1 and lung protection

Conclusion: The physiological and pathological implications of uPA-mediated tissue factor

expression will require extensive studies, but recent data show that the pathway is operative in normal lung tissue. Interestingly, the binding of PAI-1 to its cofactor vitronectin increases the half-life of the active form of PAI-1. Due to the importance of the plasminogen activation system (PAS) in human health and disease, it has been extensively studied in mammals and been a popular target for drug development. Although only a limited number of studies of this system exist in other vertebrates.

It can be suggested the uPA and its receptors in conjunction regulate physiological and pathophysiological processes that the determination of serum suPAR levels can be helpful in the follow-up of COPD and in the monitoring of the treatment response, potentially making suPAR a valuable biomarker in the prognosis of COPD. Serum suPAR levels correlated with plasma fibrinogen levels, both markers have the potential to predict COPD.

The uPA/uPAR system modulates antigen processing and presentation, lymphocyte activation , generation of pro- and anti-inflammatory signals , activation of intracellular signaling pathways , and cytotoxicity , all of which are critical steps in cell-mediated immune responses. Involvement of the uPA/uPAR system in derangements of immune responses for e.g in HIV disease via an IFN-like mechanism has also been demonstrated demonstrate that uPA stimulates expression of TF by lung ECs in culture and *in vivo*. This newly identified pathway is, to our knowledge, the first observation that uPA regulates the expression of components involved in coagulation in any cell type. Due to the importance of the plasminogen activation system (PAS) in human health and disease, it has been extensively studied in mammals and been a popular target for drug development. Although only a limited number of studies of this system exist in other vertebrates. All together current evidence strongly suggests that the urokinase and its receptors contribute effectively in pulmonary function and interact with Serpins to regulate physiological function.

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