Cell. Mol. Life Sci. 64 (2007) 1033 – 1037
1420-682X/07/091033-5
DOI 10.1007/s00018-007-6498-z

Cellular and Molecular Life Sciences

Visions & Reflections (Minireview)

Does the urokinase receptor exist in a latent form?

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Received 22 November 2006; received after revision 8 January 2007; accepted 7 February 2007
Online First 19 March 2007

Abstract. Multiple cellular functions of urokinase and its receptor are associated with the receptor's capability to interact with a number of ligands at the molecular level.The presence of urokinase is generally needed for the urokinase receptor to acquire this capability. Recent X-ray studies of the structure of the urokinase receptor in complex with either its ligand or peptide inhibitors demonstrate the flexibility of the domain organization of the receptor, suggesting that unliganded urokinase receptor may exist in a latent form that has a conformation different from its ligand-binding form.

Keywords. Urokinase receptor, urokinase, latent form.

Introduction

Urokinase-type plasminogen activator receptor (uPAR) is expressed mainly on the surface of peripheral blood cells: monocytes, granulocytes, activated lymphocytes, and many non-hematopoietic cells, such as vasal endothelial cells, fibroblasts, keratinocytes, and several tumor cell lines. Together, urokinase plasminogen activator (uPA) and uPAR constitute one of the two primary endogenous systems that mediate plasminogen activation. uPA-uPAR is also involved in other general cellular functions and in subsequent diverse pathophysiological processes, such as tissue remodeling, arteriosclerosis, and tumor biology [1]. uPAR can bind with uPA at high affinity (K_d 0.1–1 nM), thus localizing the generation of plasmin from plasminogen to pericellular regions of a variety of cells. Besides uPA, uPAR is also capable of interacting with several other ligands, including vitronectin (VN), integrins, G-protein-coupled receptors, and uPAR-associated protein (uPARAP) [1–4]. This capacity to interact with multiple ligands may be related to its broad general cellular function. Recent crystal structures of uPAR-uPA complexes show that uPA plays an important role in stabilizing uPAR into its ligand-binding conformation, and also suggest that unliganded uPAR may have a distinct conformation and may exist in a latent form. This result is consistent with previous functional studies of uPAR.

Structure of uPAR in its uPA-binding form

The uPAR gene consists of seven exons and six introns with a length of 23 kb [5]. The primary translated product includes a polypeptide of 313 amino acid residues and a signal peptide of 21 amino acid residues. The mature uPAR is a highly glycosylated glycoprotein anchored on the cell surface through a glycophosphatidylinositol (GPI) that is linked to residue Gly283 of uPAR [6, 7]. Soluble uPAR variants

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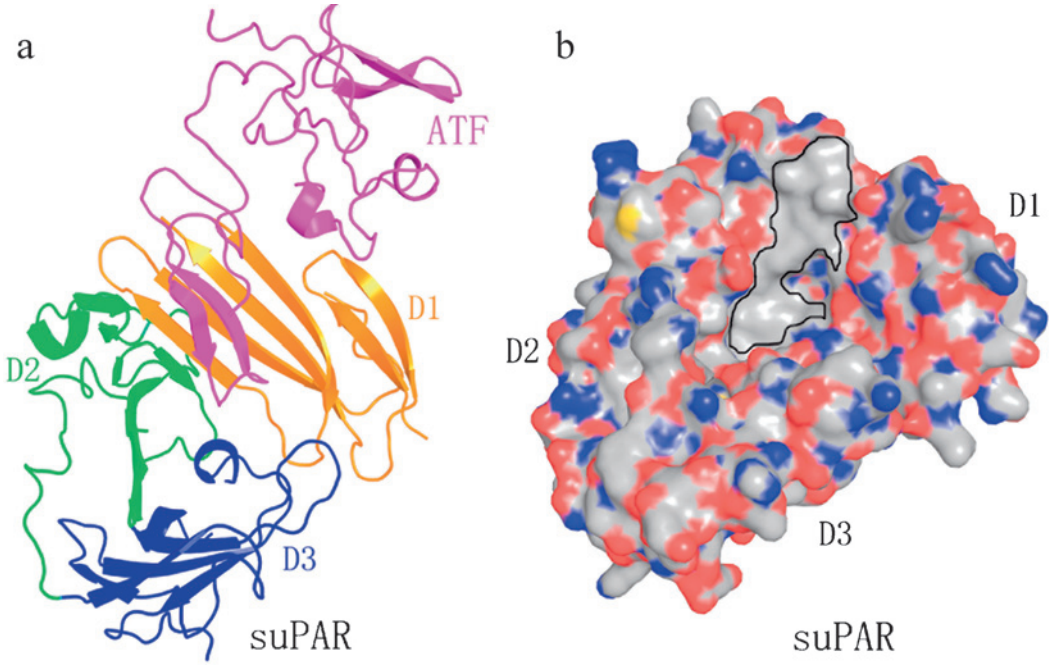

Figure 1. (a) The structure of the suPAR-ATF complex. The DI domain of suPAR is shown in orange, the DII domain in green, and the DIII domain in blue. The ATF is shown in magenta. (b) Surface representation of the suPAR. The circled areas are high hydrophobic region in the DI domain. Carbon, oxygen, nitrogen and sulfur atoms are shown in grey, red, blue, and yellow, respectively.

(suPAR), consisting of residues 1–277 without the GPI anchor, have been identified under physiologic conditions and in pathological conditions such as in patients with malignancy [8] or paroxysmal nocturnal hemoglobinuria [9]. Some differences between suPAR and GPI-uPAR have been noted. For example, purified GPI-anchored uPAR is much more susceptible to uPA-mediated cleavage than suPAR, and an antibody to the peptide AVTYSRSRYLE, amino acids 84–94 of uPAR, recognizes GPI-uPAR but not suPAR [10]. However, suPAR binds to exogenous uPA with a Kd in the sub-nanomolar range, indistinguishable from the cell surface GPI-anchored full-length uPAR [11].

The recently determined structures of suPAR in complex with peptide inhibitor [12], the urokinase amino-terminal fragment (ATF) and an antibody [13], and ATF [14] show that the structure of suPAR consists of 17 antiparallel β strands and three short α helices to form three domains (DI, DII, DIII) connected by short peptides (Fig. 1a). Each domain has almost 90 amino acid residues and forms a so-called three-finger fold [11], which has three adjacent loops forming a flat antiparallel β sheet, emerging from a small globular core stabilized by four conserved disulfide bonds (the DI domain has only three disulfide bonds). DI and DII of suPAR have six β strands, whereas DIII has five β strands. The three consecutive domains of suPAR pack closely to each other, resulting in a cone-shaped cavity surrounded by these three domains. The cavity has a wide opening (25 Å) and significant depth (14 Å). ATF inserts its growth factor domain into the uPAR cavity and makes contact with all three uPAR domains.

Effect of uPA on the structure of suPAR

The structural studies also reveal the flexibility of the suPAR structure. Comparison of suPAR between the suPAR-ATF-Fab [13] and suPAR-peptide [12] structures indicates that the DI domain rotates by 20.5°, while the DII and DIII domains remain in the same orientations, suggesting the flexibility of suPAR interdomain organization. The individual domains of suPAR also show some degree of local flexibility, even though no global changes of the individual uPAR domains were observed between these two structures. Residues 131–136 in the βIIC-βIID loop (see [12] for the nomenclature of the secondary structural elements of uPAR) and the connection peptide residues 84–90 between DI and DII were disordered in suPAR in the suPAR-ATF-Fab and suPAR-peptide structures. Residues 35–37 in the βIC-βID loop and 249–251 in the βIIIC-βIIID loop of suPAR were also not found in the ternary complex, suggesting flexibility in those areas.

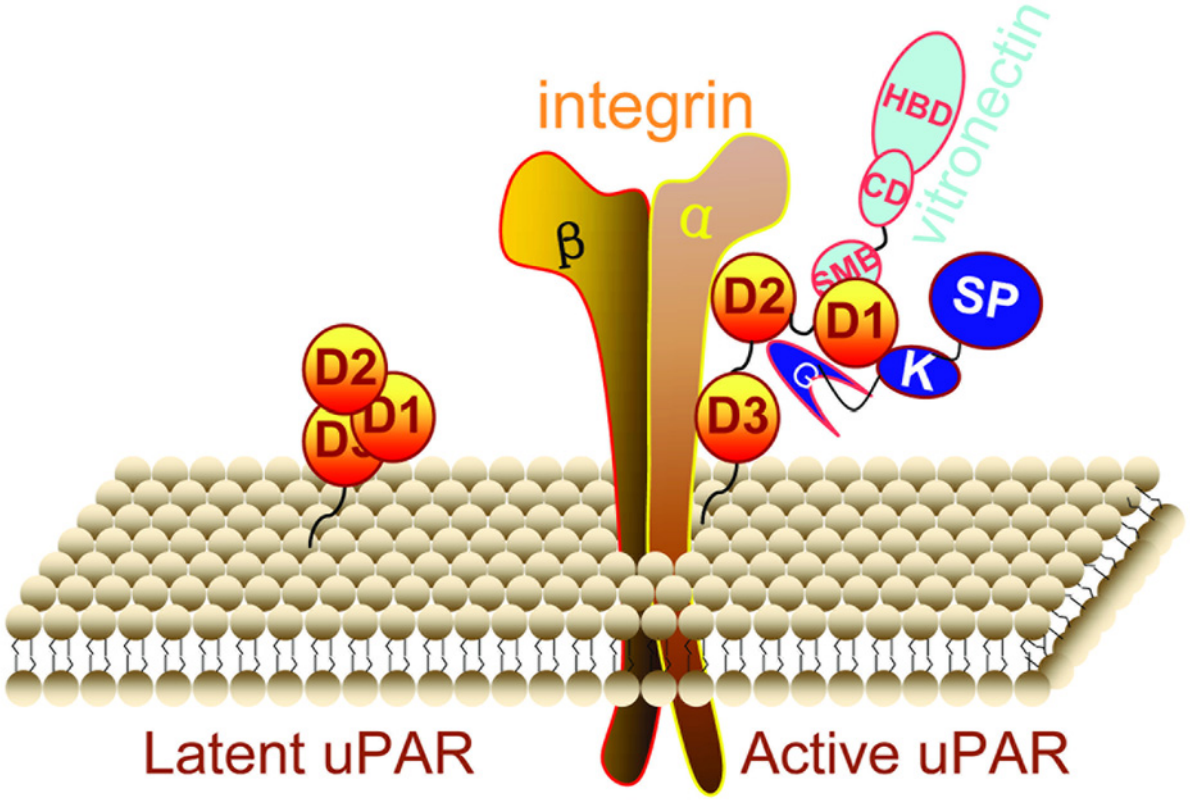

Figure 2. The existence of ligand (uPA) is required in many instances for urokinase receptor (uPAR) to interact with other uPAR ligands (e.g., vitronectin and integrin, right panel). Unliganded uPAR may adopt a very distinct conformation and exist in a latent form (left panel), not fully functional. Soluble uPAR has three domains (DI–DIII); uPA also consists of three domains: growth factor domain (G), kringle domain (K), and serine protease domain (SP).

The interface between suPAR and ATF is quite large (1171 Å^2) and composed of six hydrogen bonds and extensive hydrophobic interactions [13]. A distinguished feature of the ATF-bound uPAR structure is a large hydrophobic patch on one side of its central ligand-binding cavity (Fig. 1b), which consists of the hydrophobic residues I28, V29, L31, L38, L40, V41, L55, I63, L66, I96, L113, V125, L144, L150, and L168. Exposure of a large hydrophobic area to aqueous environment is energetically disfavored. In the ATF-bound form, this hydrophobic patch is covered up by interacting with ATF. How will this hydrophobic patch be sequestered in the absence of uPA? Most likely, suPAR undergoes rearrangement in its ligand-free conformation and differs from its ligand-binding conformation (Fig. 2). Thus, the ligand may play an important role in stabilizing the structure of suPAR into its active conformation.

Effect of uPA on the interaction of uPAR with other ligands.

uPA binding increases the interaction between uPAR and vitronectin.

Vitronectin is a multifunction glycoprotein produced in the liver and present at high concentration in plasma [15]. It binds to uPAR through its NH2-terminal somatomedin B-domain (SMB) [16]. Interaction between uPAR and vitronectin is necessary for endothelial cell invasion and migration at vitronectin-rich extracellular matrix sites.

Many cellular experiments showed that binding of uPA increases the interaction between uPAR and vitronectin [17–20]. This enhancement depends on the presence of the ATF of uPA [17–20]. Recent biochemical experiments [21] also show that vitronectin binding to suPAR can be increased more than 1000-fold in the presence of pro-uPA.

uPA binding increases the interaction between uPAR and integrin.

uPAR lacks a transmembrane domain but is capable of mediating cellular signal transduction events, leading to its functions of cell proliferation, chemotaxis, adhesion, and migration [1]. It was thought that the signaling functions of uPAR are mediated by the interaction with other cellular partners, e.g., integrins. Indeed, it has been shown that uPAR can interact with β1, β2, β3, and β5 integrins [2, 22, 23].

Some *in vivo* studies suggest that uPAR-mediated recruitment of lymphocytes that contain β2 integrin to mice lung was independent of [24, 25] or even inhibited [26] by the presence of its natural ligand uPA. However, more studies point to the positive role of receptor occupancy on enhancing interaction between uPAR and integrins. uPA promotes coprecipitation of β1 integrins with uPAR [27]. Soluble uPAR binds to purified α3β1 integrin only in the presence of uPA [28]. uPAR on monocyte surfaces was found to bind directly to an immobilized peptide M25 derived from αMβ2, but such binding was significantly enhanced in the presence of uPA [29]. Treatment of HT1080 human fibrosarcoma and MCF-7 human breast adenocarcinoma cell lines with uPA promotes the physical association of uPAR with αvβ5, which is required for uPAR-directed cell migration [23]. It was shown that uPA may make direct contact with integrins besides uPAR [30, 31]. However, these experiments also show that the growth factor domain of uPA, which binds to uPAR, increases the binding capability of uPAR to integrins, confirming the positive role of receptor occupancy by uPA on integrin binding.

Interaction between uPAR and thrombospondin [32] is enhanced in the presence of uPA, leading to promotion of tumor cell invasion and development of metastasis in different adenocarcinoma models [33, 34]. Likewise, interaction of uPAR with uPARAP is mediated by uPA [3]. The addition of uPA to MDA-MB-231 breast cancer cells containing uPARAP leads to a clustering of uPARAP and uPAR at the leading edge of the polarized cells [35].

Binding of uPA to uPAR is also needed for receptor internalization. Only when bound to uPA and PAI-1 can uPAR interact with LDL receptor-related protein (LRP) through the uPAR DIII domain and induce endocytosis of the quaternary complex, leading to clearance of uPA-PAI-1 and regeneration of unoccupied uPAR [36]. In the presence of uPA, uPAR interacts with uPARAP and may be involved in clearance of the uPAR-uPA complex [37].

Structure of uPAR in ligand-free form

There is as yet no crystal structure of uPAR in its ligand free form. However, several lines of evidence from structural and functional studies suggest that unliganded uPAR may adopt a conformation so distinct from its ligand-bound conformation that it may be worthwhile to describe unliganded uPAR as a 'latent' conformation. This also highlights the effect of uPA on the structure and function of uPAR.

The structural mechanism of the uPA effect on uPAR-ligand interaction is unknown. Structurally, unliganded uPAR may undergo a major conformational change to sequester the hydrophobic patch and thus may lose its central cavity. This could change the conformation of the outer surface of uPAR, and thus its ligand-binding properties. In the absence of uPA, other partners with hydrophobic features could also occupy the central cavity of uPAR to sequester the hydrophobic patch. A crystal structure of unliganded soluble uPAR or GPI-uPAR will facilitate understanding of the structural mechanism of the uPA effect on its receptor.

Acknowledgements. We thank the NSFC (30430190) for financial support.

1 Blasi, F. and Carmeliet, P. (2002) uPAR: a versatile signalling orchestrator. Nat. Rev. Mol. Cell Biol. 3, 932 – 943.
2 Kugler, M. C., Wei, Y. and Chapman, H. A. (2003) Urokinase receptor and integrin interactions. Curr. Pharm. Des. 9, 1565 – 1574.
3 Behrendt, N. (2004) The urokinase receptor (uPAR) and the uPAR-associated protein (uPARAP/Endo180): membrane proteins engaged in matrix turnover during tissue remodeling. Biol. Chem. 385, 103 – 136.
4 Ploug, M. (2003) Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. Curr. Pharm. Des. 9, 1499 – 1528.
5 Casey, J. R., Petranka, J. G., Kottra, J., Fleenor, D. E. and Rosse, W. F. (1994) The structure of the urokinase-type plasminogen-activator receptor gene. Blood 84, 1151 – 1156.
6 Ploug, M., Kjalke, M., Ronne, E., Weidle, U., Hoyerhansen, G. and Dano, K. (1993) Localization of the disulfide bonds in the NH2-terminal domain of the cellular receptor for human urokinase-type plasminogen-activator – a domain-structure belonging to a novel superfamily of glycolipid-anchored membrane-proteins. J. Biol. Chem. 268, 17539 – 17546.
7 Roldan, A. L., Cubellis, M. V., Masucci, M. T., Behrendt, N., Lund, L. R., Dano, K., Appella, E. and Blasi, F. (1990) Cloning and expression of the receptor for human urokinase plasminogen activator, a central molecule in cell surface, plasmin dependent proteolysis. EMBO J. 9, 467 – 474.
8 Pappot, H., Hoyer-Hansen, G., Ronne, E., Hansen, H. H., Brunner, N., Dano, K. and Grondahl-Hansen, J. (1997) Elevated plasma levels of urokinase plasminogen activator receptor in non-small cell lung cancer patients. Eur. J. Cancer 33, 867 – 872.
9 Ronne, E., Pappot, H., Grondahl-Hansen, J., Hoyer-Hansen, G., Plesner, T., Hansen, N. E. and Dano, K. (1995) The receptor for urokinase plasminogen activator is present in plasma from healthy donors and elevated in patients with paroxysmal nocturnal haemoglobinuria. Br. J. Haematol. 89, 576 – 581.

10 Hoyer-Hansen, G., Pessara, U., Holm, A., Pass, J., Weidle, U., Dano, K. and Behrendt, N. (2001) Urokinase-catalysed cleavage of the urokinase receptor requires an intact glycolipid anchor. Biochem. J. 358, 673 – 679.
11 Ploug, M. and Ellis, V. (1994) Structure-function relationships in the receptor for urokinase-type plasminogen-activator – comparison to other members of the Ly-6 family and snake-venom alpha-neurotoxins. FEBS Lett. 349, 163 – 168.
12 Llinas, P., Le Du, M. H., Gardsvoll, H., Dano, K., Ploug, M., Gilquin, B., Stura, E. A. and Menez, A. (2005) Crystal structure of the human urokinase plasminogen activator receptor bound to an antagonist peptide. EMBO J. 24, 1655 – 1663.
13 Huai, Q., Mazar, A. P., Kuo, A., Parry, G. C., Shaw, D. E., Callahan, J., Li, Y. D., Yuan, C., Bian, C. B., Chen, L. Q., et al. (2006) Structure of human urokinase plasminogen activator in complex with its receptor. Science 311, 656 – 659.
14 Barinka, C., Parry, G., Callahan, J., Shaw, D. E., Kuo, A., Bdeir, K., Cines, D. B., Mazar, A. and Lubkowski, J. (2006) Structural basis of interaction between urokinase-type plasminogen activator and its receptor. J. Mol. Biol. 363, 482 – 495.
15 Schvartz, I., Seger, D. and Shaltiel, S. (1999) Vitronectin. Int. J. Biochem. Cell Biol. 31, 539 – 544.
16 Deng, G., Curriden, S. A., Wang, S. J., Rosenberg, S. and Loskutoff, D. J. (1996) Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? J. Cell Biol. 134, 1563 – 1571.
17 Chang, A. W., Kuo, A., Barnathan, E. S. and Okada, S. S. (1998) Urokinase receptor-dependent upregulation of smooth muscle cell adhesion to vitronectin by urokinase. Arterioscler. Thromb. Vasc. Biol. 18, 1855 – 1860.
18 Wei, Y., Waltz, D. A., Rao, N., Drummond, R. J., Rosenberg, S. and Chapman, H. A. (1994) Identification of the urokinase receptor as all adhesion receptor for vitronectin. J. Biol. Chem. 269, 32380 – 32388.
19 Stahl, A. and Mueller, B. M. (1997) Melanoma cell migration on vitronectin: regulation by components of the plasminogen activation system. Int. J. Cancer 71, 116 – 122.
20 Kanse, S. M., Kost, C., Wilhelm, O. G., Andreasen, P. A. and Preissner, K. T. (1996) The urokinase receptor is a major vitronectin-binding protein on endothelial cells. Exp. Cell Res. 224, 344 – 353.
21 Sidenius, N., Andolfo, A., Fesce, R. and Blasi, F. (2002) Urokinase regulates vitronectin binding by controlling urokinase receptor oligomerization. J. Biol. Chem. 277, 27982 – 27990.
22 Chapman, H. A. (1997) Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. Curr. Opin. Cell Biol. 9, 714 – 724.
23 Carriero, M. V., Del Vecchio, S., Capozzoli, M., Franco, P., Fontana, L., Zannetti, A., Botti, G., D'Aiuto, G., Salvatore, M. and Stoppelli, M. P. (1999) Urokinase receptor interacts with alpha(v)beta(5) vitronectin receptor, promoting urokinase-dependent cell migration in breast cancer. Cancer Res. 59, 5307 – 5314.
24 May, A. E., Kanse, S. M., Lund, L. R., Gisler, R. H., Imhof, B. A. and Preissner, K. T. (1998) Urokinase receptor (CD87) regulates leukocyte recruitment via beta 2 integrins in vivo. J. Exp. Med. 188, 1029 – 1037.
25 Gyetko, M. R., Sud, S., Sonstein, J., Polak, T., Sud, A. and Curtis, J. L. (2001) Antigen-driven lymphocyte recruitment to the lung is diminished in the absence of urokinase-type plasminogen activator (uPA) receptor, but is independent of uPA. J. Immunol. 167, 5539 – 5542.
26 Waltz, D. A., Fujita, R. M., Yang, X., Natkin, L., Zhuo, S., Gerard, C. J., Rosenberg, S. and Chapman, H. A. (2000) Nonproteolytic role for the urokinase receptor in cellular migration in vivo. Am. J. Respir. Cell Mol. Biol. 22, 316 – 322.
27 Yebra, M., Goretzki, L., Pfeifer, M. and Mueller, B. M. (1999) Urokinase-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. Exp. Cell Res. 250, 231 – 240.
28 Wei, Y., Eble, J. A., Wang, Z., Kreidberg, J. A. and Chapman, H. A. (2001) Urokinase receptors promote β1 integrin function through interactions with integrin α3β1. Mol. Biol. Cell 12, 2975 – 2986.
29 Simon, D. I., Wei, Y., Zhang, L., Rao, N. K., Xu, H., Chen, Z., Liu, Q., Rosenberg, S. and Chapman, H. A. (2000) Identification of a urokinase receptor-integrin interaction site. Promiscuous regulator of integrin function. J. Biol. Chem. 275, 10228 – 10234.
30 Pluskota, E., Soloviev, D. A. and Plow, E. F. (2003) Convergence of the adhesive and fibrinolytic systems: recognition of urokinase by integrin alpha(M)beta(2) as well as by the urokinase receptor regulates cell adhesion and migration. Blood 101, 1582 – 1590.
31 Franco, P., Vocca, I., Carriero, M. V., Alfano, D., Cito, L., Longanesi-Cattani, I., Grieco, P., Ossowski, L. and Stoppelli, M. P. (2006) Activation of urokinase receptor by a novel interaction between the connecting peptide region of urokinase and alpha v beta 5 integrin. J. Cell. Sci. 119, 3424 – 3434.
32 Higazi, A. A. R., Upson, R. H., Cohen, R. L., Manuppello, J., Bognacki, J., Henkin, J., McCrae, K. R., Kounnas, M. Z., Strickland, D. K., Preissner, K. T., et al. (1996) Interaction of single-chain urokinase with its receptor induces the appearance and disappearance of binding epitopes within the resultant complex for other cell surface proteins. Blood 88, 542 – 551.
33 Albo, D. and Tuszynski, G. P. (2004) Thrombospondin-1 upregulates tumor cell invasion through the urokinase plasminogen activator receptor in head and neck cancer cells. J. Surg. Res. 120, 21 – 26.
34 Albo, D., Berger, D. H., Rothman, V. L. and Tuszynski, G. P. (1999) Role of urokinase plasminogen activator receptor in thrombospondin 1-mediated tumor cell invasion. J. Surg. Res. 82, 331 – 338.
35 Sturge, J., Wienke, D., East, L., Jones, G. E. and Isacke, C. M. (2003) GPI-anchored uPAR requires Endo180 for rapid directional sensing during chemotaxis. J. Cell Biol. 162, 789 – 794.
36 Czekay, R.-P., Kuemmel, T. A., Orlando, R. A. and Farquhar, M. G. (2001) Direct binding of occupied urokinase receptor (uPAR) to LDL receptor-related protein is required for endocytosis of uPAR and regulation of cell surface urokinase activity. Mol. Biol. Cell 12, 1467 – 1479.
37 Nielsen, B. S., Rank, F., Engelholm, L. H., Holm, A., Dano, K. and Behrendt, N. (2002) Urokinase receptor-associated protein (uPARAP) is expressed in connection with malignant as well as benign lesions of the human breast and occurs in specific populations of stromal cells. Int. J. Cancer 98, 656 – 664.