The Role of the Pericellular Fibrinolytic System in Angiogenesis

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Angiogenesis, the formation of new blood vessels by branching from extant vessels, is an important biological activity of vascular endothelial cells during wound healing and cancer invasion. Angiogenesis is controlled in vascular endothelial cells mainly by the cellular regulation of the fibrinolytic system. Cell-associated fibrinolysis is defined as the "pericellular fibrinolytic system." The fibrinolytic system in the blood involves an activation process in which zymogen plasminogen is converted to the active enzyme plasmin by plasminogen activators. The plasmin then degrades fibrin, the main component of a thrombus. However, recent studies have shown that a variety of physiological and pathological processes in tissues are initiated by the localized and effective activation of plasminogen on the cell surface. Plasmin generated on the cell surface not only degrades several matrix proteins but also induces activation of the latent form of matrix metalloproteinases, thus resulting in pericellular proteolysis. The assembly of fibrinolytic components on the cell surface is mediated by the expression and functions of cellular receptors. This review focuses on the pericellular fibrinolytic system involved in the actions of endothelial cells in angiogenesis. The mechanism of the sequential enzymatic activation cascade, several fibrinolytic factors (ligands and receptors) responsible for the mechanism, and the possible contributions of these components to angiogenesis are described.

Fibrinolytic System

1. Mechanism of fibrinolysis in plasma

The fibrinolytic system in the blood (Fig. 1) is initiated through the activation of plasminogen [1] by plasminogen activators (PAs) [2] to plasmin, which preferably degrades fibrin (lysis of fibrin; fibrinolysis [3]). Thus, this system plays an important role in the dissolution of thrombi and hemostatic plugs mainly composed of fibrin [4]. PAs are serine proteases that are immunologically classified into tissue-type PA (t-PA) [5] and urokinase-type PA (u-PA) [6]. t-PA has a high affinity for fibrin, reflecting high thrombolytic efficacy [7]. Since t-PA is synthesized by vascular endothelial cells [8] and secreted into the blood, it functions effectively in intravascular fibrinolysis. In contrast, u-PA is mainly synthesized by kidney cells [9], as well as by many normal and carcinoma tissues. Thus, u-PA functions as a fibrinolytic activator in extravascular fibrinolysis.

The inactivation of the fibrinolytic system includes two types of specific inhibitors: α2-antiplasmin (α2-AP) [10] and PA inhibitors (PAl) [11]. α2-AP is a fast-acting plasmin inhibitor that rapidly binds to plasmin via its active and lysine binding sites and specifically inactivates plasmin activity. There are several species of PAlS; type-1 PAI (PAI-1) [12], type-2 PAI (PAI-2) [13], type-3 PAI (PAI-3) [14], and protease nexin I [15]. PAI-1 is a major physiological inhibitor of t-PA and u-PA in the blood. PAI-1 is synthesized by vascular endothelial cells [16] and secreted into the blood. PAI-1 is also released from the α-granules of platelets [17]. Therefore, the balance of functional activities between t-PA and PAI-1 basically determines the fibrinolytic state in the blood [18].

2. Mechanism of pericellular fibrinolysis

In addition to plasminogen activation in the fluid phase, plasminogen is also activated by PAs on the surface of a variety of cells. This cell-associated plasmin generation contributes to various physiological
and pathological phenomena. This mechanism, defined as “pericellular fibrinolysis,” involves several factors, among which the crucial molecules are the cell surface receptors for u-PA, plasminogen and t-PA. The u-PA receptor (u-PAR) is the best characterized of the receptors [19]. The binding of u-PA to u-PAR and the activation of plasminogen on the cell surface by the u-PAR-bound u-PA (u-PA/u-PAR system) are essential for pericellular fibrinolysis. Plasmin generated by the u-PA/u-PAR system activates the proenzyme form of matrix metalloproteinases (MMPs) [20] such as procollagenase (Fig. 2). This proteolytic cascade enhances pericellular proteolysis, which predominantly contributes to several cellular functions such as tumor invasion and/or metastasis, malignant transformation, cell migration, tissue repair, ovulation, spermatogenesis, development, or atherosclerosis, as well as angiogenesis.

1) u-PA. The ligand for u-PAR, u-PA, is a member of the family of serine proteases which converts plasminogen to plasmin. u-PA is found in urine and plasma but is frequently detected in many kinds of cultured cells including malignant cells. u-PA is a key enzyme that initiates the pericellular fibrinolytic process by binding to u-PAR in the cells of both normal and tumor tissues. u-PA is now being studied for its role as an angiogenic factor together with u-PAR in endothelial cells.

u-PA is a 55,000 Da glycoprotein consisting of 411 amino acids. It has several functional regions: an epidermal growth factor-like (EGF) domain, a Kringle (K) domain, and a catalytic or protease (P) domain [21]. Since three amino acid residues (His204, Asp255, and Ser355) form a catalytic triad in the P domain, u-PA belongs to the serine protease family. The EGF and K domains, and the P domain are located on the A and B chains, respectively. Though u-PA has one glycosylated site in the B-chain, the addition of fucose...
residue is observed in the A-chain by an unusual post-synthetic modification [22]. There are two structurally different forms of u-PA. These are single-chain u-PA (scu-PA), which is an intact form of this protein synthesized and secreted from cells, and two-chain u-PA (tcu-PA or UK), which is proteolytically cleaved by kallikrein, Factor XIIa, cathepsins (B, D, G), or plasmin at Lys158-Ile159, but its A and B chains are connected by an intramolecular disulfide bond. scu-PA has little or no plasminogen activator activity and is occasionally called pro-u-PA (pro-u-PA or pro-UK) [23]. However, it has an affinity for fibrin [24] which may be due to its structural conformation. The high affinity of scu-PA and t-PA for fibrin can be advantageous in thrombolytic therapy [25]. In contrast, although tcu-PA lacks the fibrin affinity, it is a fully active enzyme that converts plasminogen to plasmin in plasma. Under some conditions, tcu-PA is further cleaved at Lys135-Lys136 by itself (autolysis), generating a low molecular weight u-PA (LMW-u-PA) (M.W. 33,000) which lacks the functional regions (EGF and K). Therefore, the native u-PA is often called high molecular weight u-PA (HMW-u-PA). Interestingly, the active coagulant factor, thrombin, cleaves Arg156-Phe157. The thrombin-cleaved u-PA no longer has enzymatic activity, indicating that the downstream end of the A-chain is critical for the expression of u-PA activity.

The binding of u-PA to its receptor, u-PAR, is mediated by the amino terminal fragment of the u-PA molecule (a.a. 1–137), in which the sequence in the EGF domain is essential for binding [26]. Therefore, since the catalytic domain of the u-PA is not involved in the binding process, both scu-PA and tcu-PA but not LMW-u-PA can equally bind to u-PAR. Thus, scu-PA bound to u-PAR can be subsequently converted to tcu-PA, and the tcu-PA bound to u-PAR is inhibitable by its inhibitors. The binding of u-PA to its cellular receptor activates the pericellular fibrinolytic system.

2) u-PAR. The specific u-PAR is expressed on the surface of a variety of cells in both normal and carcinoma tissues. The receptor was first identified and isolated from a human monocytoid cell line, U-937 [27], which induced a significant amount of u-PAR when it was stimulated by phorbol ester. This receptor was classified as monococyte-membrane antigen CD87 [28] and identified as monococyte activation antigen Mo3 [29]. u-PAR has thus been studied as one of the key factors involved in cell adhesion [30–32] and the chemotaxis [33] of monococytes, and in the complement system by neutrophil [34]. The structure and function of u-PAR in the fibrinolytic system have been well characterized, especially in human carcinoma cells [19]. However, recent studies have revealed that u-PAR plays a pivotal role in the actions of vascular endothelial cells in angiogenic activities as well as in their antithrombotic properties.

The gene for human u-PAR (PLAUR) is located on chromosome 19q13.3–qter [35]. cDNA encoding human u-PAR (1.4 kb) shows that u-PAR has a signal peptide with 22 amino acids, a mature protein with 313 amino acids, a M.W. of 35,000, and that the protein contains 28 cysteines forming 14 disulfide bonds [36]. u-PAR is comprised of three amino acid homologous repeats: domains I (a.a. 1–92), II (a.a. 93–191), and III (a.a. 192–282) [37] (Fig. 3). Domain I is located at the N-terminus, and it involves the u-PAR binding region (a.a. 1–87) that recognizes the amino terminal fragment (ATF) of u-PA. Thus, u-PAR binds u-PA stoichiometrically at 1:1, mediated by ATF and domain I, respectively. The u-PAR protein is heavily glycosylated at 5 asparagine residues (52, 162, 172, 200, and 233), the nature of which is modified by cell type. Thus, the molecular weight of the whole molecule ranges between 45,000 and 60,000. The mature u-PAR protein lacks a transmembrane sequence, but the C-terminus is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) moiety [38]. The sequences at Ser282-Gly283-Ala284 and Arg290 are responsible for signaling the formation of a GPI anchor [39]. Thus u-PAR protein can be cleaved into a soluble form by phosphatidylinositol-specific phospholipase C (PI-PLC) [40].

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Fig. 3. The structure of u-PAR and its binding to u-PA. There are three sequence-homologous repeats in u-PAR, Domain I, II, and III, among which the N-terminal Domain I binds the amino terminal fragment (ATF) region of u-PA. The u-PAR–pallated molecule attaches to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. The whole ATF includes epidermal growth factor (EGF) and Kringle (K) domains. The catalytic site of u-PA (P) is free from the binding site, thus expressing u-PA activity on u-PAR.
The binding of u-PA to u-PAR has been extensively investigated by chemical cross-linking using N,N'-disuccinimidyl suberate [41]. Since u-PAR recognizes the ATF of u-PA, both scu-PA and tcu-PA but not LMW u-PA bind u-PAR with high affinity ($K_d = 0.4 \text{nM}$) in the monocytoid cell line U937 [42]. In particular, scu-PA and tcu-PA bind with $K_d$ of $2.8 \pm 0.1 \text{nM}$ ($B_{max} = (2.2 \pm 0.1) \times 10^5 \text{cell}^{-1}$) and $2.1 \pm 1.7 \text{nM}$ ($B_{max} = (2.9 \pm 2.9) \times 10^5 \text{cell}^{-1}$), respectively, in endothelial cells [43]. The expression of endothelial u-PAR is thus implicated in the angiogenic action of the vasculature.

The u-PAR-bound scu-PA can be directly converted to tcu-PA by plasmin, cathepsin D or other proteases around the cells [44], thus exposing the catalytic domain of u-PA. Therefore, the u-PAR-bound tcu-PA can activate plasminogen to plasmin. To examine whether the enzymatic activity of u-PA is modulated by the binding to u-PAR, the recombinant soluble form of u-PAR (su-PAR), which lacks the GPI anchor, was employed. The su-PA was expressed by mouse fibroblast (LB6) introduced by human u-PAR cDNA, which codes amino acids Leu to Glu [40], thus lacking the signal function to form the GPI anchor by Ser-Gly-Ala and Arg [39]. Although a mutant scu-PA with slight intrinsic plasminogen activation activity but uncleavable to tcu-PA showed considerably enhanced plasminogen activation upon binding to u-PAR [45], a recent study demonstrated that the enhancement of cellular plasminogen activation by u-PAR-bound scu-PA is mainly due to a cooperative effect with plasmin (ogen) and the specific cellular receptor for plasminogen (Plg-R), and that its binding to u-PAR has no influence on the zymogenic property of scu-PA [46]. If plasminogen is also bound to a plasminogen binding site on the same cell surface, the activation of plasminogen is further accelerated. Moreover, inhibition of the receptor-bound tcu-PA activity by PAI-1 is moderately reduced as compared with the tcu-PA activity in the fluid phase [47]. These characteristics of the u-PA/u-PAR system enhance the level of cellular fibrinolysis, which subsequently triggers the pericellular proteolytic cascade [48].

3) PAI-1. Plasminogen activator inhibitor 1 (PAI-1) is a member of the family of serine protease inhibitors (serpin), which are largely expressed by endothelial cells and stored in platelets. PAI-1 is highly expressed in certain carcinoma cells [49] and regulates the fibrinolytic potential of carcinoma cells [19].

PAI-1 is a single-chain peptide consisting of 379 amino acids (M.W. 54,000) that is glycosylated at 3 asparagine residues (209, 265, and 329). Though human PAI-1 is encoded by a 12.2 kb-gene, two species of PAI-1 mRNAs (3.2 and 2.4 kb) are generated by alternative polyadenylation during splicing [50]. PAI-1 specifically inactivates both t-PA and u-PA. PAI-1 exists mainly in two forms: an active form that inhibits t-PA and u-PA, and a latent form that does not inhibit the PAs due to its conformational change [51]. In addition, there is a substrate form of PAI-1 that does not inactivate t-PA but is cleaved by t-PA, behaving like a substrate of the enzyme [52]. The active form of PAI-1 interacts with PAs at its reactive center, Arg$_{346}$Met$_{347}$ (P1-P1'), and potential binding sites Val$_{10}$-Lys$_{806}$, Asp$_{102}$-Leu$_{103}$-Lys$_{104}$, and Glu$_{105}$ [53]. In addition to the catalytic domains of the PAs, PAI-1 binds the finger domain Lys$_{896}$-Gly$_{902}$ or the Kringle 2 domain of t-PA [54], and the Kringle domain of u-PA. The inhibitory activity of PAI-1 is stabilized by binding to glycosaminoglycan, heparin, or extracellular matrix proteins such as vitronectin, suggesting that PAI-1 functions more effectively in the pericellular space than in the fluid phase.

Though PAI-2 predominantly inhibits u-PA in monocytes [55], PAI-1 preferably inhibits the u-PAR-bound u-PA activity in many cells. Therefore, PAI-1 is the major inhibitor of the u-PA/u-PAR system in pericellular fibrinolysis. In addition, when a complex between u-PAR-bound u-PA and PAI-1 is formed, it is internalized and degraded by the cellular uptake mechanism mediated by low-density lipoprotein receptor/α$_2$-macroglobulin receptor-related protein (LRP), which is described in the following section.

4) Other fibrinolytic receptors (Plg-R, t-PAR, and LRP). Though plasminogen binds the surface of platelets via integrin GPIIb/IIIa, the specific cellular receptor for plasminogen (Plg-R) has been found in various cell types, including peripheral blood cells (platelets, monocytes, granulocytes, and lymphocytes), endothelial cells, smooth muscle cells and fibroblasts, as well as in some malignant cells. The affinity of plasminogen to Plg-R on the cell surface is relatively low ($K_d = 0.3-2.8 \mu M$) compared with that of u-PA to u-PAR, but the binding is also a key event for cellular fibrinolysis. The receptor-bound plasminogen is activated by u-PAR-bound u-PA far more efficiently than in the soluble phase. Since plasminogen binds Plg-R via a lysine-binding site (LBS) in the Kringle 1–3 domains, it can be activated to plasmin on the receptor, and occupancy of the LBS, one of the recognition sites by α$_2$-AP, results in partial protection against inhibition by α$_2$-AP [56].

Plg-R is completely different from u-PAR, and the expression of Plg-R and u-PAR on the same cell surface has often been observed [57]. Some of the Plg-Rs share their plasminogen binding sites with t-PA bind-
ing sites. One candidate for Plg-R, α-enolase [58], and others possess both plasminogen and t-PA binding sites at a common location [59]. An analog of the calcium- and phospholipid-binding protein annexin II, which is a specific receptor for t-PA (t-PAR), has distinct binding regions for each ligand [60, 61]. One binding site, located at the N-terminal region, can bind t-PA; the other, located at the C-terminal region, binds plasminogen. Thus, annexin II is a dual-ligand receptor which may induce considerably enhanced plasminogen activation by t-PA on the cell surface [62]. Our study, however, demonstrated that t-PAR, which binds t-PA but not plasminogen, is expressed on the surface of normal endothelial cells [63, 64]. Purified t-PAR enhances the plasminogen activation by t-PA, suggesting that this t-PAR is an intact form of the receptor for t-PA expressed in endothelial cells. Though the other type of t-PAR that recognizes the C-terminal region with a catalytic domain is found in endothelial cells [65, 66], the function of t-PAR remains unknown. It seems that these fibrinolytic t-PARs function as anti-thrombotic regulators in the endothelium by binding t-PA and expressing its enzymatic activity more efficiently on the cell surface. In contrast, several studies have shown that t-PA is internalized and degraded in hepatocytes [67] by metabolic receptors for t-PA such as the mannan receptor [68]. Thus, t-PAR plays a pivotal role in the regulation of t-PA levels in the blood through the catabolic t-PARs in hepatocytes, and in the regulation of cellular fibrinolysis through fibrinolytic t-PARs in vascular endothelial cells.

Low-density LRP participates in the regulation of u-PA and t-PA clearance in cells [69]. LRP is a multi-ligand receptor distributed in hepatocytes, macrophages, smooth muscle cells, and neurons, and it plays an important role in the catabolism of lipoproteins, proteasines, and proteinase-inhibitor complexes. LRP binds not only t-PA and u-PA but also t-PA/PAI-1 and u-PA/PAI-1, followed by the cellular uptake of these ligands. It is notable that u-PA, which is bound by pro-u-PA or u-PA/PAI-1, may be endocytosed along with LRP and that u-PAR and LRP may be recycled [70]. Although LRP has not been identified in endothelial cells, the clearance of u-PA and/or t-PA may proceed through other LRP-like receptors such as the very-low-density lipoprotein receptor (VLDL-R) [71, 72]. In contrast, the formation of a complex of u-PA and PAI-2 generates a new epitope which can be recognized by cellular receptor(s), and the uptake of the complex seems not to be mediated by the u-PAR system in monocytes [73]. Therefore, several types of cellular uptake and degradation of fibrinolytic compo-

ments may possibly be regulated by receptors which have not yet been identified.

**Pericellular Fibrinolysis in Angiogenesis**

Angiogenesis plays an important role in the process of various physiological and pathological events, including normal embryogenesis and growth, wound healing, tumor development, atherosclerosis, and rheumatoid arthritis. Angiogenesis is achieved by the following serial biological stages: 1) the degradation of vascular basement membrane by endothelial cells; 2) the migration of endothelial cells with digesting fibrin deposits or interstitial components; 3) the proliferation of endothelial cells; and 4) the formation of new tubular structures with de novo basement membranes. The pericellular proteolytic activities are essential for the progression of the first two steps of angiogenesis; namely, local proteolytic remodeling of the matrix and the migration of endothelial cells [74, 75] (Fig. 4).

Since endothelial cells express u-PAR [76] and as well as Plg-R [77] on their cell surfaces and secrete both u-PA and t-PA as well as their inhibitor PAI-1 [18], the cellular fibrinolysis by the u-PA/u-PAR system in endothelial cells may play an important role in the regulation of the early stage of angiogenesis. Therefore, the initiation of angiogenesis depends on the upregulation of cellular fibrinolysis in endothelial cells. The regulation of cellular fibrinolysis may be controlled by stimulation with a number of biologically active substances. The contact of fibrin with endothelial cells enhances the secretion of t-PA but considerably reduces the secretion of PAI-1 [16] via the downregulation of protein kinase C [78], resulting in enhanced fibrinolytic activity around the cells. The damaged tissues may have a high temperature due to the heat produced by inflammatory substances. The hyperthermia also promotes the fibrinolytic potential of endothelial cells by upregulating u-PAR as well as u-PA and t-PA [79]. Plasmin generated on the cell surface by the u-PA/u-PAR system then degrades the basement membrane and interstitial matrix by itself, or activates a panel of proenzymes of MMPs such as collagenases (MMP-1, -8), stromelysins (MMP-3, -4), and gelatins (MMP-2, -9) [80–83]. Thus, plasmin initiates pericellular proteolysis by MMPs.

Angiogenesis is possibly modulated by the growth and migration of endothelial cells mediated by cytokines such as interleukin-6 (IL-6) [84], IL-8, and tumor necrosis factor α (TNF-α) [85], and by growth factors including fibroblast growth factor (FGF) [86, 87], platelet-derived endothelial cell growth factor (PD-ECGF) [88], vascular endothelial cell growth fac-
Fig. 4. Pericellular fibrinolysis by u-PA/u-PAR system and pericellular proteolysis in angiogenesis [75]. Angiogenesis is initiated when pericellular fibrinolysis is enhanced by the u-PA/u-PAR system, which subsequently activates pericellular proteolysis by plasmin and MMPs. Basic fibroblast growth factor (bFGF) stimulates the expression of both u-PA and u-PAR as well as the proliferation of endothelial cells (EC) in an autocrine fashion. In the later stage of angiogenesis, these activities in EC are downregulated by transforming growth factor-β (TGF-β) to prevent the proliferation of EC. TGF-β stimulates the expression of PAI-1 and tissue inhibitors of metalloproteinases (TIMPs), which inhibits the u-PA/u-PAR system and pericellular proteolysis by blocking u-PAR-bound u-PA and matrix metalloproteinase (MMPs), respectively. Abbreviations: Plg, plasminogen; Plm, plasmin; Plg-R, plasminogen receptor; u-PAR, urokinase receptor; PAI-1, plasminogen activator inhibitor-1.

tor (VEGF), which was previously identified as vascular permeability factor (VPF) [89, 90], placenta growth factor (PLGF) [91], hepatocyte growth factor (HGF) [92], epidermal growth factor (EGF) [93], insulin-like growth factor (IGF-1) [94], and other growth factors that induce angiogenesis [95]. Interestingly, VEGF and PLGF exist as heterodimers [96] and exhibit more potent mitogenic activity than VEGF homodimers do [97], which is suggested to occur in signal transduction by VEGF receptor Flt-1 [98]. Some of these angiogenic growth factors promote not only proliferation and/or migration but also plasminogen activation on endothelial cells. The potent growth factor basic FGF (bFGF) upregulates both u-PA activity [99] and u-PAR levels [100] but suppresses PAI-1 release [101]. Furthermore, TNF-α, bFGF, and VEGF cooperatively enhance the formation of tubular structures by upregulating u-PA activity [102]. Therefore, in addition to the proliferative and migratory effects of cytokines and growth factors, endothelial cells can more effectively proliferate and migrate after degrading extracellular matrix by local proteolysis, which is induced by the increased potential for cellular fibrinolysis in the u-PA/u-PAR system in response to these growth factors. In contrast, transforming growth factor-β (TGF-β), which is produced by various normal cells including platelets as well as malignant cells, suppresses the proliferation and migration of endothelial cells [103, 104]. TGF-β also reduces the bFGF-mediated increase in the plasminogen activation activity of endothelial cells partially due to the increase of PAI-1 expression in these cells stimulated by TGF-β [105]. TGF-β prevents the expression of MMP-1 and MMP-3 and is thus regarded as an anti-angiogenic factor. TGF-β is secreted in an inactive (latent) form, but it is proteolytically converted to the active form by plasmin and cathepsin D. Therefore, TGF-β is activated by the enhanced generation of plasmin via the u-PA/u-PAR system. Active TGF-β stimulates the production of tissue inhibitors of metalloproteinases (TIMPs) [106–108] that selectively inactivate MMPs. Thus, activated TGF-β blocks enhanced cellular fibrinolysis/pericellular proteolysis during the early stages of angiogenesis, and then induces reduced cellular fibrinolysis/pericellular proteolysis in the late stages of angiogenesis. At this point, pericellular proteolytic activities must be suppressed so that the endothelial cells can establish new matrices and form tubular structures.

Fibrin, which is a final product of the coagulation system, is also involved in the angiogenic process [109]. Since one of the inflammatory responses of endothelial cells is the formation of a fibrin deposit, it has been reported that fibrin itself activates endothe-
Pericellular Fibrinolytic System in Angiogenesis

Pericellular fibrinolysis induced by the activation of plasminogen on the cell surface, which triggers a proteolytic cascade in the pericellular space, plays a pivotal role in the regulation of many biological processes of both normal and cancerous cells. Cellular fibrinolysis is induced by the u-PA/u-PAR system, in which u-PA binds to its specific receptor (u-PAR) anchored on the cell membrane and initiates the cell surface-associated activation of plasminogen. This system functions more efficiently when binding sites for plasminogen are co-expressed on the same cell surface. Plasminogen binds not only Plg-R but also t-PAR; thus, these fibrinolytic receptors contribute to acceleration of the u-PA/u-PAR system.

Angiogenesis is a feature of vascular endothelial cells during several physiological and pathological processes. The u-PA/u-PAR system in endothelial cells is upregulated during the early stages of angiogenesis, in which enhanced proteolytic activity is required for the migration of cells by digesting vascular basement membrane and interstitial matrix. In the later stages of angiogenesis, the u-PA/u-PAR system is downregulated by PAI-1 and endothelial cells adhere to matrix to form tubular structures.

Various angiogenic factors (e.g., growth factors and cytokines) regulate the expression of these receptor systems as well as inhibitor systems (i.e., PAI-1 and TIMPs). Therefore, cellular fibrinolysis on endothelial cells is upregulated in the early stage of angiogenesis to enable the cells to degrade basement membrane and migrate through the extracellular matrix. Thereafter, cellular fibrinolysis is downregulated in the later stage of angiogenesis to enable the migrated cells to reorganize and form tubular structures.

Thus, the pericellular fibrinolytic system regulated by u-PA and u-PAR is intimately involved in the process of angiogenesis.

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