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UROKINASE RECEPTORS, AND ITS STRUCTURE, SIGNAL TRANSDUCTION AND IMPORTANCE IN 'PULMONARY RENAL CASCADE'

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Abstract

Aim: Urokinase is an important and critical enzyme of the Pulmonary renal cascade. The urokinase-type plasminogen activator (uPA) receptor (uPAR), was identified, isolated and cloned as the plasma membrane high-affinity binding-site of the serine protease uPA. Plasma membrane urokinase-type plasminogen activator (uPA)-receptor (uPAR) is a glycosyl phosphatidylinositol (GPI)-anchored protein that binds with high-affinity and activates the serine protease uPA, thus regulating proteolytic activity at the cell surface. uPA and its receptor may be important for respiratory rhythmogenesis and may contribute in respiratory disorders. This mini-review outlines the importance of uPA and uPAR structure, receptor function and roles in respiratory disorders.

Introduction

The Urokinase receptor is a GPI-anchored receptor molecule, the signaling activity of uPAR relies on its interaction with other proteins. Since its discovery about two decades ago, a wide variety of uPAR interactors have been reported in the literature. Based on the level of evidence available, these interactors may be divided into two groups [1]. The first group is formed by the serine protease uPA and the extracellular matrix protein VN, which may be considered the "core" uPAR ligands for which extensive, independent and coherent biological, biochemical and structural evidence is available[2]. The human uPAR cDNA encodes a polypeptide of 335 amino acids including a N-terminal 22-residue secretion signal peptide and a C-terminal segment (30 amino acids) removable with the attachment of a (GPI)-anchor [3](Fig.2). The mature protein (283 residues) is highly glycosylated and composed of three similarly sized (about 90 residues each) homologous domains hereby referred to as DI, DII and DIII and belonging to the Ly-6/uPAR protein domain family [4].

The main components of urokinase-type plasminogen activator (uPA) system, including uPA urokinase-type plasminogen activator receptor (uPAR) and urokinase-type plasminogen activator inhibitor (PAI-1), are important constituents of activated immune system and inflammation [5]. uPAR (CD87) is a cysteine-rich, glycosylphosphatidylinositol (GPI)-anchored cell membrane protein [6]**.** uPAR is on the outer parts of several cell types, such as monocytes and macrophages, polymorphonuclear neutrophils, vascular endothelial, smooth muscle and epithelial cells [7-9] (Fig.1). suPAR arises from proteolytic cleavage of the GPI anchor by various proteases, is released from cell membrane-bound uPAR and is detected in various body fluids, such as blood, urine and pleural [10,11]. The release of suPAR is influenced by various immune and inflammatory effectors, such as bacterial products, cytokines and growth factors [12]. Robert Medcalf & Dear designated Urokinase as a pleiotropic molecule [13]. uPAR protein is composed of three different sites, called D1, D2 and D3 between the amino and the carboxy termini of molecules, each including a preserved organization of disulfide bonds and separated by among short linker arrays [14]. This mini-review outlines the significantce of uPA and uPAR structure, receptor function and roles in respiratory disorders.

Gpi anchored protein and biosynthesis:

The biosynthesis of GPI-protein complexes, includes GPI precursors and post-translational modification of proteins with GPI take place in the endoplasmic reticulum [15]. The actual process starts on the cytoplasmic side of this membrane and is completed on the lumenal side, so the intermediate glycophospholipid must be flipped across the membrane. In

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mammalian cells, the lipid precursor is a conventional phosphatidylinositol molecule, which is first attached to an *N*-acetylglucosamine residue. The GPI proteins all contain a characteristic carboxyl-terminal signal peptide with a hydrophobic tail, which is split off before the protein with a new carboxyl-terminal is combined with the amino group of the ethanolamine residue of the GPI moiety. A GPI-transamidase complex catalyses the entire process of cleavage and GPI attachment. The palmitate attached to inositol may then be removed before the GPI-anchored proteins are transported to the Golgi. At this stage, the unsaturated fatty acid in position *sn*-2 of the glycerol moiety is removed by the action of phospholipase A_2 to form a lyso-GPI-protein, and this is re-acylated with a saturated acid (26:0 in yeast and mainly 18:0 in mammalian cells). The remodelling of the glycan side-chain by removal of an ethanolamine phosphate residue and addition of an *N*-acetylgalactosamine can also occur [16,17]. The re-modelled GPI-anchored protein containing two saturated fatty acids is finally transferred to the outer leaflet of the plasma membrane.

uPAR anchoring to glycosylphosphatidylinositol (GPI):

uPAR is anchored in the plasma membrane by a glycosylphosphatidylinositol (GPI) moiety and it contains 283 amino acids in its processed form, which is designated GPI-anchored uPAR (GPI-uPAR) in some research articles. The protein consists of three domains, and each domain contains approximately 90 amino acids connected by linker regions of 15–20 amino acids each [18]. The N-terminal domain 1 is needed for the binding of uPA as well as vitronectin, but the entire uPAR is required for highaffinity binding of both ligands. The GPI anchor is composed of a lipid moiety linked to the protein through a phosphodiester bond and a carbohydrate moiety. Therefore, suPAR found in blood is thought to be shed from the cell surface by either cleavage of the glycolipid anchor by GPI specific phospholipase D or polypeptide cleavage mediated by proteases cleaving the protein proximal to the C-terminus [19].

Fig.1 : uPA and receptor actions

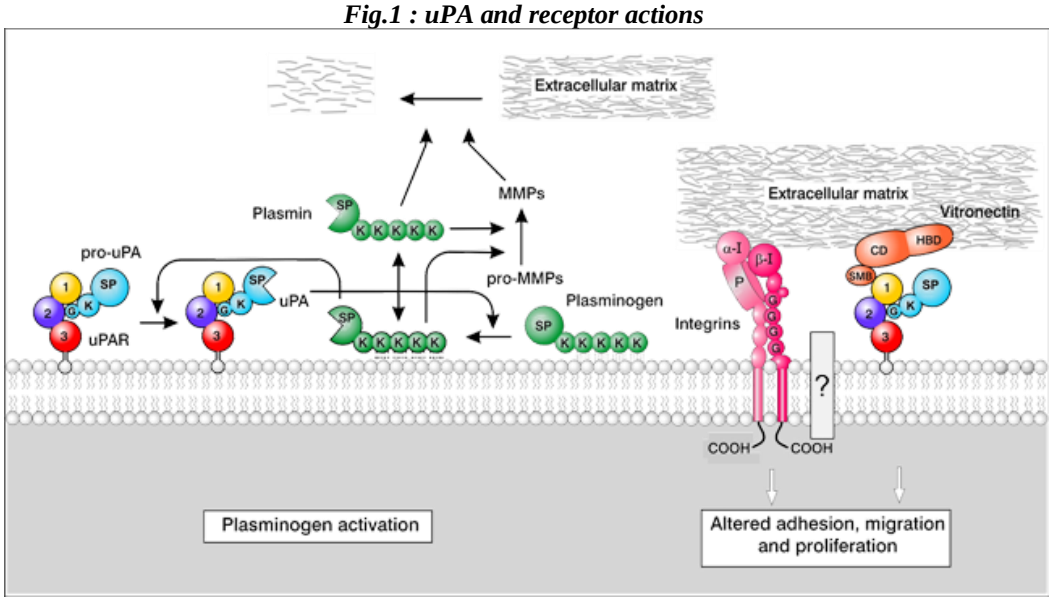

Fig.1 Courtesy : Kjaergaard M, Hansen LV, Jacobsen B, Gardsvoll H, Ploug M. Front Biosci. 2008 May 1;13:5441-61.

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Fig.2 Sequence of GPI anchored uPAR (Length: 335, Molecular mass: 36.97 kDa), Courtesy UnitProKB, EMBL, Hiedelberg, Germany

10	20	30	40	50
MGHPPLLPLL	LLLHTCVPAS	WGLRCMQCKT	NGDCRVEECA	LGQDLCRTTI
60	70	80	90	100
VRLWEEGEEL	ELVEKSCTHS	EKTNRTLSYR	TGLKITSLTE	VVCGLDLCNQ
110	120	130	140	150
GNSGRAVTYS	RSRYLECISC	GSSDMSCERG	RHQSLQCRSP	EEQCLDVVTH
160	170	180	190	200
WIQEGEEGRP	KDDRHLRGCG	YLPGCPGSNG	FHNNDTFHFL	KCCNTTKCNE
210	220	230	240	250
GPILELENLP	QNGRQCYSCK	GNSTHGCSSE	ETFLIDCRGP	MNQCLVATGT
260	270	280	290	300
HEPKNQSYMV	RGCATASMCQ	HAHLGDAFSM	NHIDVSCCTK	SGCNHPDLDV
310		320		330
QYRSGAAPQP GPAHLSLTIT LLMTARLWGG TLLWT				

Urokinase and its role in multiple respiratory diseases:

Urokinase and its receptor may be critical in the pathophysiology of respiratory disease and in general the regulation of respiration and its rhythmogenesis. Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation, proliferation and apoptosis require transmembrane signaling, which is mediated by direct contacts of uPAR with a variety of extracellular proteins and membrane receptors. There is enough evidence suggesting its role in a variety of respiratory diseases for e.g COPD, asthma, pneumonia and even pulmonary tuberculosis. In a study conducted by Wang *et al* it was found that the markers of active epithelial-mesenchymal transition (EMT) and existence of uPAR were highly elevated in the small airway epithelium of patients with COPD than those of controls and also witnessed a significant association of uPAR[20]. It was reported that suPAR is increased in the sputum in such pulmonary diseases as asthma, COPD and cystic fibrosis. In another study performed by Brooks *et al*., lower molecular weight structures of uPAR were demonstrated to be in peripheral neutrophils, while higher molecular weight structures are seen more frequently in lung eosinophils [21]. In a study performed by Zhang *et al*., it was found that uPA, uPAR and PAI-1 are at a significantly higher rate in lung cells and pulmonary macrophages in patients with COPD than those of controls, and significant inverse correlations were observed between lung function, and uPA, uPAR and PAI-1 [22]. This study also demonstrated that the system of uPA is seen at different levels in lung tissues of COPD patients from those of control smokers and nonsmokers. In a study by Rijneveld *et al*., it was determined that mice deficient in uPAR are more sensitive to pneumococcal pneumonia owing to an inhibition of neutrophil recruitment into the inflamed lung. uPAR is needed to recruit adequate neutrophils into alveoli and lungs during pneumonia led by S. pneumonia [23].

Urokinase a critical molecule of the 'pulmonary-renal cascade':

This author alongwith his post graduate students had proposed the existence of a neurohumoral cascade in humans which was named the 'Pulmonary renal cascade' (Tyagi *et al*, 2004) [24,25]. This cascade comprises mainly of Urokinase, Erythropoietin, Heparin binding growth factors [26,27] and Protease nexin-1 secreted into the blood working in tandem with pulmonary system and the modulation by pulmonary disease and hypo or hyperventilation, gasping etc., affecting the function of this neurohumoral cascade regulating respiration, coagulation, hemopoiesis and skeletal muscle contractility. The recent research on Urokinase, Erythropoietin and heparin binding growth factors has only substantially strenghthened the existence of this circuitry and contribution to the various pathophysiological roles in human body.

Conclusion

It is now evident from the recent studies that Urokinase is not just a plasminogen activator and is important for maintaining the pulmonary/renal function but has multifaceted roles to play as an important constituent of the 'Pulmonary-renal cascade'. The plethora of evidence now suggest to its important role in patho-physiology of various disorders. The characterization of the different Urokinase receptors has added a new dimension and the possibility of targeting these membrane bound or soluble receptors with either specific antagonists or antibodies is a distinct possibility.

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