CMLS, Cell. Mol. Life Sci. 57 (2000) 25–40
1420-682X/00/010025-16 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

The plasminogen activation system in tumor growth, invasion, and metastasis

P. A. Andreasen*, R. Egelund and H. H. Petersen

Cellular Protein Science Laboratory, Department of Molecular and Structural Biology, Aarhus University, 10C Gustav Wied's Vej, 8000 Aarhus C (Denmark), Fax + 45 8612 3178, e-mail: pa@mbio.aau.dk

Abstract. Generation of the serine proteinase plasmin from the extracellular zymogen plasminogen can be catalyzed by either of two other serine proteinases, the urokinase- and tissue-type plasminogen activators (uPA and tPA). The plasminogen activation system also includes the serpins PAI-1 and PAI-2, and the uPA receptor (uPAR). Many findings, gathered over several decades, strongly suggest an important and causal role for uPA-catalyzed plasmin generation in cancer cell invasion through the extracellular matrix. Recent evidence suggests that the uPA system is also involved in cancer cell-directed tissue remodeling. Moreover, the system also supports cell migration and invasion by plasmin-independent mechanisms, including multiple interactions between uPA, uPAR, PAI-1, extracellular matrix proteins, integrins, endocytosis receptors, and growth factors. These interactions seem to allow temporal and spatial reorganizations of the system during cell migration and a selective degradation of extracellular matrix proteins during invasion. The increased knowledge about the plasminogen activation system may allow utilization of its components as targets for anti-invasive therapy.

Key words. Serine proteinases; serpins; integrins; cell migration; anti-invasive therapy.

Introduction

For several decades, it has been assumed that plasminogen activation plays an important role in tumor invasion and metastasis. The basic idea was that plasminogen activators released from cancer cells catalyze the proteolytic conversion of the inactive zymogen plasminogen to the active proteinase plasmin, which in turn catalyzes degradation of proteins in basement membranes and extracellular matrix (ECM) and thus facilitates cancer cell invasion into the surrounding tissue [for reviews see refs 1–3]. There are two types of plasminogen activators, the urokinase-type (uPA) and the tissue-type (tPA). uPA is generally agreed to be the enzyme of most relevance to tumor biology, while the primary role of tPA is generation of plasmin for fibrinolysis in blood vessels. There are two main plasminogen activator inhibitors, PAI-1 and PAI-2. The uPA receptor (uPAR) is a cell membrane-anchored uPA-binding protein, concentrating plasminogen activation activity at the cell surfaces [for a review see ref. 2].

Based on evidence from experimental invasion and metastasis models and on expression patterns for components of the uPA system in tumors and normal tissues, it now seems beyond reasonable doubt that this enzyme system plays a central role in tumor biology. The system appears to be involved not only in cancer cell migration and invasion, but also in other processes in tumors which may collectively be called cancer cell-directed tissue remodeling. Examples of such processes are angiogenesis [for reviews see refs 4, 5] and desmoplasia, i.e., stimulation of fibroblast proliferation and ECM protein synthesis [for reviews see refs 6, 7]. Such processes, although involving migration and invasion by noncancer cells, may strongly influence tumor growth, invasion, and metastasis. It has also been realized that the system has plasmin-independent functions, related to signal transduction and interactions of PAI-1 and uPAR with integrins and the ECM protein vitronectin and of plasminogen activator-inhibitor complexes with endocytosis receptors of the low-density lipoprotein receptor (LDLR) family. This evidence was to a large extent obtained with noncancer cells, but no

* Corresponding author.

qualitative differences exist between cancer cells and normal cells such as endothelial cells with respect to the basic processes of migration and invasion [for reviews see refs 1, 3].

In this review, we will describe the recent rapid increase in knowledge about the biochemistry, molecular biology, and cell biology of plasminogen activation in relation to tumor growth, invasion, and metastasis. Rather than being comprehensive, our review will cover specific areas undergoing rapid development. For other and earlier developments in the field, the reader is referred to other reviews [1–3, 8–16].

Biochemical properties of plasmin, uPA, and tPA

The serine proteinase plasmin (M_r approximately 90,000) consists of two disulfide bond-linked polypeptide chains. The N-terminal A chain contains five so-called kringle domains. The C-terminal B chain contains a typical serine proteinase domain, which is responsible for catalytic activity (fig. 1). Plasmin catalyzes the hydrolysis of peptide bonds on the C-terminal side of Lys and Arg residues. Plasminogen is the one-chain zymogen counterpart of plasmin, with an activity 10^4- to 10^6-fold lower than that of plasmin. Conversion of plasminogen to plasmin, comprising proteolytic cleavage of a single peptide bond (Arg561-Val562 in human plasminogen), can be catalyzed by either uPA or tPA. The concentration of plasminogen in blood plasma is approximately 2 μM, but there is also a large pool of extravascular plasminogen [for reviews see refs 1, 2, 17].

Angiostatin, reportedly an inhibitor of angiogenesis (see below), is a plasmin fragment consisting of kringles 1–3 or kringles 1–4 [18]. It can be generated from plasminogen and plasmin in vitro by pancreatic elastase, a variety of metalloproteinases, and by plasmin autoproteolysis [18–24]. Angiostatin has been reported to contain at least one free sulfhydryl group [22].

The serine proteinase uPA (M_r approximately 55,000) consists of two disulfide bridge-linked polypeptide chains, a C-terminal B chain, containing a serine proteinase domain, and an N-terminal A chain (amino acids 159–411 and 1–158, respectively, in human uPA). The A chain contains a growth factor domain (amino acids 1–49), a kringle domain (amino acids 50–131), and an interdomain linker region (amino acids 132–158) (fig. 1). The one-chain zymogen form of uPA, pro-uPA, has an activity about 250-fold less than that of two-chain uPA [25]. Conversion of pro-uPA to two-chain uPA occurs by proteolytic cleavage of a single peptide bond (Lys158-Ile159 in human uPA). The con-

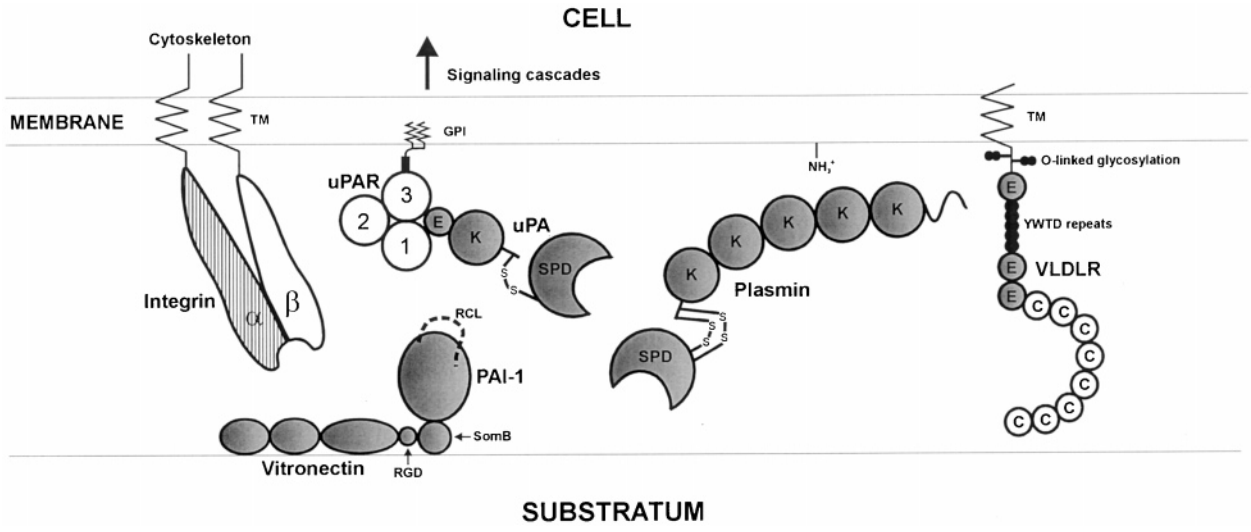

Figure 1. Schematic presentation of uPA and some of its pericellular interacting partners. uPA consists of a serine proteinase domain (SPD), a linker, a kringle (K), and a uPAR-binding epidermal growth factor-like domain (E). uPAR has three domains and is anchored to the membrane by a GPI anchor. Domains 1 and 3 make contact with uPA. Plasmin has a serine proteinase domain (SPD) and five kringles (K) and binds to pericellular proteins with C-terminal lysines. The RCL of PAI-1 is able to bind to the active site of uPA. Vitronectin, in the substratum, has an N-terminal PAI-1- and uPAR-binding somatomedin B domain (SomB) next to an integrin-binding RGD sequence. The α and β integrin subunits have transmembrane helices (TM) and are anchored to the cytoskeleton. Integrins can interact with uPAR. As a representative of the uPA-PAI-1 complex-binding endocytosis receptors of the LDLR family, VLDLR is indicated with a transmembrane domain (TM), an O-linked sugar domain, two epidermal growth factor-like domains (E) which are separated from a third one by a YWTD spacer region, and a ligand-binding cluster of eight complement-type repeats (C).

version can be catalyzed by plasmin or several other proteinases [for reviews see refs 1, 13]. Proteolytic cleavage of uPA in the linker region gives rise to the amino-terminal fragment (ATF), i.e., the growth factor domain and the kringle, and low molecular-weight uPA forms (LMW-uPA), i.e., the serine proteinase domain and part of the linker. uPA has a restricted substrate specificity. Besides the conversion of plasminogen to plasmin, uPA catalyzes conversion of the inactive one-chain proforms of hepatocyte growth factor/scatter factor (HGF/SF) and macrophage-stimulating protein (MSP) to their active two-chain forms. HGF/SF and MSP have sequence similarity to plasmin, but are devoid of proteolytic activity and act by binding to receptor tyrosine kinases [for a review see ref. 26]. uPA also catalyzes intradomain cleavage of uPAR (see below).

The serine proteinase tPA (M_r approximately 70,000) is also synthesized and released from cells in a single-chain form, which can be proteolytically converted to a two-chain form by cleavage of a single polypeptide bond (Arg275-Ile276 in human tPA). The two chains are held together by a disulfide bond. The N-terminal A chain contains, starting from the N terminus, a fibronectin type II domain, a growth factor domain, and two kringle domains, while the C-terminal B chain contains the serine proteinase domain. The plasminogen activation activity of single-chain tPA is 10- to 50-fold lower than that of the two-chain form. The activity of both single-chain and two-chain tPA is stimulated considerably by fibrin, in agreement with the role of tPA as a fibrinolytic enzyme [for reviews see refs 9, 12, 17].

Biochemical properties of uPAR

The amino acid sequence of uPAR [27–31] exhibits three repeats, each of approximately 90 amino acids, suggesting the existence of three homologous, independently folded domains (domains 1, 2, and 3, as numbered from the N terminus) (fig. 1). Accordingly, stable receptor fragments with internal disulfide bridges can be produced by limited proteolysis [32–35]. Based on the similarity of the disulfide pattern of domain 1 to that of snake venom α neurotoxins [33, 34], a model for the unknown three-dimensional structure of the uPAR domains was proposed [36]. uPAR is bound to cell membranes by a glycosyl phosphatidyl inositol (GPI) anchor [37]. Anchorless, soluble uPAR variants have also been identified in conditioned medium from various cell lines [38] and in body fluids from cancer patients [39–42] and may arise by differential splicing [28, 30, 43], by proteolysis, or by phospholipase C cleavage of the GPI anchor.

Pro-uPA and active uPA bind to uPAR with the same affinity, with a K_d in the subnanomolar range [44]. uPAR binds the growth factor domain of uPA [45], the uPAR-binding residues being localized at one side of a seven residue-long Ω loop [46–49]. uPA binds to a site composed of amino acids from both domain 1 and domain 3 of uPAR [32, 35, 48, 50–52] (fig. 1). Based on site-directed mutagenesis, two subregions within domain 1 of human uPAR, spanning residues 2–10 and 47–53, seemed critical for uPA binding [53]. By chemical modification and photoaffinity labeling of human uPA and human uPAR, Arg53, Tyr57, and Leu66 in uPAR domain 1, and His251 in uPAR domain 3 appeared to come in close contact with the above-mentioned Ω loop in uPA [46, 52]. In vitro, uPA is able to cleave the Arg83-Ala84 and Arg89-Ser90 bonds between domains 1 and 2 of human uPAR. The cleavage abolishes uPA binding [54, 55]. N-terminally truncated variants of uPAR, consisting of domains 2 and 3, have also been demonstrated in vivo [42, 56].

uPAR also binds vitronectin, with a K_d in the subnanomolar range. The binding is stimulated by pro-PA, uPA, ATF, and the uPA-PAI-1 complex, but is inhibited by PAI-1 [57–60]. Interdomain proteolytic cleavage of uPAR prevented its binding to vitronectin [61].

uPAR binds $\beta 2$ integrins in a purified system [62] and may also be able to associate with $\beta 1$ and $\beta 3$ integrins (see below).

Serpin inhibitors in the plasminogen activation system

PAI-1 and PAI-2 belong to the serpin (serine proteinase inhibitor) family. Of decisive importance for the inhibitory mechanism of serpins is the surface-exposed, approximately 20 amino-acid-long reactive center loop (RCL) (fig. 2). The reactive center peptide bond (the P_1-P_1' bond) in the RCL interacts with the active site of the proteinase, resulting in a stable complex of 1:1 stoichiometry [for reviews see refs 63, 64]. The three-dimensional structure of serine proteinase-serpin complexes has not been determined. Nevertheless, complex formation seems to comprise P_1-P_1' bond cleavage and coupling of the P_1 residue to the active-site serine of the proteinase by an ester bond [65], the N-terminal part of the RCL becoming inserted as strand 4 in β-sheet A, translocation of the proteinase across the plane of β-sheet A towards the other pole of the molecule [66, 67], and conformational changes occurring in the so-called flexible-joints region, i.e., the region around α-helices D and E [68, 69] (fig. 2).

PAI-1 (M_r approximately 50,000) reacts quickly with both uPA and tPA [for a review see ref. 13]. PAI-1 binds to vitronectin with high affinity, while plasminogen activator-complexed PAI-1 does not [for a review see ref. 70]. Vitronectin-binding residues were localized to α-helix E, β-stand 1A and α-helix F of PAI-1,

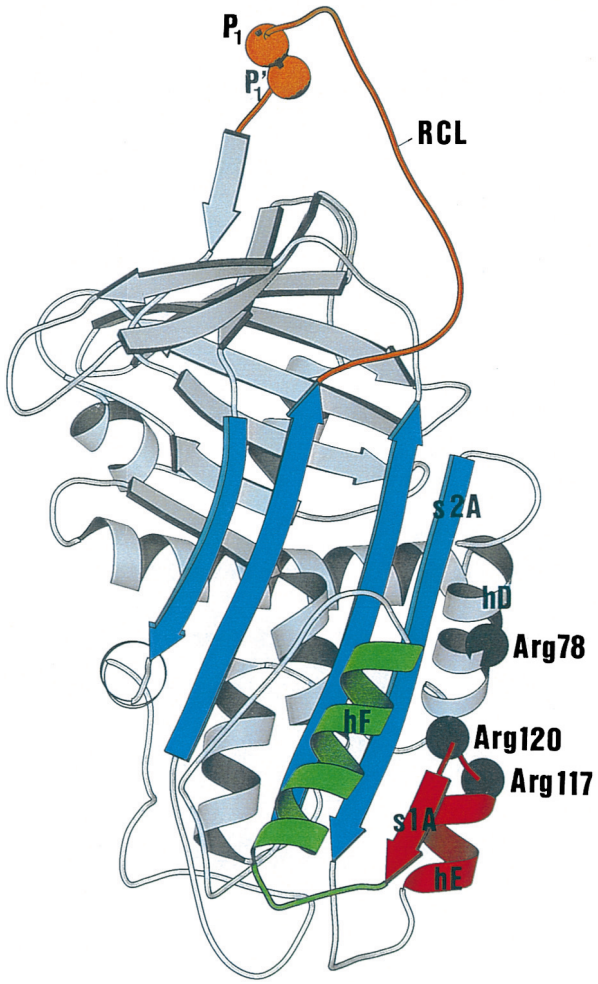

Figure 2. A model of the three-dimensional structure of PAI-1. The RCL is shown in orange. The P_1 and the P'_1 residues are indicated by orange spheres. The large central β-sheet A is shown in blue, except β-strand 1A. The regions implicated in binding to vitronectin are shown in green (α-helix F) or red (α-helix E, β-strand 1A) [71–73]. Activity-neutralizing antibodies bind to α-helix F and the flanking sequences [189]. The black spheres indicate basic residues, which were implicated in binding of anthranilic acid derivatives to PAI-1 [191]. The figure is a Molscript display of the model of Aertgeerts et al. [197].

overlapping with the flexible-joints region [71–73] (fig. 2). PAI-2 (M_r approximately 50,000) is also able to inhibit both uPA and tPA, although it reacts more slowly than PAI-1 with both proteinases [for reviews see refs 8, 12, 16].

The serpin α2-antiplasmin is the primary inhibitor of plasmin and ensures a short half-life of plasmin in blood and tissues [for a review see ref. 17].

Biochemical properties of vitronectin

Vitronectin is a M_r 78,000 glycoprotein, present in blood plasma and extravascularly in many forms of ECM [for reviews see refs 74, 75]. The N-terminal somatomedin B domain (amino acids 1–44) of vitronectin binds both PAI-1 [for a review see ref. 70] and uPAR [58] (fig. 1), which, accordingly, compete for binding [58, 59]. Amino acids 45–47 constitute an RGD sequence (fig. 1), binding the integrins $\alpha V\beta 1$, $\alpha V\beta 3$, $\alpha V\beta 5$, $\alpha IIb\beta 3$, and $\alpha 8\beta 1$ [74, 75]. PAI-1 and integrins compete for binding to vitronectin [76, 77].

Pericellular localization of components of the uPA system in cell cultures

Binding to uPAR causes accumulation of pro-uPA and uPA on cell surfaces. Immunocytochemical studies with cell cultures showed that uPAR and its complexes with pro-uPA and uPA often accumulate at specific sites at cell-substratum contact areas. In several cell types, mostly of fibroblastic origin, the accumulation sites had the characteristics of focal contacts, where integrins cluster and interact with actin filaments [for a review see ref. 13]. uPAR-integrin and/or uPAR-vitronectin interactions seemed to contribute to the accumulation at these sites. Thus, uPAR coimmunoprecipitated with $\beta 1$ integrins from human 293 embryonic kidney cells [62]. In human HT-1080 fibrosarcoma cells, uPAR-bound uPA was localized to focal contacts in cultures with a vitronectin substratum, but not with a fibronectin substratum [78]. In the same cells, uPAR consistently colocalized with the $\beta 1$ integrin subunit, but displayed varying colocalization with $\beta 3$, $\alpha 3$, $\alpha 5$, $\alpha 6$, and αV integrin subunits, depending on whether the cells were cultured on vitronectin, fibronectin, or laminin substrata [79]. Given the affinity of uPAR to vitronectin, but not to other ECM proteins, and the differential binding specificity of different combinations of integrin subunits, this observation suggests very specific uPAR-integrin interactions, which are regulated by binding of uPAR to vitronectin and by ligation of the integrins. In addition, uPAR and $\beta 2$ integrins colocalized on human neutrophils and monocytes [80–82]. With reference to the biochemical evidence for uPAR-$\beta 2$ integrin binding (see above), the colocalization may be caused by direct binding of the extracellular domains of the two proteins.

During cell migration, uPAR usually becomes concentrated at the leading cell edge [for a review see ref. 13]. Evidence obtained with monoclonal antibodies with differential affinity to free and uPA-ligated uPAR suggested that the ligation contributes to the accumulation of uPAR at specific sites [83]. Antibody-induced polarization of human neutrophils led to reversible dissocia-

tion of uPAR and $\alpha M\beta 2$, uPAR becoming accumulated in lamellipodia [84]. Thus, the pericellular localization of uPAR seems to be dynamic in nature.

Many GPI-linked proteins, integrins, and signal transmitters are accumulated in so-called rafts, which are specific membrane subdomains with a high content of cholesterol and glycosphingolipids, resistant to extraction with Triton X-100 at 4 °C, and often forming membrane invaginations, so-called caveolae, together with membrane-embedded proteins of the caveolin family [for reviews see refs 85, 86]. Accordingly, uPAR is found in caveolae in several cell types [62, 87–89], together with $\beta 1$ integrins [62].

In cell cultures, PAI-1 is bound to vitronectin at the substratum [for a review see ref. 13], while plasminogen seems to be localized at the ventral cell surface [10].

Pericellular plasminogen activation and plasmin activity in cell cultures

Activation of pro-uPA bound to uPAR at cell surfaces proceeds much faster than activation of fluid-phase pro-uPA. Active uPA bound to uPAR at the cell surface catalyzes plasminogen activation much more efficiently than fluid-phase uPA. The enhancement of pro-uPA activation and of uPA-catalyzed plasminogen activation seems to be due to cell surface coaccumulation with plasminogen. Plasminogen presumably binds to pericellular proteins with C-terminal lysines [for reviews see refs 13, 90].

The most obvious consequence of pericellular plasmin generation is degradation of fibrin and other ECM proteins [for a review see ref. 13]. It should be noted, however, that native collagens are resistant to plasmin [for reviews see refs 1, 2]. The pericellular localization of PAI-1 on vitronectin may selectively protect vitronectin against uPA-mediated degradation [78]. Plasmin also catalyzes activation of latent transforming growth factor-β and release of basic fibroblast growth factor from its ECM-binding sites [for a review see ref. 2]. In addition, plasmin may contribute to activation of zymogens of matrix metalloproteinases (MMPs) [for a review see ref. 91]. Experiments with mice with disruptions of the genes for plasminogen, uPA, and tPA ($plasminogen^{-/-}$, $uPA^{-/-}$, and $tPA^{-/-}$ mice, respectively) confirmed the physiological importance of the plasminogen activation system in activation of MMP-3, MMP-9, and MMP-13 [92–94], but suggested additional activation mechanisms for MMP-2 [93, 94]. Coaccumulation with uPA on cell surfaces accelerates activation of MMP-2 and MMP-9 [95].

Pericellular plasminogen activation in cell cultures can be followed by plasmin autoproteolysis and generation of angiostatin, through the concomitant action of a reductase catalyzing formation of one or more free sulfhydryl groups [20, 22, 96].

Activation of intracellular signal transduction cascades through uPAR

Much attention has been focused on the possibility that uPA-uPAR binding activates intracellular signal transduction cascades without plasmin generation and proteolytic activation of growth factor precursors. This is most simply demonstrated by showing activation of signaling cascades by proteolytically inactive uPA variants, also excluding effects of minor contaminations of proteolytically active uPA. The possibility of uPAR becoming occupied by the added inactive variant, leading to an increase in fluid-phase uPA activity due to endogenous uPA, should also be excluded [11]. The most easily interpretable results will therefore be obtained with cells with low endogenous uPA production, which are also expected to have a low background in uPA-stimulated signal transduction pathways.

Despite the technical difficulties, evidence is increasing for uPA-initiated signaling through uPAR. In a variety of cell lines, uPAR coimmunoprecipitated and/or colocalized with tyrosine kinases, including hck, fyn, lck, lyn, fgr, Jak1, and Tyk2 [81, 89, 97–99]. In some cases, the colocalization depended on uPA ligation of uPAR [89, 97]. Moreover, varying between cell lines, uPA ligation of uPAR stimulated tyrosine phosphorylation of hck, focal adhesion kinase, paxillin, $p130^{CAS}$, mitogen-activated protein kinase (MAPK), and several unidentified proteins, and activated the kinase activity of hck and MAPK and the DNA-binding ability of Stat1 [97–102]. Signaling via G protein-regulated enzymes, i.e., protein kinase Cε [103], and G protein-regulated second messengers, i.e., cAMP [104] and diacylglycerol [105–107], have also been implicated in uPAR-activated signaling cascades. The relative order of the different molecules in uPAR-stimulated signaling cascades and the possibility of cell type specificities in the activated pathways have not yet been clarified.

Initiation of intracellular signals from the GPI-anchored uPAR would be expected to require extracellular interaction with membrane-spanning proteins but until now, putative signal transducers remain unidentified. It is interesting, however, that ATF-induced signaling in THP-1 cells was mimicked by addition of soluble uPAR, provided that it was proteolytically cleaved between domains 1 and 2 [97], by uPAR fragments harboring residues 88–92 of the domain 1-domain 2 linker, and by peptides covering this region [108]. On this basis, uPA was proposed to activate signaling cascades by cleaving the interdomain linker [108]. The apparent discrepancy between this hypothesis and the fact that

signaling can be initiated with proteolytically inactive uPA variants was resolved by suggesting that uPA can also activate uPAR by inducing a conformational change in uPAR, exposing proteolytic cleavage sites to endogenous proteinases [108].

Pericellular reaction of uPA with inhibitors and endocytosis of uPA-inhibitor complexes

uPAR-bound uPA reacts with PAI-1 at approximately the same rate as fluid-phase uPA [109, 110]. The uPAR-bound uPA-PAI-1 complex is endocytosed and degraded much faster than PAI-1 and uPAR-bound pro-uPA and uPA. The endocytosis occurs by binding of the complex to clathrin-coated pit-localized endocytosis receptors of the LDLR family, either α_2MR/LRP, gp330/megalin, or VLDLR [for reviews see refs 13, 111–113]. uPAR is, as a consequence of its binding to the uPA moiety of the complex, coendocytosed [114], but later recirculated to the cell surface [115].

Ligand binding by receptors of the LDLR family is critically dependent on clusters of complement-type repeats. The receptors also contain epidermal growth factor precursor homology domains (fig. 1). Each receptor binds several structurally unrelated ligands, including lipoproteins and proteinase-inhibitor complexes [for reviews see refs 112, 113]. The receptor-associated protein (RAP) functions as a chaperone for folding of the endocytosis receptors in endoplasmatic reticulum [for a review see ref. 116]. RAP is an invaluable tool for studying ligand-endocytosis receptor binding, since it inhibits the binding of all known ligands to the receptors [for a review see ref. 112].

The plasminogen activation system in adhesion in cell cultures

The classical role of plasminogen activation is one of counteracting cell-substratum and cell-cell adhesion, as pericellular plasmin generation leads to degradation of adhesion receptors and their ECM ligands [for reviews see refs 1, 2].

Under some conditions, however, binding of uPA to uPAR promotes cell-substratum adhesion. Thus, integrin-independent adhesion of several human cell lines to vitronectin was stimulated by binding of uPA or uPA fragments to uPAR [60, 62, 117–123]. PAI-1 inhibited uPAR-dependent adhesion to vitronectin [76, 122]. These findings are consistent with the biochemical evidence for uPA-stimulated uPAR-vitronectin binding (see above) and show that uPAR can act as an adhesion receptor. Conceivably, initiation of signaling cascades by uPA-uPAR binding may also contribute to regulation of cell adhesion.

β1-integrin-dependent adhesion of 293 cells to fibronectin was inhibited by transfection with uPAR cDNA or by addition of soluble uPAR [62]. Anti-uPAR antibodies and uPAR antisense oligonucleotides reduced adhesion of monocytes to ligands for $\alpha M\beta 2$, while activation or ligation of $\alpha M\beta 2$ promoted uPAR-mediated adhesion of monocytes to vitronectin [82, 124]. Addition of soluble uPAR restored adhesion of uPAR-depleted human HL-60 monocytic cells to endothelial cells, the adhesion being mediated by binding of $\beta 2$ integrins ($\alpha M\beta 2$ and $\alpha L\beta 2$) on the HL-60 cells to intercellular adhesion molecule-1 (ICAM-1) on the endothelial cells [125]. Interestingly, binding of uPA to uPAR counteracted $\alpha M\beta 2$-dependent adhesion [82, 124, 125]. Together with the findings concerning uPAR-integrin colocalizations (see above), these observations show that uPAR and uPA may also affect cell adhesion by regulating the functions of integrins, in an integrin-, ECM-, and cell type-specific manner, probably depending on direct extracellular uPAR-integrin contacts.

The plasminogen activation system in cell migration in cell cultures

Cell migration, defined as locomotion of a cell over an ECM substratum, involves extension and binding of integrins and other adhesion receptors to their ECM ligands at the leading cell edge. The cell-ECM adhesions at the leading edge provide guidance and traction for pulling the cell forward, mechanical force being generated by contraction of the actin cytoskeleton. Dissociation of integrin/ligand complexes allows retraction of the trailing edge [for a review see ref. 126]. Cell migration may also involve transport of membrane proteins from the trailing to the leading edge through a polarized endocytic cycle [for a review see ref. 127].

There are many reports of uPA stimulating cell migration by catalyzing plasmin generation [for a review see ref. 13], in good agreement with the expectation that plasmin-catalyzed degradation of ECM proteins and adhesion receptors helps release the trailing cell edge. In endothelial cells, generation of the plasmin fragment angiostatin by plasmin autoproteolysis could provide a negative feedback on plasmin-dependent cell migration, as an antimigratory effect, specific for endothelial cells, was ascribed to angiostatin [128, 129]. Limited proteolysis of uPAR, followed by activation of signaling cascades, is another conceivable proteolytic mechanism allowing uPA to stimulate cell migration. Thus, soluble uPAR with a proteolytic cleavage between domains 1 and 2 mimicked uPA in stimulating chemotaxis of THP-1 cells in parallel with tyrosine kinase hck activation [108].

There is also evidence for nonproteolytic stimulatory effects of uPA-uPAR binding on cell migration. Thus, enzymatically inactive, but uPAR-binding uPA variants stimulated migration of a variety of cell types [62, 97, 101, 103, 105, 106, 117, 118, 130–137]. These promigratory effects of uPA may depend on enhancement of adhesion of the leading edge by stimulation of uPAR-vitronectin binding. Accordingly, transfection of 293 cells with uPAR cDNA conferred on them uPAR-dependent migration towards vitronectin [62]. uPA may also act by activating intracellular signal transduction cascades at the leading edge. Thus, pro-uPA or ATF stimulation of migration of MCF-7 cells was inhibited by a synthetic inhibitor of MAPK [101]. In addition, nonproteolytic uPA and uPAR effects on cell migration may depend on regulation of integrin-binding activity at both the leading and the trailing edge. Thus, transfection of 293 cells with uPAR cDNA inhibited their integrin-dependent migration towards fibronectin, in parallel with uPAR and $\beta 1$ integrin colocalization [62].

PAI-1 inhibited integrin-dependent migration of rabbit vascular smooth muscle cells and of two human epithelial cell lines [76, 77], stimulated integrin-dependent migration of a melanoma cell line [122], inhibited migration of bovine aortic endothelial cells, and stimulated migration of bovine aortic smooth muscle cells [138]. Both inhibitory and stimulatory effects may be related to PAI-1 inhibition of integrin binding to vitronectin (see above), the net effect of PAI-1 depending on the relative importance of integrin-vitronectin binding at the leading and the trailing cell edges. In this scenario, uPA may regulate cell migration by complexing PAI-1, thereby removing it from vitronectin and relieving integrin-vitronectin binding from PAI-1 inhibition [76]. PAI-1 is also expected to inhibit stimulation of cell migration by uPA-catalyzed plasmin generation (see above).

Mutually conflicting results were reported concerning the possible role in cell migration of endocytosis of plasminogen activator-inhibitor complexes by receptors of the LDLR family. Plasmin-dependent migration of human umbilical vein smooth muscle cells was inhibited by RAP [136, 139], in agreement with the expectation that maintenance of a high cell surface plasminogen activation activity in the presence of PAI-1 must require constant endocytosis of uPAR-bound uPA-PAI-1 complexes and recirculation of uPAR to the cell surface. Conceivably, endocytosis could also be necessary for uPA stimulation of migration by nonproteolytic mechanisms, by participating in a polarized endocytic cycle [127], thereby removing uPAR-mediated adhesion at the trailing edge and allowing uPAR to enhance new rounds of adhesion at the leading edge. On the other hand, migration of murine embryonic fibroblasts on vitronectin was accelerated by genetic deficiency of α_2MR/LRP, in parallel with increased cell surface plasminogen activation activity but, paradoxically, was not affected by RAP [140]. However, since the endocytosis receptors have many ligands, effects of RAP and endocytosis receptor deficiency may be caused by inhibition of the binding of ligands other than plasminogen activator-inhibitor complexes.

It seems possible to arrive at a model unifying the many observations by assuming that proteolytic and nonproteolytic mechanisms of uPA action on cell migration are operating simultaneously in individual migrating cells. If pro-uPA is converted to active uPA at the ventral surface of the cells, nonproteolytic mechanisms could dominate at the leading edge, and proteolytic mechanisms at the trailing edge. The relative importance of the proteolytic and the nonproteolytic elements and the net effect of (pro-)uPA and PAI-1 would be expected to depend on the level of expression by the migrating cells of uPAR, endocytosis receptors, and integrins, of the composition of the ECM, of the pericellular localization of (pro-)uPA and PAI-1, of mechanisms for pro-uPA activation, and of the stimuli that induce cell motility.

The plasminogen activation system in cell invasion in cell culture model systems

When progressing from migration to invasion, an additional complexity is added, as invasion comprises not only cell locomotion, but also the active penetration of cells into ECM. A variety of cell culture invasion assays are available, including assays with invasion into isolated human amniotic membrane, into fibrin and collagen gels, and into other types of reconstituted ECM, so-called Matrigel. Experiments with such assays have shown that plasmin generation, as catalyzed by uPAR-bound uPA on the surface of the invading cells, is a rate-limiting factor for invasion by many cell types [for a review see ref. 13].

PAI-2 was consistently found to inhibit invasion [for a review see ref. 13]. The findings with PAI-1 were more variable. PAI-1 inhibited uPA- and uPAR-dependent invasiveness of human A549 and Calu-1 lung carcinoma cells [141], while coexpression of uPA, uPAR, and PAI-1 correlated with optimal invasiveness of human lung cancer cells [142], and transfection of human PC-3 prostate carcinoma cells with PAI-1 cDNA did not change their invasiveness [143]. The variability of the observed effects of PAI-1 may be due to an inhibitory effect on invasion of excessive plasminogen activation. Although high levels of PAI-1 may protect ECM against plasminogen activation-mediated proteolysis so that invasion becomes inhibited, low levels of PAI-1 may be needed to protect ECM proteins for traction.

The divergent possibilities for the role of PAI-1 in cell migration (see above) may also contribute to the variable observations concerning the role of PAI-1 in invasion. All in all, the effect of PAI-1 on invasion is expected to depend on expression by the invading cell of the other components of the uPA system, of endocytosis receptors, of integrins, and on the composition of the ECM.

The endocytosis receptors may or may not play a role in cell invasion. Thus, RAP was reported to inhibit invasion of human umbilical vein smooth muscle cells into Matrigel [139], but to be without effect on invasion of the same cells into a collagen gel [136].

The plasminogen activation system in some physiological and nonneoplastic pathophysiological processes—evidence from mice with specific gene deficiencies

Mice with targeted disruption of the genes for uPA and tPA [144], PAI-1 [145], uPAR [146], plasminogen [147], and vitronectin [148] develop to term, grow to adulthood, and are fertile. A variety of studies with these mice resulted in findings of high significance for understanding the basic functions of the system, and therefore also of its role in cancer.

plasminogen$^{-/-}$ mice and *uPA*$^{-/-}$-*tPA*$^{-/-}$ mice developed disseminated intra- and extravascular fibrin deposits, resulting in widespread organ damage, a low body weight, and a short life span [147]. These defects were corrected by concomitant *fibrinogen* gene deficiency [149]. Therefore, fibrinolysis was suggested to be the critical and perhaps only essential physiological role of plasminogen activation [149]. It should be noted, nevertheless, that laminin is also an in vivo plasmin substrate [150]. There was much less abnormal fibrin deposition in *tPA*$^{-/-}$ mice and *uPA*$^{-/-}$ mice than in *plasminogen*$^{-/-}$ mice and *uPA*$^{-/-}$-*tPA*$^{-/-}$ mice, implying that uPA can substitute for tPA in fibrinolysis [144]. There was also much less disseminated fibrin deposition in *uPAR*$^{-/-}$-*tPA*$^{-/-}$ mice than in *plasminogen*$^{-/-}$ and *uPA*$^{-/-}$-*tPA*$^{-/-}$ mice, suggesting that binding of uPA to uPAR is less important for uPA-mediated fibrinolysis [151]. *PAI-1*$^{-/-}$ mice had a mild hyperfibrinolytic state and a greater resistance to venous thrombosis [145] and displayed decreased fibrin deposition after lung injury [152–154].

uPA$^{-/-}$ mice had increased susceptibility to lung infection with *Cryptococcus neoformans* and a reduced recruitment of leukocytes to the infection sites [155] and increased susceptibility to staphylococcal botryomycosis [156]. *plasminogen*$^{-/-}$ mice had impaired recruitment of leukocytes to inflammatory sites [157]. The accumulation of leukocytes, mainly neutrophils and eosinophils, in peritoneum during thioglycollate-induced peritonitis was significantly reduced in *uPAR*$^{-/-}$ mice compared to wild-type mice [125].

The fact that *plasminogen*$^{-/-}$, *uPA*$^{-/-}$, *uPAR*$^{-/-}$, *PAI-1*$^{-/-}$, and *vitronectin*$^{-/-}$ mice develop to term shows that the uPA system is not essential in development, in apparent contradiction to predictions from cell culture experiments of a general regulatory role of the system in cell migration and invasion (see above) and from studies of the expression of the components of the system [for reviews see refs 1, 2, 13]. It is unknown whether this is due to functional overlap with and compensatorily increased expression of other enzyme systems, and/or to the essential functions of the system being restricted to specific processes like fibrinolysis and cell migration and invasion in inflammation.

Levels of plasminogen activation system components in tumors and patient prognosis

The levels of uPA, PAI-1, and uPAR in human malignant tumors are significantly higher than in the corresponding normal tissues [for reviews see ref. 13, 158], but their levels in a given tumor type vary considerably between individual patients. In a variety of malignancies, patients with high tumor uPA levels have a shorter disease-free interval and a shorter overall survival than patients with a low level [for reviews see refs 13, 14]. In contrast, tPA levels are correlated with a good prognosis [for a review see ref. 158]. The finding that high tumor levels of PAI-1 also predict a poor prognosis [for reviews see refs 13, 14] first came as a surprise, since it is in apparent contradiction to the original idea of cancer cell dissemination being promoted by pericellular uPA-catalyzed plasmin generation, and it has been an important motive for searching for functions of PAI-1 other than inhibition of uPA. High levels of uPAR in tumors are also correlated with a poor prognosis [for reviews see refs 13, 14].

Recently, interesting correlations between poor patient prognosis and high levels of soluble uPAR in blood plasma and other body fluids were reported [41, 42]. In addition, expression of uPAR and uPA in disseminated cancer cells in bone marrow of patients with gastric and breast cancers, respectively, predicted an early relapse [159, 160].

The plasminogen activation system in experimental metastasis in animals

Many experiments with animal model systems have shown convincingly that uPA-catalyzed plasmin generation is an important determinant of the rate of the overall process of tumor metastasis [for reviews see refs

1, 2, 13]. Recently, mice with specific gene disruptions were used for evaluating the role of the uPA system in tumor biology. The induction of blue nevi by 7,12-dimethylbenz(a)anthracene/croton oil occurred with equal efficiency in control and $uPA^{-/-}$ mice, but there was less local invasion in $uPA^{-/-}$ mice than in control mice, and $uPA^{-/-}$ mice had a drastically reduced progression to malignant melanomas [161]. Formation of vascular tumors after injection of polyoma middle T-transformed endothelial cells was reported to be highly dependent on the expression of uPA by the injected cells as well as by the host [162]. $plasminogen^{-/-}$ mice displayed a slower growth of transplanted Lewis lung carcinoma and fewer regional lymph node metastases and lived longer after the transplantation than control mice, but did not have fewer metastatic foci in the lungs [163]. The incidence of lung metastases from mammary tumors induced by mouse mammary tumor virus and polyoma middle T antigen was up to ten-fold lower in $plasminogen^{-/-}$ mice than in control mice, while tumorigenesis and primary tumor progression were unaffected [164]. These results are also in agreement with the idea that uPA-catalyzed plasminogen activation is rate limiting for tumor growth, local tumor invasion, and formation of distant metastases.

Most experiments with animal model systems showed that to support tumor metastasis, uPA must be bound to uPAR [for reviews see refs 1, 2, 13]. Exceptionally, transfection of *murine* B16 melanoma cells with *human* uPA cDNA was reported to increase the ability of these cells to metastasize [165]. Since human uPA binds with only low affinity to murine uPAR, this report suggests that unbound uPA is active in metastasis. Metastasis studies with $uPAR^{-/-}$ mice should be able to throw more light on this issue, but remain to be reported.

Experiments with two animal model systems both gave results showing that PAI-2 expression by cancer cells suppress metastasis [166, 167]. In contrast, no consistent picture has emerged concerning PAI-1. Transfection of PC-3 cells with PAI-1 cDNA impaired their ability to metastasize in nude mice [143]. Transduction with adenovirus vector carrying PAI-1 cDNA of uveal melanoma cells, transplanted into the eyes of nude mice, resulted in a reduction both in the number of animals developing liver metastasis and in the metastatic burden in animals with metastasis [168]. Intravenous injection of PAI-1 inhibited the growth of xenografts of human DU145 and LNCaP prostate carcinoma cells in *scid/scid* mice [169]. Metastasis of murine B16 melanoma was indistingushable in control mice and in mice overexpressing PAI-1 [170]. Expression of PAI-1 by human melanomas implanted onto nude mice correlated with metastatic behavior [171]. Bajou et al. [172] transplanted malignant keratinocytes onto a collagen I gel in the skin of control and $PAI\text{-}1^{-/-}$ mice. Upward migration of host-derived endothelial cells and stromal cells and downward migration of malignant cells were severely impaired in $PAI\text{-}1^{-/-}$ mice, but could be brought back to control levels by injection of adenovirus carrying PAI-1 cDNA. The latter results provide convincing evidence for a causal involvement of host PAI-1 in cancer cell invasion and angiogenesis.

Angiostatin has been reported to inhibit primary tumor growth and metastasis in animals by inhibition of tumor angiogenesis [18, 173–176].

Processes in tumors involving the plasminogen activation system

Most of the experiments described in the previous section gave little information about the actions of the plasminogen activation system at individual steps in tumor growth, invasion, and metastasis, and the situation in tumors in vivo is far more complex than in the cell cultures used to provide evidence for important roles of the system in cell migration and invasion. In tumors, not only cancer cells, but also nonmalignant cells are migratory and invasive, during processes of cancer cell-directed tissue remodeling. The system may contribute to tumor growth, invasion, and metastasis by being involved in such processes. Most information is available about the early steps in the metastatic process, e.g., invasion and intravasation, and the later steps, e.g., extravasation and colonization, will not be dealt with here.

A strategy widely used in attempts to identify tumor processes involving the uPA system has been determination of cells expressing its components by immunohistochemistry and in situ hydridization. There are limitations to this approach, however. An inherent difficulty is that there is not necessarily a correlation between the amount of a certain protein in a certain localization and its functional importance there. Moreover, there are discrepancies between the results reported by different groups [for a review see ref. 13]. Nevertheless, the localization studies published so far do demonstrate some important points. It was a general finding that each of the components is expressed not only by a single cell type, and that the expression pattern varies between different types of tumors [for a review see ref. 13]. The localization of the components may also vary between different tumors in the same tissue, depending on their degree of differentiation [177–179]. Some studies suggested collaboration between different cell types, each producing different components of the system [180]. The localization of the components in multiple cell types points to the system having multiple functions contributing to tumor growth, invasion, and metastasis. Thus, the presence of

the components of the system on cancer cells suggests a function in cancer cell migration and invasion, their presence on stromal fibroblasts suggests a role in desmoplasia and stroma remodeling, and their presence on endothelial cells suggests a role in angiogenesis. Differential expression of PAI-1 by different cell types and in different tissue areas may contribute to cancer cell-directed tissue remodeling by allowing deposition of new ECM, e.g., during angiogenesis, in tissue areas with a high uPA activity.

The hypothesis of a role for the uPA system in angiogenesis is supported by findings with angiogenesis model systems [for a review see ref. 181], with observations of an antagonist of uPA-uPAR binding inhibiting neovascularization of the murine B16 melanoma [182], and with a correlation between microvessel density and uPA levels in breast carcinomas [183]. On the other hand, plasminogen and plasminogen activators were not essential for outgrowth of endothelial cells from murine tissue explants into a fibrin gel [184], and an indistinguishable vascularization of Lewis lung carcinomas in *plasminogen*$^{-/-}$ and control mice argued against a role of plasmin in angiogenesis in this tumor [163]. Angiostatin inhibits angiogenesis, in agreement with its ability to inhibit endothelial cell migration (see above) and to inhibit endothelial cell proliferation [18, 24, 128, 129, 174, 175, 185]. Since angiostatin is derived from plasminogen, the finding of indistinguishable vascularization of Lewis lung carcinomas in *plasminogen*$^{-/-}$ and control mice [163] argues against angiostatin as an essential, naturally occurring regulator of angiogenesis. The findings of Bajou et al. [172] (see above) provided strong evidence for PAI-1 being necessary for tumor angiogenesis.

In view of the finding that fibrinogen deficiency corrects many effects of plasminogen deficiency in mice [149] (see above), the importance of fibrin and fibrinolysis for the role of the uPA system in tumor biology must be considered. Cancer cell spread may require invasion into extravascular fibrin deposits, which occur in many tumors and which may also provide a matrix for desmoplasia and angiogenesis [4, 7]. From the evidence for uPAR-independent actions of uPA in fibrinolysis (see above), it may also be speculated that uPAR-independent uPA-mediated fibrinolysis contributes to tumor invasion. The apparent paradox that tumor levels of tPA, the classical fibrinolytic enzyme, are correlated with a good rather than a poor prognosis (see above) may be resolved by assuming that tPA is not produced by the right cell types. More conclusive information about the role of fibrinolysis in tumor growth, invasion, and metastasis may be obtained with *fibrinogen*$^{-/-}$ and *plasminogen*$^{-/-}$-*fibrinogen*$^{-/-}$ mice.

The plasminogen activation system as a therapeutic target

The components of the uPA system are potential targets for anti-invasive and anti-metastatic therapy. The most obvious target is the enzyme activity of uPA. Most classical proteinase inhibitors inhibit both uPA and tPA and are therefore also expected to inhibit fibrinolysis, but peptide sequences have recently been identified which may form the basis for development of selective uPA inhibitors [186]. The binding of uPA to uPAR is another potential target [for a review see ref. 13]. The best-characterized antagonist is a peptide selected from a phage-displayed peptide library [187], binding to a site composed of regions in uPAR domains 1 and 3, and most likely competing directly with uPA for binding [48, 52].

Several compounds have been found to inhibit the reaction of PAI-1 with uPA and tPA. Some monoclonal anti-PAI-1 antibodies inhibit the reaction [188], many of these having epitopes near α-helix F [189]. PAI-1-neutralizing derivatives of diketopiperazine also bind near α-helix F [190]. PAI-1-neutralizing derivatives of anthranilic acid are believed to insert in a hydrophobic pocket lined by α-helices D, E, and F and β-strand 2A and to form salt bridges to basic residues in α-helices D and E [191]. PAI-1-neutralizing peptides corresponding to portions of the RCL probably insert in the cleft between β-strands 3 and 5 [192]. These compounds may therefore act by changing the conformation of the flexible-joints region and/or by preventing the loop insertion associated with complex formation (fig. 2). Sodium dodecyl sulfate [193] and peptides selected from a phage-displayed peptide library [194] also neutralized PAI-1, but their binding sites are unknown. Importantly, in order to neutralize the functions of PAI-1 in tumor invasion, it may also be necessary to neutralize its ability to inhibit the binding of uPAR and integrins to vitronectin.

An alternative approach is inhibition of biosynthesis of the components of the uPA system by antisense oligonucleotide therapy [for a review see ref. 14]. The potential use of angiostatin as a generally applicable anticancer drug, based on its ability to inhibit angiogenesis, has recently attracted much interest [195, 196].

Conclusions and perspectives

The original idea of plasminogen activation as a rate-limiting factor in tumor invasion and metastasis has been supported by many results with in vitro and in vivo model systems and by demonstration of correlations between patient prognosis and tumor levels of components of the uPA system. Most recently, experiments with mice with targeted disruption of the genes

for these components have given results supporting this idea. But the increased knowledge about the system has also led to the realization that the system works in a far more complex way than described by the original hypothesis. Much evidence suggests that the system also has plasmin-independent functions, consisting in intracellular signal transduction cascades being initiated by binding of uPA to uPAR, in uPAR acting as a vitronectin receptor and as a regulator of integrin function, in PAI-1 acting as a regulator of uPAR and integrin binding to vitronectin, in interactions with endocytosis receptors, and in an interplay with other proteolytic enzyme systems. The dynamic state of the system allows spatial and temporal rearrangements of its components at cell surfaces during cell migration and invasion. Moreover, it has become clear that the system has a multifunctional role in tumor biology. The system seems to function not only in cancer cell migration and invasion, but also in remodeling of the tissue surrounding the cancer cells, which may contribute decisively to the overall process of metastasis. As the biologies of individual tumors are different, different processes may be rate limiting for the endpoint of metastasis in different tumors, and the importance of the uPA system may therefore vary.

Special problems are associated with understanding the role of PAI-1 in tumor biology. The unexpected finding that PAI-1 is a marker for a poor prognosis has been the impetus for a variety of studies with in vitro and in vivo model systems, but they have not yet provided one unifying hypothesis for the role of PAI-1. Some observations suggest that PAI-1 may counteract migration and invasion by inhibiting uPA, while other observations support the hypothesis that PAI-1 is needed for the optimal function of the uPA system in these processes, by regulating cell adhesion and by restricting proteolysis in time and space. The recent results with $PAI\text{-}1^{-/-}$ mice [172] strongly suggest that PAI-1 expressed by noncancer cells does play a causal role in tumor growth and spread by protecting new ECM in tissue remodeling events like angiogenesis.

1 Danø K., Andreasen P. A., Grøndahl-Hansen K., Kristensen P., Nielsen L. S. and Skriver L. (1985) Plasminogen activators, tissue degradation and cancer. Adv. Cancer Res. **44:** 139–266

2 Mignatti P. and Rifkin D. B. (1993) Biology and biochemistry of proteinases in tumor invasion. Physiol. Rev. **73:** 161–195

3 Werb Z. (1997) ECM and cell surface proteolysis: regulating cellular ecology. Cell **91:** 439–442

4 Dvorak H. F., Brown L. F., Detmar M. and Dvorak A. M. (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am. J. Pathol. **146:** 1029–1039

5 Hanahan D. and Folkman J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell **86:** 353–364

6 Tremblay G. (1979) Stromal aspects of breast carcinoma. Exp. Mol. Pathol. **31:** 248–260

7 Nagy J. A., Brown L. F., Senger D. R., Lanir N., Water L. van de, Dvorak A. M. et al. (1989) Pathogenesis of tumor stroma generation: a critical role for leaky blood vessels and fibrin deposition. Biochim. Biophys. Acta **948:** 305–326

8 Andreasen P. A., Georg B., Lund L. R., Riccio A. and Stacey S. N. (1990) Plasminogen activator inhibitors: hormonally regulated serpins. Mol. Cell. Endocrinol. **68:** 1–19

9 Andreasen P. A., Petersen L. C. and Danø K. (1991) Diversity in catalytic properties of single-chain and two-chain tissue-type plasminogen activator. Fibrinolysis **5:** 207–215

10 Pöllänen J., Stephens R. W. and Vaheri A. (1991) Directed plasminogen activation at the surface of normal and malignant cells. Adv. Cancer Res. **57:** 273–328

11 Vassalli J.-D. (1994) The urokinase receptor. Fibrinolysis **8[suppl. 1]:** 172–181

12 Rijken D. C. (1995) Plasminogen activators and plasminogen activator inhibitors: biochemical aspects. Bailliéres Clin. Haematol. **8:** 291–312

13 Andreasen P. A., Kjøller L., Christensen L. and Duffy M. J. (1997) The urokinase-type plasminogen activator system in cancer metastasis: a review. Int. J. Cancer **72:** 1–22

14 Schmitt M., Harbeck N., Thomssen C., Wilhjelm O., Magdolen V., Reuning U. et al. (1997) Clinical impact of the plasminogen activation system in tumor invasion and metastasis: prognostic relevance and target for therapy. Thromb. Haemostasis **78:** 285–296

15 Behrendt N. and Stephens R. W. (1998) The urokinase receptor. Fibrinolysis Proteolysis **12:** 191–204

16 Dear A. E. and Medcalf R. L. (1995) The cellular and molecular biology of plasminogen activator inhibitor type-2. Fibrinolysis **9:** 321–330

17 Collen D. (1980) On the regulation of fibrinolysis. Thromb. Haemostasis **43:** 77–89

18 O'Reilly M. S., Holgren L., Shing Y., Chen C., Rosenthal R. A., Moses M. et al. (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell **79:** 315–328

19 Dong Z., Kumar R., Yang X. and Fidler I. J. (1997) Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell **88:** 801–810

20 Gately S., Twardowski P., Stack M. S., Cundiff D. L., Grella D., Castellino F. J. et al. (1997) The mechanism of cancer-mediated conversion of plasminogen to the angiogenesis inhibitor angiostatin. Proc. Natl. Acad. Sci. USA **94:** 10868–10872

21 Patterson B. C. and Sang Q. A. (1997) Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J. Biol. Chem. **272:** 28823–28825

22 Stathakis P., Fitzgerald M., Matthias L. J., Chesterman C. N. and Hogg P. J. (1997) Generation of angiostatin by reduction and proteolysis of plasmin: catalysis by a plasmin reductase secreted by cultured cells. J. Biol. Chem. **272:** 20641–20645

23 Lijnen H. R., Ugwu F., Bini A. and Collen D. (1998) Generation of an angiostatin-like fragment from plasminogen by stromelysin-1 (MMP-3). Biochemistry **37:** 4699–4702

24 Lucas R., Holmgren L., Garcia I., Jimenez B., Mandriota S. J., Borlat F. et al. (1998) Multiple forms of angiostatin induce apoptosis in endothelial cells. Blood **92:** 4730–4741

25 Petersen L. C., Lund L. R., Nielsen L. S., Dano K. and Skriver L. (1988) One-chain urokinase-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J. Biol. Chem. **263:** 11189–11195

26 Birchmeier C. and Gherardi E. (1998) Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. Trends Cell Biol. **8:** 404–410

27 Roldan A. L., Cubellis M. V., Masucci M. T., Behrendt N., Lund L. R., Danø K. et al. (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
28 Kristensen P., Eriksen J., Blasi F. and Danø K. (1991) Two alternatively spliced mouse urokinase receptor mRNAs with different histological localization in the gastrointestinal tract. J. Cell Biol. **115:** 1763–1771
29 Krätzschmar J., Haendler B., Kojima S., Rifkin D. B. and Schleuning W. D. (1993) Bovine urokinase-type plasminogen activator and its receptor: cloning and induction by retinoic acid. Gene **125:** 177–183
30 Rabbani S. A, Rajwans N., Achbarou A., Murthy K. K. and Goltzman D. (1994) Isolation and characterization of multiple isoforms of the rat urokinase receptor in osteoblasts. FEBS Lett. **338:** 69–74
31 Fowler B., Mackman N., Parmer R. J. and Miles L. A. (1998) Binding of human single chain urokinase to chinese hamster ovary cells and cloning of hamster uPAR. Thromb. Haemostasis **80:** 148–154
32 Behrendt N., Ploug M., Patthy L., Houen G., Blasi F. and Danø K. (1991) The ligand-binding domain of the cell surface receptor for urokinase-type plasminogen activator. J. Biol. Chem. **266:** 7842–7847
33 Ploug M., Kjalke M., Rønne E., Weidle U., Høyer-Hansen G. and Danø 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
34 Ohkura N., Inoue S., Ikeda K. and Hayashi K. (1994) The two subunits of a phospholipase A2 inhibitor from the plasma of Thailand cobra having structural similarity to urokinase-type plasminogen activator receptor and LY-6 related proteins. Biochem. Biophys. Res. Commun. **204:** 1212–1218
35 Behrendt N., Rønne E. and Danø K. (1996) Domain interplay in the urokinase receptor: requirement for the third domain in high affinity ligand binding and demonstration of ligand contact sites in distinct receptor domains. J. Biol. Chem. **271:** 22885–22894
36 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 α-neurotoxins. FEBS Lett. **349:** 163–168
37 Ploug M., Rønne E., Behrendt N., Jensen A. L., Blasi F. and Danø K. (1991) Cellular receptor for urokinase plasminogen activator: carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidyl inositol. J. Biol. Chem. **266:** 1926–1933
38 Lau H. K. and Kim M. (1994) Soluble urokinase receptor from fibrosarcoma HT-1080 cells. Blood Coagul. Fibrinolysis **5:** 473–478
39 Pedersen N., Schmitt M., Rønne E., Nicoletti M. I., Høyer-Hansen G., Conese M. et al. (1993) A ligand-free, soluble urokinase receptor is present in the ascitic fluid from patients with ovarian cancer. J. Clin. Invest. **92:** 2160–2167
40 Sillem M., Prifti S., Monga B., Buvari P., Shamia U. and Runnebaum B. (1997) Soluble urokinase-type plasminogen activator receptor is over-expressed in uterine endometrium from women with endometriosis. Mol. Hum. Reprod. **3:** 1101–1105
41 Sier C. F., Stephens R., Bizik J., Mariani A., Bassan M., Pedersen N. et al. (1998) The level of urokinase-type plasminogen activator receptor is increased in serum of ovarian cancer patients. Cancer Res. **58:** 1843–1849
42 Wahlberg K., Høyer-Hansen G. and Casslén B. (1998) Soluble receptor for urokinase plasminogen activator in both full-length and a cleaved form is present in high concentration in cystic fluid from ovarian cancer. Cancer Res. **58:** 3294–3298
43 Pyke C., Eriksen J., Solberg H., Nielsen B. S., Kristensen P., Lund L. R. et al. (1993) An alternatively spliced variant for the human receptor for urokinase plasminogen activator. FEBS Lett. **326:** 69–74
44 Cubellis M. V., Nolli M. L., Cassani G. and Blasi F. (1986) Binding of single chain pro-urokinase to the urokinase receptor of human U937 cells. J. Biol. Chem. **261:** 15819–15822
45 Appella E., Robinson E. A., Ullrich S. J., Stoppelli M. P., Corti A., Cassani G. et al. (1987) The receptor-binding sequence of urokinase: a biological function for the growth-factor module of proteases. J. Biol. Chem. **262:** 4437–4440
46 Ploug M., Rahbek-Nielsen H., Ellis V., Roepstorff P. and Danø K. (1995) Chemical modification of the urokinase-type plasminogen activator and its receptor using tetranitromethane: evidence for the involvement of specific tyrosine residues in both molecules during receptor-ligand interaction. Biochemistry **34:** 12524–12534
47 Magdolen V., Rettenberger P., Koppitz M., Goretzki L., Kessler H., Weidle U. H. et al. (1996) Systematic mutational analysis of the receptor-binding region of the human urokinase-type plasminogen activator. Eur. J. Biochem. **237:** 743–751
48 Ploug M., Østergaard S., Hansen L. B., Holm A. and Danø K. (1998) Photoaffinity labeling of the human receptor for urokinase-type plasminogen activator using a decapeptide antagonist: evidence for a composite ligand-binding site and a short interdomain separation. Biochemistry **37:** 3612–3622
49 Quax P. H., Grimbergen J. M., Lansink M., Bakker A. H., Blatter M. C., Belin D. et al. (1998) Binding of human urokinase-type plasminogen activator to its receptor: residues involved in species specificity and binding. Arterioscler. Thromb.Vasc. Biol. **18:** 693–701
50 Ploug M., Ellis V. and Danø K. (1994) Ligand interaction between urokinase-type plasminogen activator and its receptor probed with 8-anilino-1-naphthalenesulfonate: evidence for a hydrophobic binding site exposed only on the intact receptor. Biochemistry **33:** 8991–8997
51 Oda M., Shiraishi A. and Hasegawa M. (1998) Analysis of the ternary complex formation of human urokinase with the separated domains of its receptor. Eur. J. Biochem. **256:** 411–418
52 Ploug M. (1998) Identification of specific sites involved in ligand binding by photoaffinity labeling of the receptor for the urokinase-type plasminogen activator: residues located at equivalent positions in uPAR domains I and III participate in the assembly of a composite ligand-binding site. Biochemistry **37:** 16494–16505
53 Pöllänen J. (1993) The N-terminal domain of human urokinase receptor contains two distinct regions critical for ligand recognition. Blood **82:** 2719–2729
54 Høyer-Hansen G., Rønne E., Solberg H., Behrendt N., Ploug M., Lund L. R. et al. (1992) Urokinase plasminogen activator cleaves its cell surface receptor, releasing the ligand binding domain. J. Biol. Chem. **267:** 18224–18229
55 Høyer-Hansen G., Ploug M., Behrendt N., Rønne E. and Danø K. (1997) Cell-surface acceleration of urokinase-catalysed receptor cleavage. Eur. J. Biochem. **243:** 21–36
56 Solberg H., Rømer J., Brünner N., Holm A., Sidenius N., Danø K. et al. (1994) A cleaved form of the receptor for urokinase-type plasminogen activator in invasive transplanted human and murine tumors. Int. J. Cancer **58:** 877–881
57 Wei Y., Waltz D. A., Rao N., Drummond R. J., Rosenberg S. and Chapman H. A. (1994) Identification of the urokinase receptor as an adhesion receptor for vitronectin. J. Biol. Chem. **269:** 32380–32388
58 Deng G., Curriden S. A., Wang S., 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
59 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
60 Carriero M. V., Del Vecchio S., Franco P., Potena M. I., Chiaradonna F., Botti G. et al. (1997) Vitronectin binding to

urokinase receptor in human breast cancer. Clin. Cancer Res. **3:** 1299–1308
61 Høyer-Hansen G., Behrendt N., Ploug M., Danø. K. and Preissner K. T. (1997) The intact urokinase receptor is required for efficient vitronectin binding: receptor cleavage prevents ligand interaction. FEBS Lett. **420:** 79–85
62 Wei Y., Lukashev M., Simon D. I., Bodary S. C., Rosenberg S., Doyle M. V. et al. (1996) Regulation of integrin function by the urokinase receptor. Science **273:** 1551–1555
63 Carrell R. W. and Stein P. E. (1996) The biostructural pathology of the serpins: critical functions of sheet opening mechanism. Biol. Chem. Hoppe-Seyler **377:** 1–17
64 Gils A. and Declerck P. J. (1998) Structure-function relationships in serpins: current concepts and controversies. Thromb. Haemostasis **80:** 531–541
65 Egelund R., Rodenburg K. W., Andreasen P. A., Rasmussen M. S., Guldberg R. E. and Petersen T. E. (1998) An ester bond linking a fragment of a serine proteinase to its serpin inhibitor. Biochemistry **37:** 6375–6379
66 Stratikos E. and Gettins P. G. (1997) Major proteinase movement upon stable serpin-protease complex formation. Proc. Natl. Acad. Sci. USA **94:** 453–458
67 Wilczynska M., Fa M., Karolin J., Ohlsson P. I., Johansson L. B. and Ny T. (1997) Structural insights into serpin-protease complexes reveal the inhibitory mechanism of serpins. Nat. Struct. Biol. **4:** 354–357
68 Stein P. E. and Chothia C. (1991) Serpin tertiary structure transformation. J. Mol. Biol. **221:** 615–621
69 Egelund R., Schousboe S. L., Sottrup-Jensen L., Rodenburg K. W. and Andreasen P. A. (1997) Type-1 plasminogen-activator inhibitor: conformational differences between latent, active, reactive-centre-cleaved and plasminogen-activator-complexed forms, as probed by proteolytic susceptibility. Eur. J. Biochem. **248:** 775–785
70 Deng G., Royle G., Seiffert D. and Loskutoff D. J. (1995) The PAI-1/vitronectin interaction: two cats in a bag? Thromb. Haemostasis **74:** 66–70
71 Lawrence D. A., Berkenpas M. B., Palaniappan S. and Ginsburg D. (1994) Localization of vitronectin binding domain in plasminogen activator inhibitor-1. J. Biol. Chem. **269:** 15223–15228
72 Van Meijer M., Gebbink R. K., Preissner K. T. and Pannekoek H. (1994) Determination of the vitronectin binding site on plasminogen activator inhibitor 1 (PAI-1). FEBS Lett. **352:** 342–346
73 Padmanabhan J. and Sane D. C. (1995) Localization of a vitronectin binding region of plasminogen activator inhibitor-1. Thromb. Haemostasis **73:** 829–834
74 Felding-Habermann B. and Cheresh D. A. (1993) Vitronectin and its receptors. Curr. Opin. Cell Biol. **5:** 864–868
75 Preissner K. T. and Seiffert D. (1998) Role of vitronectin and its receptors in haemostasis and vascular remodeling. Thromb. Res. **89:** 1–21
76 Stefansson S. and Lawrence D. A. (1996) The serpin PAI-1 inhibits cell migration by blocking integrin $\alpha_v\beta_3$ binding to vitronectin. Nature **383:** 441–443
77 Kjøller L., Kanse S., Kirkegaard T., Rodenburg K. W., Rønne E., Goodmann S. L. et al. (1997) Plasminogen activator inhibitor-1 represses integrin- and vitronectin-mediated cell migration independently of its function as an inhibitor of plasminogen activation. Exp. Cell Res. **232:** 420–429
78 Ciambrone G. J. and McKeown-Longo P. J. (1990) Plasminogen activator inhibitor type I stabilizes vitronectin-dependent adhesions in HT-1080 cells. J. Cell Biol. **111:** 2183–2195
79 Xue W., Mizukami I., Todd R. F. and Petty H. R. (1997) Urokinase-type plasminogen activator receptors associate with β_1 and β_3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. Cancer Res. **57:** 1682–1689
80 Xue W., Kindzelskii A. L., Todd R. F. and Petty H. R. (1994) Physical association of complement receptor type 3 and urokinase-type plasminogen activator receptor in neutrophil membranes. J. Immunol. **152:** 4630–4640
81 Bohuslav J., Horejsi V., Hansmann C., Stöckl J., Weidle U. H., Majdic O. et al. (1995) Urokinase plasminogen activator receptor, β_2-integrins, and Src-kinases within a single receptor complex of human monocytes. J. Exp. Med. **181:** 1381–1390
82 Sitrin R. G., Todd R. F., Petty H. R., Brock T. G., Shollenberger S. B., Albrecht E. et al. (1996) The urokinase receptor (CD87) faciliates CD11b/CD18-mediated adhesion of human monocytes. J. Clin. Invest. **97:** 1942–1951
83 Myöhänen H. T., Stephens R. W., Hedman K., Tapiovaara H., Rønne E., Høyer-Hansen G. et al. (1993) Distribution and lateral mobility of the urokinase-receptor complex at the cell surface. J. Histochem. Cytochem. **41:** 1291–1301
84 Kindzelskii A. L., Laska Z. O., Todd R. F. and Petty H. R. (1996) Urokinase-type plasminogen activator receptor reversibly dissociates from complement receptor type 3 ($\alpha_M\beta_2$, CD11b/CD18) during neutrophil polarization. J. Immunol. **156:** 297–309
85 Simons K. and Ikonen E. (1997) Functional rafts in cell membranes. Nature **387:** 569–572
86 Porter J. C. and Hogg N. (1998) Integrins take partners: cross-talk between integrins and other membrane receptors. Trends Cell Biol. **8:** 390–396
87 Stahl A. and Mueller B. M. (1995) The urokinase-type plasminogen activator receptor, a GPI-linked protein, is localized in caveolae. J. Cell Biol. **129:** 335–344
88 Okada S. S., Tomaszewski J. E. and Barnathan E. S. (1995) Migrating vascular smooth muscle cells polarize cell surface urokinase receptors after injury in vitro. Exp. Cell Res. **217:** 180–187
89 Koshelnick Y., Ehart M., Hufnagl P., Heinrich P. C. and Binder B. R. (1997) Urokinase receptor is associated with the components of the JAK1/STAT1 signaling pathway and leads to activation of this pathway upon receptor clustering in the human kidney epithelial tumor cell line TCL-598. J. Biol. Chem. **272:** 28563–28567
90 Felez J. (1998) Plasminogen binding to cell surfaces. Fibrinolysis Proteolysis **12:** 183–189
91 Matrisian L. M. (1992) The matrix-degrading metalloproteinases. Bioessays **14:** 455–463
92 Carmeliet P., Moons L., Lijnen R., Baes M., Lemaitre V., Tipping P. et al. (1997) Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat. Genet. **17:** 439–444
93 Lijnen H. R., Van Hoef B., Lupu F., Moons L., Carmeliet P. and Collen D. (1998) Function of the plasminogen/plasmin and matrix metalloproteinase systems after vascular injury in mice with targeted inactivation of fibrinolytic system genes. Arterioscler. Thromb. Vasc. Biol. **18:** 1035–1045
94 Lijnen H. R., Silence J., Lemmens G., Frederix L. and Collen D. (1998) Regulation of gelatinase activity in mice with targeted inactivation of components of the plasminogen/plasmin system. Thromb. Haemost. **79:** 1171–1176
95 Mazzieri R., Masiero L., Zanetta L., Monea S., Onisto M., Garbisa S. et al. (1997) Control of type IV collagenase activity by components of the urokinase system: a regulatory mechanism with cell-bound reactants. EMBO J. **16:** 2319–2332
96 Gately S., Twardowski P., Stack M. S., Patrick M., Boggio L., Cundiff D. L. et al. (1996) Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor, angiostatin. Cancer Res. **56:** 4887–4890
97 Resnati M., Guttinger M., Valcamonica S., Sidenius N., Blasi F. and Fazioli F. (1996) Proteolytic cleavage of the urokinase receptor substitutes for the agonist-induced chemotactic effect. EMBO J. **15:** 1572–1582
98 Konakova M., Hucho F. and Schleuning W.-D. (1998) Downstream targets of urokinase-type plasminogen-activator-mediated signal transduction. Eur. J. Biochem. **253:** 421–429
99 Dumler I., Weis A., Mayboroda O. A., Maasch C., Jerke U., Haller H. et al. (1998) The Jak/Stat pathway and urokinase

receptor signaling in human aortic vascular smooth muscle cells. J. Biol. Chem. **273:** 315–321
100 Dumler I., Petri T. and Schleuning W.-D. (1993) Interaction of urokinase-type plasminogen activator (uPA) with its cellular receptor (uPAR) induces phosphorylation on tyrosine of a 38 kDa protein. FEBS Lett. **322:** 37–40
101 Nguyen D. H., Hussaini I. M. and Gonias S. L. (1998) Binding of urokinase-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal-regulated kinase 1 and 2 which is required for increased cellular motility. J. Biol. Chem. **273:** 8502–8507
102 Tang H., Kerins D. M., Hao Q., Inagami T. and Vaughan D. E. (1998) The urokinase-type plasminogen activator receptor mediates tyrosine phosphorylation of focal adhesion proteins and activation of mitogen-activated protein kinase in cultured endothelial cells. J. Biol. Chem. **273:** 18268–18272
103 Busso N., Masur S. K., Lazega D., Waxman S. and Ossowski L. (1994) Induction of cell migration by pro-urokinase binding to its receptor: possible mechanisms for signal transduction in human epithelial cells. J. Cell Biol. **126:** 259–270
104 Li C., Liu J.-N. and Gurewich V. (1995) Urokinase-type plasminogen activator-induced monocyte adhesion requires a carboxyl-terminal lysine and cAMP-dependent signal transduction. J. Biol. Chem. **270:** 30282–30285
105 Del Rosso M., Anichini E., Pedersen N., Blasi F., Fibbi G., Pucci M. et al. (1993) Urokinase-urokinase receptor interaction: non-mitogenic signal transduction in human epidermal cells. Biochem. Biophys. Res. Commun. **190:** 347–352
106 Anichini E., Fibbi G., Pucci M., Caldini R., Chevanne M. and Del Rosso M. (1994) Production of second messengers following chemotactic and mitogenic urokinase-receptor interaction in human fibroblasts and mouse fibroblasts transfected with human urokinase receptor. Exp. Cell Res. **213:** 438–448
107 Fibbi G., Caldini R., Chevanne M., Pucci M., Schiavone N., Morbidelli L. et al. (1998) Urokinase-dependent angiogenesis in vitro and diacylglycerol production are blocked by antisense oligonucletodies against the urokinase receptor. Lab. Invest. **78:** 1109–1119
108 Fazioli F., Resnati M., Sidenius N., Higashimoto Y., Appella E. and Blasi F. (1997) A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. EMBO J. **16:** 7279–7286
109 Cubellis M. V., Andreasen P. A., Ragno P., Mayer M., Danø K. and Blasi F. (1989) Accessibility of receptor-bound urokinase to type-1 plasminogen activator inhibitor. Proc. Natl. Acad. Sci. USA **86:** 4828–4832
110 Ellis V., Wun T.-C., Behrendt N., Rønne E. and Danø K. (1990) Inhibition of receptor-bound urokinase by plasminogen activator inhibitors. J. Biol. Chem. **265:** 9904–9908
111 Andreasen P. A., Sottrup-Jensen L., Kjøller L., Nykjær A., Moestrup S. K., Munch Petersen C. et al. (1994) Receptor-mediated endocytosis of plasminogen activators and activator/inhibitor complexes. FEBS Lett. **338:** 239–245
112 Gliemann J. (1998) Receptors of the low density lipoprotein (LDL) receptor family in man: multiple functions of the large family members via interaction with complex ligands. Biol. Chem. **379:** 951–964
113 Neels J. G., Horn I. R., Berg B. M. M. van den, Pannekoek H. and Zonneveld A.-J. van (1998) Ligand-receptor interactions of the low density lipoprotein receptor-related protein, a multiligand endocytic receptor. Fibrinolysis Proteolysis **12:** 219–240
114 Conese M., Nykjær A., Petersen C. M., Cremona O., Pardi R., Andreasen P. A. et al. (1995) α_2-Macroglobulin receptor/LDL receptor related protein (LRP)-dependent internalization of the urokinase receptor. J. Cell Biol. **131:** 1609–1622
115 Nykjær A., Conese M., Christensen E. I., Olson D., Cremona O., Gliemann J. et al. (1997) Recycling of the urokinase receptor upon internalization of uPA:serpin complexes. EMBO J. **16:** 2610–2620
116 Bu G. and Schwartz A. L. (1998) RAP, a novel type of ER chaperone. Trends Cell Biol. **8:** 272–276
117 Franco P., Iaccarino C., Chiaradonna F., Brandazza A., Iavarone C., Mastronicola M. R. et al. (1997) Phosphorylation of human pro-urokinase on Ser138/303 impairs its receptor-dependent ability to promote myelomonocytic adherence and motility. J. Cell Biol. **137:** 779–791
118 Franco P., Massa O., Garcia-Rocha M., Chiaradonna F, Iaccarinom C., Correas I. et al. (1998) Protein kinase C-dependent in vivo phosphorylation of prourokinase leads to the formation of a receptor competitive antagonist. J. Biol. Chem. **273:** 27734–27740
119 Nusrat A. R. and Chapman H. A. (1991) An autocrine role for urokinase in phorbol ester-mediated differentiation of myeloid cell lines. J. Clin. Invest. **87:** 1091–1097
120 Waltz D. A., Sailor L. Z. and Chapman H. A. (1993) Cytokines induce urokinase-dependent adhesion of human myeloid cells: a regulatory role for plasminogen activator inhibitors. J. Clin. Invest. **91:** 1541–1552
121 Waltz D. and Chapman H. (1994) Reversible cellular adhesion to vitronectin linked to urokinase receptor occupancy. J. Biol. Chem. **269:** 14746–14750
122 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
123 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
124 Simon D. I., Rao N. K., Xu H., Wei Y., Majdic O., Rønne E. et al. (1996) Mac-1 (CD11b/CD18) and the urokinase receptor (CD87) form a functional unit on monocytic cells. Blood **88:** 3185–3194
125 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 β_2 integrins in vivo. J. Exp. Med. **188:** 1029–1037
126 Sheetz M. P., Felsenfeld D. P. and Galbraith C. G. (1998) Cell migration: regulation of force on extracellular-matrix-integrin complexes. Trends Cell Biol. **8:** 51–54
127 Bretscher M. S. (1996) Moving membrane up to the front of migrating cells. Cell **85:** 465–467
128 Ji W. R., Castellino F. J., Chang Y., Deford M. E., Gray H., Villarreal X. et al. (1998) Characterization of kringle domains of angiostatin as antagonists of endothelial cell migration, an important process in angiogenesis. FASEB J. **12:** 1731–1738
129 Claesson-Welsh L., Welsh M., Ito N., Anand-Apte B., Soker S., Zetter B. et al. (1998) Angiostatin induces endothelial cell apoptosis and activation of focal adhesion kinase independently of the integrin-binding motif RGD. Proc. Natl. Acad. Sci. USA **95:** 5579–5583
130 Gudewicz P. W. and Gilboa N. (1987) Human urokinase-type plasminogen activator stimulates chemotaxis of human neutrophils. Biochem. Biophys. Res. Commun. **147:** 1176–1181
131 Fibbi G., Ziche M., Morbidelli L., Magnelli L. and Del Rosso M. (1988) Interaction of urokinase with specific receptors stimulates mobilization of bovine adrenal capillary endothelial cells. Exp. Cell Res. **179:** 385–395
132 Del Rosso M., Fibbi G., Dini G., Grappone C., Pucci M., Caldini R. et al. (1990) Role of specific membrane receptors in urokinase-dependent migration of human keratinocytes. J. Invest. Dermatol. **94:** 310–316
133 Odekon L. E., Sato Y. and Rifkin D. B. (1992) Urokinase-type plasminogen activator mediates basic fibroblast growth factor-induced bovine endothelial cell migration independent of its proteolytic activity. J. Cell. Physiol. **150:** 258–263
134 Gyetko M. R., Todd R. F., Wilkinson C. C. and Sitrin R. G. (1994) The urokinase receptor is required for human monocyte chemotaxis in vitro. J. Clin. Invest. **93:** 1380–1387
135 Stahl A. and Mueller B. M. (1994) Binding of urokinase to its receptor promotes migration and invasion of human melanoma cells in vitro. Cancer Res. **54:** 3066–3071

136 Okada S. S., Grobmyer S. R. and Barnathan E. S. (1996) Contrasting effects of plasminogen activators, urokinase receptor, and LDL receptor-related protein on smooth muscle cell migration and invasion. Arterioscler. Thromb. Vasc. Biol. **16:** 1269–1276

137 Sandberg T., Casslen B., Gustavsson B. and Benraad T. J. (1998) Human endothelial cell migration is stimulated by urokinase plasminogen activator:plasminogen activator inhibitor 1 complex released from endometrial stromal cells stimulated with transforming growth factor beta1: possible mechanism for paracrine stimulation of endometrial angiogenesis. Biol. Reprod. **59:** 759–767

138 Petzelbauer E., Springhorn J. P., Tucker A. M. and Madri J. A. (1996) Role of plasminogen activator inhibitor in the reciprocal regulation of bovine aortic endothelial and smooth muscle cells migration by TGF-β1. Am. J. Pathol. **149:** 923–931

139 Wijnberg M. J., Quax P. H. A., Niewenbroek N. M. E. and Verheijen J. H. (1997) The migration of human smooth muscle cells in vitro is mediated by plasminogen activation and can be inhibited by α_2-macroglobulin receptor associated protein. Thromb. Haemostasis **78:** 880–886

140 Weaver A. M., Hussaini I. M., Mazar A., Henkin J. and Gonias S. L. (1997) Embryonic fibroblasts that are genetically deficient in low density lipoprotein receptor-related protein demonstrate increased activity of the urokinase receptor system and accelerated migration on vitronectin. J. Biol. Chem. **272:** 14372–14379

141 Brückner A., Filderman A. E., Kirchheimer J. C., Binder B. R. and Remold H. G. (1992) Endogenous receptor-bound urokinase mediates tissue invasion of the human lung carcinoma cell line A549 and Calu-1. Cancer Res. **52:** 3043–3047

142 Liu G., Shuman M. A. and Cohen R. L. (1995) Co-expression of urokinase, urokinase receptor and PAI-1 is necessary for optimum invasiveness of cultured lung cancer cells. Int. J. Cancer **60:** 501–506

143 Soff G. A., Sanderowitz J., Gately S., Verrusio E., Weiss I., Brem S. et al. (1995) Expression of plasminogen activator inhibitor type 1 by human prostate carcinoma cells inhibits primary tumor growth, tumor-associated angiogenesis, and metastasis to lung and liver in an athymic mouse model. J. Clin. Invest. **96:** 2593–2600

144 Carmeliet P., Schoojans L., Kiekens L., Ream B., Degen J. L., Bronson R. et al. (1994) Physiological consequences of loss of plasminogen activator gene function in mice. Nature **368:** 419–424

145 Carmeliet P., Stassen J.-M., Schoojans L., Ream B., Van den Oord J. J., De Mol M. et al. (1993) Plasminogen activator inhibitor-1 gene-deficient mice. II. Effects on hemostasis, thrombosis, and thrombolysis. J. Clin. Invest. **92:** 2756–2760

146 Bugge T. H., Suh T. T., Flick M. J., Daugherty C. C., Rømer J., Solberg H. et al. (1995) The receptor for urokinase-type plasminogen activator is not essential for mouse development or fertility. J. Biol. Chem. **270:** 16886–16894

147 Bugge T. H., Flick M. J., Daugherty C. C. and Degen J. L. (1995) Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. Genes Dev. **9:** 794–807

148 Zheng X., Saunders T. L., Camper S. A., Samuelson L. C. and Ginsburg D. (1995) Vitronectin is not essential for normal mammalian development and fertility. Proc. Natl. Acad. Sci. USA **92:** 12426–12430

149 Bugge T. H., Kombrinck K. W., Flick M. J., Daugherty C. C., Danton M. J. and Degen J. L. (1996) Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. Cell **87:** 709–719

150 Chen Z.-L. and Strickland S. (1997) Neuronal death in the hippocampus is promoted by plasmin-catalysed degradation of laminin. Cell **91:** 917–925

151 Bugge T. H., Flick M. J., Danton M. J., Daugherty C. C., Rømer J., Danø K. et al. (1996) Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. Proc. Natl. Acad. Sci. USA **93:** 5899–5904

152 Barazzone C., Belin D., Piguet P. F., Vassalli J. D. and Sappino A. P. (1996) Plasminogen activator inhibitor-1 in acute hyperoxic mouse lung injury. J. Clin. Invest. **98:** 2666–2673

153 Eitzman D. T., McCoy R. D., Zheng X., Fay W. P., Shen T., Ginsburg D. et al. (1996) Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. J. Clin. Invest. **97:** 232–237

154 Pinsky D. J., Liao H., Lawson C. A., Yan S. F., Chen J., Carmeliet P. et al. (1998) Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. J. Clin. Invest. **102:** 919–928

155 Gyetko M. R., Chen G. H., McDonald R. A., Goodman R., Huffnagle G. B., Wilkinson C. C. et al. (1996) Urokinase is required for the pulmonary inflammatory response to *Cryptococcus neoformans*: a murine transgenic model. J. Clin. Invest. **97:** 1818–1826

156 Shapiro R. L., Duquette J. G., Nunes I., Roses D. F., Harris M. N., Wilson E. L. et al. (1997) Urokinase-type plasminogen activator-deficient mice are predisposed to staphylococcal botryomycosis, pleuritis, and effacement of lymphoid follicles. Am. J. Pathol. **150:** 359–369

157 Ploplis V. A., French E. L., Carmeliet P., Collen D. and Plow E. F. (1998) Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. Blood **91:** 2005–2009

158 Duffy M. J., Reilly D., O'Grady P., O'Higgins N., Fennelly J. J., and Andreasen P. A. (1995) Tissue-type and urokinase-type plasminogen activator as prognostic markers in breast cancer. In: Fibrinolysis in Disease, pp. 14–18, Glass-Greenwalt P. (ed.), CRC Press, New York

159 Heiss M. M., Allgayer H., Gruetzner K. U., Funke I., Babic R., Jauch K.-W. et al. (1995) Individual development and u-PA receptor expression of disseminated tumour cells in bone marrow: a reference to early disease in solid cancer. Nat. Med. **1:** 1035–1038

160 Solomayer E. F., Diel I. J., Wallwiener D., Bode S., Meyberg G., Sillem M. et al. (1997) Prognostic relevance of urokinase plasminogen activator detection in micrometastatic cells in the bone marrow of patients with primary breast cancer. Br. J. Cancer **76:** 812–818

161 Shapiro R. L., Duquette J. G., Roses D. F., Nunes I., Harris M. N., Kamino H. et al. (1996) Induction of primary cutaneous melanocytic neoplasms in urokinase-type plasminogen activator (uPA)-deficient and wild-type mice: cellular blue nevi invade but do not progress to malignant melanoma in uPA-deficient animals. Cancer Res. **56:** 3597–3604

162 Sabapathy K. T., Pepper M. S., Kiefer F., Mohle-Steinlein U., Tacchini-Cottier F., Fetka I. et al. (1997) Polyoma middle T-induced vascular tumor formation: the role of the plasminogen activator/plasmin system. J. Cell Biol. **137:** 953–963

163 Bugge T. H., Kombrinck K. W., Xiao Q., Holmback K., Daugherty C. C., Witte D. P. et al. (1997) Growth and dissemination of Lewis lung carcinoma in plasminogen-deficient mice. Blood **90:** 4522–4531

164 Bugge T. H., Lund L. R., Kombrinck K. K., Nielsen B. S., Holmback K., Drew A. F. et al. (1998) Reduced metastasis of polyoma virus middle T antigen-induced mammary cancer in plasminogen-deficient mice. Oncogene **16:** 3097–3104

165 Yu H. and Schultz R. M. (1990) Relationship between secreted urokinase plasminogen activator activity and metastatic potential in murine B16 cells transfected with human urokinase sense and antisense genes. Cancer Res. **50:** 7623–7633

166 Evans D. M. and Lin P. L. (1995) Suppression of pulmonary metastases of rat mammary cancer by recombinant urokinase plasminogen activator inhibitor. Am. Surg. **61:** 692–696

167 Mueller B. M., Yu Y. B. and Laug W. E. (1995) Overexpression of plasminogen activator inhibitor 2 in human melanoma cells inhibits spontaneous metastasis in *scid*/*scid* mice. Proc. Natl. Acad. Sci. USA **92:** 205–209

168 Ma D., Gerard R. D., Li X. Y., Alizadeh H. and Niederkorn J. Y. (1997) Inhibition of metastasis of intraocular melanomas by adenovirus-mediated gene transfer of plasminogen activator inhibitor type 1 (PAI-1) in an athymic mouse model. Blood **90:** 2738–2746

169 Jankun J., Keck R. W., Skrzypczak-Jankun E. and Swiercz R. (1997) Inhibitors of urokinase reduce size of prostate cancer xenografts in severe combined immunodeficient mice. Cancer Res. **57:** 559–563

170 Eitzman D. T., Krauss J. C., Shen T., Cui J. and Ginsburg D. (1996) Lack of plasminogen activator inhibitor-1 effect in a transgenic mouse model of metastatic melanoma. Blood **87:** 4718–4722

171 Quax P. H., Van Muijen G. N. P, Weening-Verhoeff E. J., Lund L. R., Danø K., Ruiter D. J. et al. (1991) Metastastic behaviour of human melanoma cell lines in nude mice correlated with urokinase-type plasminogen activator, its type-1 inhibitor, and urokinase-mediated matrix degradation. J. Cell Biol. **115:** 191–199

172 Bajou K., Noel A., Gerard R. D., Masson V., Brünner N., Holst-Hansen C. et al. (1998) Absence of host plasminogen activator inhibitor-1 prevents cancer invasion and vascularization. Nat. Med. **4:** 923–928

173 O'Reilly M. S., Holmgren L., Chen C. and Folkman J. (1996) Angiostatin induces and sustains dormancy of human primary tumors in mice. Nat. Med. **2:** 689–692

174 Sim B. K., O'Reilly M. S., Liang H., Fortier A. H., He W., Madsen J. W. et al. (1997) A recombinant human angiostatin protein inhibits experimental primary and metastatic cancer. Cancer Res. **57:** 1329–1334

175 Wu Z., O'Reilly M. S., Folkman J. and Shing Y. (1997) Suppression of tumor growth with recombinant murine angiostatin. Biochem. Biophys. Res. Commun. **236:** 651–654

176 Cao Y., O'Reilly M. S., Marshall B., Flynn E., Ji R. W. and Folkman J. (1998) Expression of angiostatin cDNA in a murine fibrosarcoma suppresses primary tumor growth and produces long-term dormancy of metastases. J. Clin. Invest. **101:** 1055–1063

177 De Vries T. J., Quax P. H. A., Denijn M., Verrijp K. N., Verheijen J. H., Verspaget H. W. et al. (1994) Plasminogen activators, their inhibitors, and urokinase receptor emerge in late stages of melanocytic tumor progression. Am. J. Pathol. **144:** 70–81

178 Christensen L., Simonsen A. C. W., Heegaard C. W., Moestrup S. K., Andersen J. and Andreasen P. A. (1996) Immunohistochemical localization of urokinase-type plasminogen activator, type-1 plasminogen activator inhibitor, urokinase receptor, and α_2-macroglobulin receptor in human breast carcinomas. Int. J. Cancer **66:** 441–452

179 Borgfeldt C., Casslén B., Liu C.-L., Hansson S., Lecander I. and Åstedt B. (1998) High tissue content of urokinase plasminogen activator (uPA) is associated with high stromal expression of uPA mRNA in poorly differentiated serous ovarian carcinoma. Int. J. Cancer **79:** 588–595

180 Danø K., Behrendt N., Brünner N., Ellis V., Ploug M. and Pyke C. (1994) The urokinase receptor: protein structure and role in plasminogen activation and cancer invasion. Fibrinolysis **8[suppl. 1]:** 189–203

181 Rifkin D. B., Moscatelli D., Bizik J., Quarto N., Blei F., Dennis P. et al. (1990) Growth factor control of extracellular proteolysis. Cell Differ. Dev. **32:** 313–318

182 Min H. Y., Doyle L. V., Vitt C. R., Zandonella C. L., Stratton-Thomas J. R., Shuman M. A. et al. (1996) Urokinase receptor antogonists inhibit angiogenesis and primary growth in syngeneic mice. Cancer Res. **56:** 2428–2433

183 Hildenbrand R., Dilger I., Horlin A. and Stutte H. J. (1995) Urokinase plasminogen activator induces angiogenesis and tumor vessel invasion in breast cancer. Pathol. Res. Pract. **191:** 403–409

184 Hiraoka N., Allen E., Apel I. J., Gyetko M. R. and Weiss S. J. (1998) Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell **95:** 365–377

185 Cao Y., Ji R. W., Davidson D., Schaller J., Marti D., Sohndel S. et al. (1996) Kringle domains of human angiostatin: characterization of the anti-proliferative activity on endothelial cells. J. Biol. Chem. **271:** 29461–29467

186 Ke S. H., Coombs G. S., Tachias K., Corey D. R. and Madison E. L. (1997) Optimal subsite occupancy and design of a selective inhibitor of urokinase. J. Biol. Chem. **272:** 20456–20462

187 Goodson R. J., Doyle M. V., Kaufman S. E. and Rosenberg S. (1994) High affinity urokinase receptor antagonists identified with bacteriophage peptide display. Proc. Natl. Acad. Sci. USA **91:** 7129–7133

188 Nielsen L. S., Andreasen P. A., Grøndahl-Hansen J., Huang J.-Y., Kristensen P. and Danø K. (1986) Monoclonal antibodies to human 54,000 molecular weight plasminogen activator inhibitor from fibrosarcoma cells: inhibitor neutralization and one-step affinity purification. Thromb. Haemostasis **55:** 206–212

189 Keijer J., Linders M., Zonneveld A. J. van, Ehrlich H. J., Boer J. P. de, and Pannekoek H. (1991) The interaction of plasminogen activator inhibitor 1 with plasminogen activators (tissue-type and urokinase-type) and fibrin: localization of interaction sites and physiologic relevance. Blood **78:** 401–409

190 Friederich P. W., Levi M., Biemond B. J., Charlton P., Templeton D., Zonneveld A. J. van, et al. (1997) Novel low-molecular-weight inhibitor of PAI-1 (XR5118) promotes endogenous fibrinolysis and reduces postthrombolysis thrombus growth in rabbits. Circulation **96:** 916–921

191 Björquist P., Ehnebom J., Inghardt T., Hansson L., Lindberg M., Linschoten M. et al. (1998) Identification of the binding site for a low-molecular-weight inhibitor of plasminogen activator inhibitor type 1 by site-directed mutagenesis. Biochemistry **37:** 1227–1234

192 Eitzman D. T., Fay W. P., Lawrence D. A., Francis-Chmura A. M., Shore J. D., Olson S. T. et al. (1995) Peptide-mediated inactivation of recombinant and platelet plasminogen activator inhibitor-1 in vitro. J. Clin. Invest. **95:** 2416–2420

193 Urano T., Strandberg L., Johansson L. B. and Ny T. (1992) A substrate-like form of plasminogen-activator-inhibitor type 1: conversions between different forms by sodium dodecyl sulphate. Eur. J. Biochem. **209:** 985–992

194 Gårdsvoll H., Zonneveld A. J. van, Holm A., Eldering E., Meijer M. van, Danø K. et al. (1998) Selection of peptides that bind to plasminogen activator inhibitor 1 (PAI-1) using random peptide phage-display libraries. FEBS Lett. **431:** 170–174

195 Harris A. L. (1998) Are angiostatin and endostatin cures for cancer? Lancet **351:** 1598–1599

196 Sato T. N. (1998) A new approach to fighting cancer? Proc. Natl. Acad. Sci. USA **95:** 5843–5844

197 Aertgeerts K., De Bondt H. L., De Ranter C. and Declerck P. J. (1994) A model of the reactive form of plasminogen activator inhibitor-1. J. Struct. Biol. **113:** 239–245