VEGF-initiated angiogenesis and the uPA/uPAR system

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Abbreviations: VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; uPA, urokinase; uPAR, urokinase receptor; PAI-1, plasminogen activator inhibitor; MMP-2, matrix metalloproteinase-2; ECM, extracellular matrix proteins; LRP, LDL receptor-related protein; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; FAK, focal adhesion kinase

Angiogenesis involves a series of tightly regulated cellular processes initiated primarily by the vascular endothelial growth factor (VEGF). The urokinase-type plasminogen activator system, consisting of the urokinase-type plasminogen activator (uPA), its cellular receptor uPAR and its inhibitor PAI-1, participates in the realization of these VEGF-induced processes by activating pericellular proteolysis, increasing vascular permeability and by supporting endothelial cell proliferation and migration.

Stimulation of endothelial cells with VEGF-A, mediated by VEGF receptor 2 (VEGFR-2), activates the plasminogen activator system and pericellular proteolysis. Subsequently, it leads to redistribution of uPAR in a complex with integrin \( \alpha_5\beta_1 \) to focal adhesions at the leading edge of endothelial cells, thereby redistributing the tools enabling matrix degradation and cell invasion. Furthermore, interaction of uPAR with additional cognate ligands and neighboring, signaling competent receptors, as, for example, epidermal growth factor receptor (EGFR) provides further angiogenesis modulating stimuli, all of which are the topic of this short review.

Urokinase Receptor

The urokinase receptor, also known as uPA receptor or uPAR or CD87, is a glycoprotein comprised of three domains\(^1,2\) and tethered to the cell membrane with a glycosylphosphatidylinositol (GPI) anchor.\(^3\) It was originally identified as a saturable binding site for urokinase on the cell surface.\(^4\) uPAR is expressed mainly on monocytes, macrophages, fibroblasts, neuronal, endothelial and smooth muscle cells.\(^5,6\)

Although the traditional role of uPAR is the activation of urokinase at the cell surface leading to plasminogen (Plg) activation, thus generating plasmin,\(^7\) uPAR now has been shown to contribute to a range of proteolysis-independent processes. It can directly bind to vitronectin\(^8-10\) using a site distinct from its uPA-binding domain,\(^11\) and in spite of lacking transmembrane and intracellular domains, it can signal upon uPA or vitronectin ligation and via lateral interactions with signaling competent surface receptors. Thereby, it influences many important processes, such as inflammation, atherosclerosis, tissue remodeling during wound healing, angiogenesis, tumorigenesis and metastasis (for a review, see refs. 12–22).

Angiogenesis

In physiological and pathological blood vessel formation from pre-existing vessels (angiogenesis), new endothelial cells penetrate avascular zones by sprouting from existing blood vessels. This tightly regulated process plays a critical role in a variety of normal physiological events, including menstrual cycle and trophoblast implantation, embryonic development and wound healing.\(^23\)

However, uncontrolled neovascularization can contribute to a number of pathological processes, including tumor growth and metastasis, psoriasis, arthritis and blindness.\(^24\) Therefore, the identification of molecules that regulate angiogenesis, and, in turn, the understanding of how these molecules function during the angiogenic cascade, are major challenges facing researchers in the field of vascular biology.

Vascular endothelial growth factor (VEGF), upregulated rapidly upon hypoxia, is the prime factor in the initiation of angiogenesis.\(^24\) It induces the expression of active proteases on the cell surface, increases vascular permeability,\(^25\) leading to extravascular accumulation of plasma derived matrix proteins, such as fibrinogen and vitronectin, and induces endothelial cell proliferation and migration. In all these aspects of the process of angiogenesis also uPAR has been implicated, as shown in numerous studies.

VEGF Initiates uPA/uPAR Activation and Pericellular Proteolysis

In general, for invasion, cells employ an elaborate, cell surface-based repertoire of proteolytic enzymes allowing cleavage of
extracellular matrix proteins (ECM). In the process of angiogenesis, cells first need to locally degrade the basement membrane to open a path for their migration and invasion. This process is highly controlled by the regulation of plasmin activity.

Urokinase (uPA), the key enzyme of the initial step of pericellular plasmin generation, is produced by cells in its inactive precursor form pro-urokinase (pro-uPA). On the cell surface, pro-uPA is bound to its receptor uPAR via its growth factor domain, allowing receptor-bound conversion of pro-uPA to active uPA. Activated uPA, in turn, cleaves the proenzyme plasminogen yielding active plasmin, which activates prometalloproteinases to active metalloproteinases and can, in a positive feedback loop, activate pro-uPA to uPA (for a review see refs. 13 and 27). Such upregulation of uPA/uPAR might, in addition, also increase vascular permeability by increased degradation of VE-cadherin. Self limitation of the above proteolytic cascade is provided by the release of inhibitors e.g., plasminogen activator inhibitor-1 (PAI-1) during degradation of PAI-1 binding matrix and by subsequent forming of a trimolecular complex of uPAR/uPA/PAI-1, which eventually will become internalized via low-density lipoprotein receptor (LDLR)-like proteins.

VEGF stimulation of endothelial cells leads to induction of surface-associated proteolytic activity within minutes. Via VEGFR-2 and phosphatidylinositol 3-kinase (PI3-kinase)-mediated inactivation of β1 integrins, this stimulation leads to type-1 transmembrane metalloproteinase (MT1-MMP)-mediated activation of matrix metalloproteinase-2 (MMP-2), which, in turn, activates pro-uPA and, thus, the uPA-dependent pericellular proteolysis that fosters cellular invasion. The activation of the proteolytic activity induced by VEGF-A is mediated by VEGFR-2, but not by VEGFR-1. Overall, stimulation of endothelial cells via VEGFR-2 leads to subsequent redistribution of uPAR to the leading edge of endothelial cells, thereby focusing the proteolytic capacity to the invasive front of the cell.

The response of endothelial cells toward VEGF-stimulation is reduced by cell density via density-enhanced, receptor-like tyrosine phosphatase DEP-1. Enhanced levels of DEP-1 expression impairs the mitogen-activated protein kinase (MAPK) pathway activation and, thus, uPAR synthesis, which reduces the angiogenic capacity of cells. Decreasing the levels of uPAR can elevate levels of soluble VEGFR-1, thereby reducing the availability of VEGF.

Besides VEGF, a variety of growth factors, including fibroblast growth factor-2 (FGF-2), epithelial growth factor (EGF) as well as hepatocyte-growth factor (HGF) were recently shown to induce a PI3-kinase-dependent activation of uPAR-bound pro-uPA, leading to endothelial cell migration/invasion. These data support the perception of uPAR as a central organizer of pericellular proteolysis induced by growth factors.

Animal Models Offering Insight to the Role of the uPAR System in Angiogenesis

Generation of mice deficient in components of the uPAR system provided for an opportunity to assess the role of individual components of this system during angiogenesis in vivo. Though embryonic and postnatal vascular development was unaffected in these mice, challenging them revealed the role of the uPAR system in neo-angiogenesis. For example, in uPA-deficient mice, in a model of myocardial infarction, postinfarction myocardial revascularization was severely impaired. In a murine fibrosarcoma model, tumor growth was significantly inhibited and vessels displayed a different neovascular morphology in uPA-deficient as well as in PAI-1-deficient mice. Similarly, tumor growth of subcutaneously injected murine prostate cancer cells was significantly retarded in uPA- or uPAR-deficient mice compared with wild-type mice. Importantly, the inhibition of the uPAR system prevented or reduced the tumor growth and metastasis in animal models. Thus, e.g., inhibition of uPAR by a fusion protein consisting of the receptor-binding N-terminal fragment of uPA and the Fc portion of human IgG, which functions as a high-affinity uPAR antagonist, inhibited basic fibroblast growth factor (bFGF)-induced angiogenesis in subcutaneously injected matrix gel. An adenovirally delivered N-terminal fragment of uPA inhibited tumor angiogenesis and metastasizing in syngeneic xenograft murine tumor models. These data are in line with numerous clinical studies where elevated uPA and uPAR levels are associated with poor prognosis and are strong independent markers in many types of cancer.

Other uPAR Interactions Affecting Angiogenesis

Even though uPAR was discovered and named as an uPA-binding cell surface receptor, ample evidence supports the perception of uPAR as a multifunctional polyvalent receptor. While its function in the regulation of pericellular proteolysis seems to be independent from signaling, other functions of uPAR, including its function as vitronectin receptor (for a review, see ref. 53), require the help of neighboring receptors, like LDL receptor-related protein (LRP), integrins, epidermal growth factor receptor (EGFR) or platelet-derived growth factor receptor (PDGFR), all of which have been implicated also in angiogenesis and whose signaling capacity, via focal adhesion kinase (FAK), Src, Rac, ERK/MAPK and JAK-STAT, uPAR can utilize and also modulate, are discussed below.

uPAR/integrin interactions. The urokinase receptor can laterally interact with adhesion receptors of the integrin family, as first observed in leukocytes for the integrin αMβ2 (Mac-1, CD11b/CD18). Later, in tumor cells, context-dependent association with integrins of both other integrin subfamilies, the β1 and the αv subfamilies, has been observed in dependence on the offered extracellular matrix.

The most convincing proof of uPAR/integrin complexes was provided by the identification of the respective interacting domains in the binding partners. It seems that depending on their specific heterodimer composition, integrins can use either the α- or β- subunit for interaction with uPAR. uPAR itself uses regions either on its domain II or on its domain III for the interaction with the respective integrins. In domain III, e.g., the amino acids S245 and H249 were implicated in the interaction with the fibronectin receptor α5β1, while the

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mutation D262A impaired binding of integrin α3β1, a laminin receptor.  

In endothelial cells, this uPAR/α5β1_interaction site in uPAR domain III is required for angiogenesis, as highlighted by recent work defining uPAR as vehicle for the crucial, VEGF-A-induced, VEGFR-2-mediated, redistribution of integrin α5β1, thus initiating VEGF-induced migration of endothelial cells (Fig. 1). UPAR-peptide aa 243–251, containing both in the uPAR/α5β1_interaction implicated residues S245 and H249, not only blocks the association of the two receptors and their co-internalization via the LRP/clathrin endocytosis pathway but also cell migration and consequently angiogenesis in vivo.  

Thus, stimulation of endothelial cells via VEGFR-2 achieves the distribution of both proteolytic capacity as well as cell matrix interaction capacity. Targeting of interaction sites between uPAR and integrin α5β1 at the cell surface seems to be a promising strategy to inhibit angiogenesis independently from the presence of VEGF.  

As mentioned, also the integrin αvβ3 has been described in an in vitro assay and in tumor cells as possible interaction partner for the urokinase receptor.  

The possible relevance of its complex

![Figure 1. UPAR interactions in angiogenesis: VEGF-A activation of endothelial cells via VEGFR-2 leads to integrin inactivation, MMP- and uPA-activation ending in internalization and redistribution of the receptors. Matrix- and VE-cadherin degradation and integrin engagement support invasion, while kininogen and endostatin interfere with uPAR-mediated endothelial migration. uPAR, urokinase receptor; uPA, urokinase; PAI-1, plasminogen activator inhibitor; VEGFR-2, vascular endothelial growth factor receptor-2; α5β1, integrin α5β1; MT1-MMP, type-1 transmembrane metalloproteinase; MMP-2, matrix metalloproteinase-2; ECM, extracellular matrix proteins; LRP, LDL receptor-related protein; clathrin; VE-cadherin, vascular endothelial cadherin.](image-url)
formation with uPAR in endothelial cells has not been clarified in
spite of the prominent, but also controversial role in angiogenesis
assigned to integrin αvβ3. On the other hand, detailed evidence characterizes integrin αvβ3 as a bona fide receptor
for urokinase-type plasminogen activator. Urokinase bound to integrin αvβ3 activates plasminogen and stimulates signaling and cell migration. Integrin αvβ3 is specifically upregulated during inflammation- and tumor-
induced angiogenesis, suggesting that its pro-angiogenic interaction with uPA and its anti-angiogenic interaction with angiostatin (an anti-angiogenic fragment of plasminogen) provide an additional layer of regulation during the formation of new blood vessels.

uPAR associations with other receptors or factors influencing angiogenesis. Besides the already mentioned interaction with the scavenger receptor LRP, responsible for the internalization of several uPAR complexes, uPAR can also interact with receptor tyrosine kinases like EGFR and PDGFR. During angiogenesis, such interaction would be expected to affect proliferation and survival of angiogenic endothelial and smooth muscle cells.

EGFR-activation and signaling via FAK and ERK was described
to occur in tumor cells, even in the absence of EGF, in response to elevated levels of uPAR expression in combination with fibronectin ligation of integrin αvβ3. Such a complex can play a role also in endothelial cells is demonstrated by a recent publication describing factor XII as an additional association partner for uPAR/integrin/EGFR. The resulting complex induces angiogenesis via ERK1/2 and Akt phosphorylation leading to endothelial growth. Since these effects can be achieved with already about 10% of the serum concentration of factor XII, the physiologic significance deserves to be elucidated.

In vascular smooth muscle cells, uPAR activation by uPA induces the non-receptor tyrosin kinase Janus and Src JAK-STAT pathway, uPAR association with PDGFR-β and its internalization via LR11, a scavenger receptor of the LDLR-family of receptors. Upregulation of uPAR and LR11 results in an increased migration in response to uPA. It is tempting to speculate that, in analogy to the process discussed above for endothelial cells, the effect on migration by uPAR recycling might be enhanced by concomitant, uPAR-mediated integrin redistribution. Yet another signaling complex of uPAR has been described in vascular smooth muscle cells. This complex is comprised of nucleolin, uPAR and casein kinase 2 and is responsible for the uPA-related mitogenic response.

As an example of a direct anti-angiogenic role of uPAR in endothelial cells, uPAR, by forming a complex with integrin αvβ3, nucleolin and endostatin, facilitates the internalization and nuclear translocation of nucleolin-bound endostatin, an anti-angiogenic fragment derived from collagen XVIII.

On the other hand, examples for indirect anti-angiogenic activities by uPAR function are the anti-angiogenic capacity of cleaved kininogen, recognized recently as uPAR mediated. By interfering with the uPA/uPAR complex, by inhibition of ERK activation and by blocking uPAR internalization cleaved kininogen leads to motility arrest and endothelial cell death. Another novel ligand for uPAR, sushi repeat protein X-linked 2 (SRPX2), has been first described as interaction partner of uPAR in the brain and later on in endothelial cells where it exerts an anti-angiogenic effect when binding to uPAR. A different mode of control is provided by soluble mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R), which binds to plasminogen rendering it inaccessible to activation by uPA/uPAR. Finally, combined downregulation of uPAR and the uPA-activatable matrix metalloproteinase-9 (MMP-9), increases sensitivity to irradiation and leads to G2/M cell cycle arrest in medulloblastoma.

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