

Theme Issue Article

The urokinase receptor and the regulation of cell proliferation

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Summary

The urokinase receptor is a multifunctional receptor modulating both proteolytic dependent and independent processes. It binds the extracellular proteolytic enzyme urokinase and engages lateral interactions with several transmembrane receptors, including integrins and the EGFR. Both, by initiating a proteolytic cascade acting on the extracellular matrix components, and by regulating the activity of important signal transducers,

uPAR participates not only in the modulation of cell-cell and cell-extracellular matrix interactions, but also in the control of extracellular signals determining the proliferative state of a cell. Alteration of such a complex and finely modulated mechanism results in unregulated cell proliferation and altered tissue organization, typically associated with tumor progression.

Keywords

Urokinase receptor, proliferation, tumors, metastasis

Thromb Haemost 2005; 93: 641-6

The uPA/uPAR system

The urokinase receptor (uPAR) was first identified as the cell surface receptor for urokinase (uPA) in 1985 (1, 2). uPAR is a heavily glycosylated GPI-anchored protein (3) formed by three cysteine-rich CD59-like extracellular domains (D1, D2, and D3) connected by short linker regions (4). The amino-terminus of the receptor, domain D1, is the primary site for the binding of uPA, whose binding affinity is increased by the presence of both D2 and D3 (5). The linker region between D1 and D2 is by far the most protease-sensitive region of uPAR. Indeed, uPAR can be cleaved by various proteases including uPA giving a shorter form of uPAR (D2D3), which no longer binds uPA (6–11). The conformational change induced by uPA binding and/or the site-specific proteolytic cleavage exposes a specific sequence between domains D1 and D2 of uPAR that can induce migration through FPRL1 (12–14).

uPA converts the zymogen plasminogen to the serine protease plasmin, which is involved in the degradation of the extracellular matrix by direct digestion or activation of other zymogens such as pro-metalloproteases. Because of its ability to bind uPA, it was first believed that uPAR only served to focus the proteolytic cascade initiated by uPA at the leading edge of a migrating cell. However, in recent years, various types of evidence have indicated that uPA binding to uPAR induces both proteolysis-dependent and independent intracellular signaling affecting cell adhesion, migration, and proliferation in a variety of cells (15). Several signaling pathways have been reported to be activated by the uPA/uPAR system. Focal adhesion kinase (FAK),

extracellular signaling regulated kinase (ERK), src-like tyrosin kinase, MLC-kinase, members of the Jak/Stat pathway and scaffolding protein p130Cas have been reported to be phosphorylated upon uPA binding to uPAR in several cell types (16). In this review, we will focus on the different mechanisms by which the uPA/uPAR system may modulate cell proliferation and in particular we will analyze it in the context of tumor progression.

uPAR and cancer

Both uPAR and uPA are overexpressed in several human tumors including leukemias, tumors of the breast, lung, bladder colon, liver, pleura, pancreas and brain (17–27). Several pieces of experimental evidence support the importance of the uPA/uPAR system in cancer with respect to its ability to modulate cell migration and cell adhesion and therefore determine the invasive and metastatic properties of tumor cells both *in vitro* and *in vivo* (28). Tumor angiogenesis, a necessary event in tumor progression to sustain tumor growth and metastasis dissemination, is also modulated by the uPA/uPAR system (29–31). It requires finely regulated cell proliferation, differentiation and migration. After activation, endothelial cells express an increased amount of uPA and uPAR at their leading migratory front to modulate extracellular matrix degradation, re-deposition and cell adhesion (15).

In addition to its role in cell migration and cell adhesion, because of its overexpression in most tumor cell lines and human tumors, and of its ability to modulate their growth, uPAR may be speculated to also play a role in modulating cell proliferation at

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Received January 11, 2005

Accepted after revision January 26, 2005

Prepublished online March 7, 2005 DOI: 10.1160/TH05-01-0021

early stages of tumor progression. Indirect evidence comes from a genetic screen for cancer-associated genes in mice bearing the combined loss of the onco-suppressors p16INK4a and p19ARF due to a genetic deficiency of the Cdkn2a locus. These mice are highly prone to lymphomas and fibrosarcomas (32) and infection with MoMuLV induces tumors at a faster rate by insertional mutagenesis. Interestingly, two viral integrations were found to target the uPAR locus in independent tumors, thus candidating uPAR as a potential cancer-associated gene (33). It is interesting to note that both inactivation of tumor suppressor genes and overexpression of uPAR commonly occur in human tumors. Indeed, increased levels of tissue and serum uPAR strongly correlate with a poor prognosis and unfavorable clinical outcome (34).

Another important observation on the role of uPAR in cancer was obtained in a clinical study on the role of micro-metastasis in the prognosis of gastric cancer. Several parameters were investigated, but only the presence and number of uPAR-positive cancer cells in the bone marrow correlated with prognosis. In patients with micro-metastatic uPAR-positive cells, the number of these cells increased in subsequent sampling and the patient had a shorter relapse-free interval. In contrast, patients with few or no uPAR-positive cells had a longer relapse-free period despite the presence of tumor cells in the bone marrow. (35, 36). These observations could suggest a growth-promoting role of uPAR associated with its high expression levels.

Finally, several studies in mice show that the use of antagonists of uPA or uPAR prevent growth, invasiveness and metastasis of tumors (37–40).

uPAR and proliferation

What are the molecular mechanisms behind the growth promoting effect of the uPA/uPAR system? We can distinguish between proteolysis-dependent and independent functions of the uPA/uPAR system. To the first group belong the proteolytic cascade initiated by uPA bound to uPAR and the consequent release and activation of growth factors stored in the extracellular matrix. To the second group can be assigned the signaling systems modulated by the lateral interactions between uPAR and several transmembrane receptors including integrins and the EGFR.

Proteolysis-dependent effects on proliferation

Growth factors release from the ECM

ECM was traditionally viewed as a relatively stable structure with the main function of determining tissue architecture. However, in recent years, the ECM has been shown to play additional roles by serving as a reservoir for growth factors (41) and contributing to processes such as organ formation, growth, and homeostasis through the modulation and/or organization of growth factor bioavailability. Ramirez and Rifkin (42) refer to this new function as the 'instructive role of the insoluble matrix' including under this definition the regulation of cellular activities through both integrin-mediated signaling, and growth factor modulation. An important mechanism for mobilizing ECM components and thereby modulating extracellular signaling

events is proteolysis (43). In the context of cancer progression, the proteolytic remodeling of the ECM is usually seen as a mechanism to remove the physical obstacle that an invasive tumor cell has to surpass, without, however, eliminating the substratum on which the migrating cell moves. High levels of extracellular proteolysis correlate with metastatic potential of cell lines *in vitro* and with tumor progression and patient survival *in vivo* (28). It is now evident that such a strong correlation *in vivo* cannot only derive from ECM degradation even if finely controlled and modulated. Remodeling of the ECM is also associated with release of matrix-bound signaling molecules, controlling several cellular processes including proliferation, survival and apoptosis (43). Examples of matrix proteolytic products modifying cell signaling include activation of latent TGF- β by cell surface localized matrix metalloproteinase-2 and -9 (MMPs-2 and -9; 44) or plasmin (45), release of perlecan-bound FGF by MMPs-1 and -3 (46), regulation of IGF activity through MMP-induced degradation of the cognate IGF-binding proteins, the IGFbps (47), and activation of HGF/SF by uPA and tPA (48–50). In particular, in the case of TGF β and HGF/SF the requirement for uPAR has been demonstrated.

TGF β

TGF β s are secreted from cells as biologically latent complexes, some of which are incorporated into the ECM. In a coculture system of vascular endothelial cells and smooth muscle cells (51) or in a culture of thioglycollate-elicited macrophages stimulated with LPS (52), activation of latent TGF β is mediated by cell surface plasmin formed from plasminogen by urokinase produced by the cells. Conversion of latent TGF β to active TGF β is blocked by adding anti-uPA antibodies to cocultures or by preventing uPA from interacting with its cell surface receptor (53). TGF β plays a dual role in tumor progression. It is a potent growth inhibitor and has tumor-suppressing activity in the early phases of carcinogenesis. During subsequent tumor progression, this essential signaling pathway becomes inactivated and the increased secretion of TGF β by both tumor cells and, in a paracrine fashion, stromal cells, is involved in the enhancement of tumor invasion, angiogenesis and immunosuppression. Furthermore, in some cells, TGF β stimulates cell proliferation rather than inhibiting cell growth. Studies of colorectal carcinomas have documented that high-level expression of TGF β 1 in the primary tumor is associated with advanced tumor stage and is an independent negative prognostic factor (54). Although TGF β 1 overexpression in transgenic mouse keratinocytes inhibits the growth of carcinogen-induced benign skin tumors, this overexpression promotes the progression of advanced lesions to the malignant phenotype (55). Thus, TGF β may play a critical role in the progression of some malignancies by stimulating tumor proliferation both in an autocrine and paracrine fashion in the tumor microenvironment.

HGF

Hepatocyte growth factor/scatter factor (HGF/SF) is a secretory product of stromal fibroblasts, sharing sequence and structural homology with plasminogen. HGF/SF promotes motility, invasion and growth of epithelial and endothelial cells (56). HGF/SF is secreted as a single-chain biologically inactive precursor

(pro-HGF/SF), mostly found in a matrix-associated form. In vitro, pro-HGF/SF is cleaved at a single site by both urokinase-type plasminogen activator and tissue-type plasminogen activator generating the active mature HGF/SF heterodimer which then can function as a mitogenic or differentiation factor by binding and activating its cell surface tyrosin kinase receptor c-Met (48–50). *In vivo*, inactivation of the urokinase plasminogen activator receptor (*uPAR*) gene leads to diminished levels of HGF/SF and a 50–65% reduction in cortical GABAergic interneurons beginning at embryonic day 16.5 (57). Moreover, *uPAR*^{-/-} mouse lacks specific subpopulations of cortical interneurons, displays increased anxiety, and exhibits a novel pattern of spontaneous myoclonic seizures (58). Aberrant HGF/SF and c-Met expression is frequently observed in a variety of human tumors, often in association with progressive disease (59). Paracrine activation of the c-Met receptor by stromal-derived HGF or autocrine activation of c-Met are closely associated with the progression of malignant tumors. Activation of c-Met signaling can lead to scattering, angiogenesis, proliferation, enhanced cell motility, invasion, and eventual metastasis (59).

Animal models: the mouse skin system uPAR overexpression

Overexpression of both uPA and uPAR, but not of either uPA or uPAR in the basal keratinocytes of murine skin resulted in several cutaneous alterations including a large increase in epidermis thickness with up to 24 cell layers compare to the 2–3 layers present in the wild type epidermis (60). Nucleated cells in the cornified layer, also indicated disturbed epidermal differentiation in the uPA/uPAR bitransgenic mice. The phenotype was due to the catalytic activity of uPA and it was accompanied by increased plasminogen activation, up-regulation and activation of MMP-2 and MMP-9 and uPAR cleavage. The requirement for uPA activity was demonstrated by the generation of a bitransgenic mouse overexpressing uPAR and a catalytically inactive uPA, which did not show epidermis hyperproliferation (60). Moreover, the contribute of plasmin-catalyzed extracellular proteolysis was shown by backcrossing the uPA/uPAR bitransgenic mice into a plasminogen-deficient (Plg^{-/-}) background, which completely recovered the skin phenotypes (61).

uPAR deletion

Surprisingly, very little has been done to study tumor growth and dissemination in mice lacking the uPAR gene. The only *in vivo* model tested so far is the transplantation of transformed keratinocytes. Malignant murine keratinocytes (PDVA cells; 62) are cultured on a collagen gel and implanted onto the dorsal muscle fascia of mice. In response to angiogenic stimuli (produced by tumor cells; 63), new blood vessels are formed in the underlying stroma, invade the collagen gel, and reach the malignant epithelial layer. Thereafter, the malignant keratinocytes form tumor sprouts that invade downwards into the granulation tissue. Using this model system, it was demonstrated that the plasminogen activator inhibitor type 1 (PAI-1) promoted tumor angiogenesis (64), not by interacting with VN, but rather by inhibiting proteolytic activity, suggesting that excessive plasmin proteolysis prevents assembly of tumor vessels. To further determine the respective role of the different members of the Plg/plasmin system,

single deficiency of Plg, uPA, tissue-type PA (tPA), uPA receptor, or VN, as well as combined deficiencies of uPA and tPA were tested and only lack of Plg reduced tumor growth and angiogenesis (65). Plasmin proteolysis is therefore essential but must be tightly controlled during tumor angiogenesis, probably to allow vessel stabilization and maturation. In this model the loss of uPAR in the host did not prevent tumor growth, however plasmin activation was required. Therefore, the uPA/uPAR system provided by the tumor cells may be sufficient to activate the host plasminogen and promote tumor angiogenesis and tumor growth.

Proteolysis-independent effects on proliferation

uPAR: a surface signaling receptor ATF

One of the first reported evidence of proteolysis-independent uPA/uPAR-induced signaling comes from *in vitro* work using the aminoterminal fragment of uPA. In addition to a catalytic domain (B-chain, aa 144–411), uPA also contains a noncatalytic N-terminal fragment (ATF, aa 1–143), which can be further subdivided into a growth factor-like domain (GFD, aa 4–43) and a kringle domain. uPA has been demonstrated to bind in a saturable high affinity manner to uPAR by the uPA GFD (66). In 1990, a growth factor-like activity for the osteosarcoma cell line, SaOS-2, was isolated from the conditioned medium of the highly invasive prostate carcinoma cell line PC-3 (67). This factor was shown to be identical to the noncatalytic N-terminal fragment of uPA (ATF) and the mitogenic activity was reproduced using a purified fucosylated growth factor domain, and fucosylation was shown to be required. DFP-uPA was also mitogenic in SaOS-2 cells. No mitogenic activity was observed in normal U-937 cells treated with the uPA forms capable of eliciting a mitogenic response in SaOS-2 tumor cells (68). Moreover, ATF increased c-myc, c-jun, and c-fos gene expression in SaOS-2 cells in a time-dependent manner. This effect of ATF was localized to its growth-factor like domain and pretreatment of SaOS2 cells with the protein tyrosine kinase inhibitor herbimycin and recombinant soluble uPA receptor (uPAR) caused a significant reduction in the ability of ATF to induce c-fos expression (69). It is interesting to note that the SaOS-2 cell line is deficient in at least the Rb tumor suppressor gene.

Integrins and EGFR

Most of the cellular responses modulated by the uPA/uPAR system, including migration, cellular adhesion, differentiation and proliferation (15) require transmembrane signaling, which cannot be mediated directly by a GPI-anchored protein such as uPAR. Several observations show that uPAR transmits an intracellular signal by interacting with extracellular domains of transmembrane proteins. Transmembrane receptors (integrins, EGF-R, caveolin and the G-protein-coupled receptor FPRL1) and cytoplasmic signaling proteins (tyrosine and ser-thr kinases, GTPases, cytoskeletal components and others) can form a complex with and/or participate in uPAR-induced signaling (15, 70).

In the context of tumor proliferation, the best-characterized pathway is the one described by Aguirre-Ghiso and colleagues. They propose that even cancer cells with multiple mutations may use surface receptors and ECM components to regulate signaling pathways that control cell cycle progression and/or arrest (71). They describe an uPAR-dependent mechanism by which the majority of tumor cells modulate the activity ratio between the proliferation inducer ERK (72) and the negative growth regulator p38 (73). Based on the study of 10 different cell lines, their results show how uPAR and $\alpha 5\beta 1$ activate the EFGFR in an EGF-independent but FAK-dependent manner (74), and generate high ERK and low p38 activity necessary for the *in vivo* growth of cancer cells. A positive loop is activated in which high ERK activity transactivates uPAR and uPA expression (75–78). High uPAR level, by activating $\alpha 5\beta 1$ maintains high ERK activity (79, 74). The cancer cell proliferation loop can be interrupted by a reduction of uPAR level, by cleavage of its domain 1 (important for the uPAR/ $\alpha 5\beta 1$ interaction and activation; 79, 74, 80) or by loss of uPA and/or FN.

Soluble uPAR and cleaved uPAR

Intact uPAR is released from the plasma membrane by glycosylphosphatidylinositol-specific phospholipase D (81) and may also be generated by alternative splicing of the uPAR mRNA (82). In humans, the levels of soluble uPAR (suPAR) found in plasma are increased in patients with malignancy and correlate with cancer progression and poor prognosis (83, 84). However, in xenograft animal model experiments, suPAR reduces the growth and metastasis of MDA-MB 231 breast cancer cells and OV-MZ-6#8 ovarian cancer cells (85, 86).

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Jo et al. (87) investigated the role of suPAR in cell proliferation of tumor cells trying to solve this paradox and proposed that suPAR in a uPA-independent manner may activate or inhibit ERK phosphorylation, depending on the state of the autocrine uPA-uPAR system. In uPAR-deficient cells (uPAR^{-/-} murine fibroblast or human embryonal kidney 293 cells) suPAR functions as a partial signaling agonist that activates ERK. On the contrary, in cells with a potent autocrine uPA-uPAR signaling system (MDA-MB 231 breast cancer cells) suPAR decreases ERK activation by competitive displacement of membrane-anchored uPA-uPAR complex from signaling adaptor proteins, and inhibits proliferation. Moreover, the authors also propose a model in which proteolytic cleavage converts suPAR into a full agonist still able to displace membrane-anchored uPA-uPAR complex, but replacing it with an active signaling molecule, which does not inhibit proliferation. This would be the first evidence involving cleaved uPAR in cell proliferation and not only in cell migration (12–14), thus introducing an additional level of complexity in the uPA/uPAR system.

Conclusions

All above reported data highly suggest a role of uPAR in cell growth regulation and cancer proliferation. Specific molecular mechanisms, which have been described in cell culture, can now be tested in animal systems. The next few years will provide exciting new information and will help to define the level at which uPAR can intervene in tumorigenesis and hence if and how can uPAR become a therapeutic target in cancer.

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