MINIREVIEW

Plasminogen and Plasminogen Activator Assembly on the Human Endothelial Cell

(43534A)

GILES C. SHIH AND KATHERINE A. HAJJAR[1]

Departments of Pediatrics and Medicine, Divisions of Hematology-Oncology, Cornell University Medical College, New York, New York 10021

Endothelial Cell Thromboregulation. The circulatory system's endothelial cell lining, uniquely situated at the interface between blood and vessel wall, plays a dynamic role in thromboregulation. Previously thought to represent a passive surface, the endothelium is now known to support a number of anticoagulant and profibrinolytic systems that serve to maintain the fluidity of blood. At least three of these systems involve the assembly of macromolecules on the endothelial cell surface.

Two distinct anticoagulant systems associated with the endothelial cell surface preserve vessel patency by modifying thrombin activity. In the first, heparin and heparin-like molecules synthesized and secreted by the endothelium enhance the anticoagulant effect of the protease inhibitor antithrombin III (1). Heparin and other mucopolysaccharides present on the endothelial cell surface serve to alter the configuration of antithrombin III, allowing it to bind to and inactivate thrombin with increased efficiency (1). Furthermore, antithrombin III exerts a heparin-mediated inhibitory effect on coagulation factors IX_a and X_a on the surface of bovine aortic endothelial cells, suggesting a broader range of action (2).

A second surface-oriented anticoagulant system on the cell surface involves thrombomodulin, a thrombin-binding glycoprotein present on all endothelial cells except those in the microcirculation of the human brain (3, 4). When thrombin and thrombomodulin complex on the cell surface, thrombin acquires the ability to activate protein C instead of clotting fibrinogen (5, 6). Activated protein C, an endothelial-cell-dependent serine protease inhibitor, then deactivates clotting factors V_a and $VIII_a$ through its interaction with the cofactor protein S, which also binds to the endothelial cell with high affinity (7, 8). Thus, expression of cell surface binding sites provides a key infrastructure for the vessel wall's anticoagulant defense, and the clinical expression of deficiency of protein C, protein S, or antithrombin III is, indeed, that of a prothrombotic state.

In the fluid phase, the endothelial cell acts to control thrombus formation by synthesizing several reactants that inhibit platelet aggregation. For example, prostaglandin (PG) D_2 and PGI_2 (prostacyclin), metabolites of arachidonic acid, inhibit platelet aggregation and induce vasodilation through a cyclic AMP-dependent mechanism (9). PGI_2 synthesis by human umbilical vein endothelial cells is stimulated by thrombin, which suggests that PGI_2 may serve to localize thrombus formation by limiting platelet aggregation at the site of thrombin activity (10). Thus, one role of PGI_2 may be to prevent overpropagation of an evolving thrombus.

Another product of vascular endothelium that promotes vasodilatation and inhibits platelet aggregation is endothelium-derived relaxing factor (EDRF), a product of vascular endothelium. EDRF is an unstable, nonprostanoid substance released constitutively and also in response to a variety of neurohormonal stimuli (11). At least one form of EDRF is now known to be nitric oxide (12), a vasodilator that also inhibits platelet aggregation by increasing cyclic GMP levels (13). Thus, EDRF and PGI_2 act through distinctly different second messengers to modulate platelet responsiveness.

[1] To whom correspondence should be addressed at Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

P.S.E.B.M. 1993, 202: 258–264

0037-9727/93/2023-0258$3.00/0

Besides releasing PGI_2 and EDRF into the circulation, the endothelial cell can also inhibit platelet aggregation through a surface-connected ecto-ADPase (14, 15). This enzyme, unlike prostacyclin and EDRF, is not secreted and mediates its antiaggregatory effect by hydrolyzing platelet-derived ADP, and eliminating its proaggregatory effect (15). The endothelial cell ADPase, unlike PGI_2, is completely insensitive to the effects of aspirin (15).

In addition to promoting anticoagulant activity, the endothelium also plays many roles in regulating the generation of plasmin. Plasmin is a serine protease responsible for a number of biological events in the human body, only one of which is the solubilization of fibrin (16, 17). Fibrin-based generation of plasmin represents a relatively late response to thrombus formation. The notion that plasmin may be formed constitutively on the endothelium as a consequence of plasminogen and plasminogen activator assembly is a relatively new concept in hemostasis and thrombosis (18), and may represent an additional defense aimed at maintaining the fluidity of blood. The following sections of this Minireview will examine the potential means by which the endothelial cell may regulate plasmin generation. These mechanisms include synthesis and secretion of plasminogen activators and their inhibitors, and assembly of plasminogen and plasminogen activators on the cell surface.

Endothelial Cell Synthesis of Plasminogen Activators and Plasminogen Activator Inhibitor 1

Plasmin activity is regulated by the endothelial cell at many levels. Expression of genes encoding tissue plasminogen activator and its physiologic inhibitor, plasminogen activator inhibitor, type 1 (PAI-1), is subject to modulation by a number of soluble mediators. Thrombin (19), phorbol myristate acetate (20), and butyric acid (21) have all been associated with elevated tissue plasminogen activator (t-PA) mRNA levels in the endothelial cell. Of these three, however, only thrombin elicited a concomitant increase in PAI-1 mRNA levels (19). In addition, shear stress from flowing blood elevated t-PA mRNA levels (22). These observations indicate that the endothelial cell increases t-PA production in response to several agents that perturb the cell, though probably through different mechanisms.

Similarly, PAI-1 synthesis may be stimulated by additional agonists that activate the endothelial cell. In recently published reports, interleukin 1, transforming growth factor-β, tumor necrosis factor, and endotoxin all induced dramatic increases in steady state PAI-1 message levels without affecting t-PA production (23–26). Similarly, treatment of endothelial cells with lipoprotein(a), a low density lipoprotein-like particle clinically associated with atherosclerosis, also induced a 2- to 4-fold increase in PAI-1 levels without affecting t-PA mRNA (27). This suggested that lipoprotein(a) regulates plasmin by a pathway separate from some other mediators (27). Because t-PA and PAI-1 mRNA levels are affected by different stimuli, it is likely that under most circumstances, t-PA production and PAI-1 production in the endothelial cell are independently regulated.

Modification of Plasmin-Forming Proteins by Plasmin

Once formed, plasmin is able to amplify its own generation by modifying proteins involved in plasminogen activation. For example, plasmin converts the circulating zymogen N-terminal glutamic acid plasminogen (Glu-PLG) to N-terminal lysine plasminogen (Lys-PLG) by releasing a 76-amino acid "preactivation" peptide. This shortened form of plasminogen is 10 to 20 times more readily activated by plasminogen activators and has higher affinity for cell surfaces (28–30). Similarly, the plasminogen activators t-PA and urokinase (u-PA) are also converted into more active forms through the action of plasmin. t-PA, synthesized by the endothelial cell as a single chain M_r 72,000 polypeptide, is converted to a two-chain molecule in the presence of plasmin (31–33). Double chain t-PA more actively cleaves plasminogen in the absence of fibrin (31), which would render it particularly efficient on a fibrin-free cell surface. Similarly, u-PA is converted from single chain to double chain form when exposed to plasmin (33). Upon conversion to the two-chain form, u-PA loses its ability to bind to fibrin, which suggests that the two-chain form of u-PA, like two-chain t-PA, may be preferentially destined for plasminogen activation on the cell surface.

Cell Surface Binding Sites for Plasminogen and Its Activators

Recent evidence has shown that plasmin-generating systems can assemble on the cell surface. In particular, assembly of plasminogen and plasminogen activators may facilitate the formation of the active protease.

Plasminogen. N-terminal glutamic acid plasminogen, the M_r 93,000 zymogen precursor of plasmin, is synthesized by the liver and circulates in plasma at a concentration of ~1.5 μM (17). Hydrolysis of the Arg 560-Val 561 peptide bond of plasminogen by either t-PA or u-PA results in the generation of the disulfide-linked, two-chain molecule, plasmin (17). Plasmin has a very broad substrate specificity, and represents the major fibrinolytic enzyme in humans (16).

In binding to the endothelial cell, circulating Glu-PLG is converted to its truncated, noncirculating form, Lys-PLG, through the proteolytic release of a 76-amino acid N-terminal preactivation peptide (34). *In vitro*, radiolabeled Glu-PLG exposed to cultured endothelial

cells or to fresh blood vessel segments yielded two molecular mass forms: the original species and an apparently truncated molecule that co-migrated with Lys-PLG on sodium dodecyl sulfate gels and reacted with a Lys-PLG-specific monoclonal antibody (34). Lys-PLG is known to be 10 to 20 times more efficiently activated by either t-PA or u-PA (28–30). This conversion of Glu-PLG to Lys-PLG provides an explanation for the observation that the efficiency of plasmin generation at the cell surface was increased over 12 times compared with the same reaction in the fluid phase (35, 36). Cell-surface-mediated conversion of Glu-PLG to Lys-PLG has also now been reported for U937 cells (37).

Recent studies have demonstrated further that a major plasminogen binding site on human endothelial cells binds Lys-PLG with 2.6 times greater affinity than Glu-PLG (34). Radiolabeled Glu-PLG bound to human umbilical vein endothelial cell monolayers in a rapid, reversible manner with high affinity (K_d 310 nM) and capacity (B_{max} 1,400,000 sites per cell) (34). Lys-PLG binding to cultured endothelial cells, although also rapid and reversible, displayed higher affinity (K_d 120 nM) and somewhat lower capacity (B_{max} 390,000) (36), which suggests that Lys-PLG may represent a form preferentially associated with cell surfaces. Cold competition studies suggest that Lys-PLG and Glu-PLG compete for the same binding site on the endothelial cell, but that Lys-PLG may represent a preferred ligand due to its higher affinity for plasminogen binding sites. Of several serine protease inhibitors, including α_2-plasmin inhibitor, only diisopropyl fluorophosphate, a low molecular weight agent that covalently targets the active site serine, blocked the conversion of Glu-PLG to Lys-PLG on the endothelial cell (34).

Approximately four distinct plasmin/plasminogen-binding proteins have been identified on various types of cells. On U937 cells, for example, α-enolase, a glycolytic enzyme, has been found to interact with plasminogen through a carboxy terminal lysine residue (38). In rat kidney glomerular and tubular epithelial cells, a large glycoprotein called the Heymann nephritis antigen (gp 330) may function as a plasminogen receptor (39). Another group has recently reported an M_r 41,000 protein that interacts with plasmin on the surface of Group A streptococci (40). Finally, we have identified an M_r 40,000 protein expressed on human endothelial cells and purified from human placenta that specifically binds plasminogen with an affinity remarkably similar to that observed on the cultured endothelial cell (41). This protein has the interesting property of independently binding tissue plasminogen activator, but not urokinase, with high affinity (41, 42). Since plasminogen and tissue plasminogen activator bound to this protein noncompetitively, this receptor may serve to colocalize enzyme and substrate in a manner that enhances the efficiency of plasmin activation.

Lipoprotein(a). Lipoprotein(a) (Lp(a)) is a low density lipoprotein-like particle that is clinically associated with atherosclerosis (43–45). Lp(a) consists of a phospholipid-cholesterol core and two associated protein moieties. One of these, apolipoprotein B100, is also found on the low density lipoprotein particle. The second, apolipoprotein(a) (apo(a)), is linked to apoB100 through a disulfide bond. Apo(a) shares remarkable homology with plasminogen, including multiple repeating domains similar to the "kringle" four unit of plasminogen, a single kringle five-like region, and a "pseudo" protease segment (46, 47). Furthermore, the finding that plasminogen and apo(a) are genetically linked on chromosome 6 suggests that they may have arisen from a common ancestral gene (48).

Because of the strong structural similarity between apo(a) and plasminogen, a number of groups have studied the effects of Lp(a) on plasminogen binding to cell surfaces. On cultured endothelial cells, Lp(a) purified from blood from two different donors inhibited 50% of Lys-PLG binding at 7- and 22-fold molar excess ratios, respectively (49). In addition, a 36-fold molar excess of purified apo(a) effectively inhibited 50% of Lys-PLG binding to cultured endothelial cells, whereas neither low density lipoprotein nor lipoprotein(−), the reductively cleaved apo(a)-free particle, inhibited Lys-PLG binding at all (49). These results implicate the kringle-containing apo(a) component of the Lp(a) molecule in competing with plasminogen for binding sites on the endothelial cell. Additional studies have shown that Lp(a) can block plasmin generation on the cell surface but not in the fluid phase (49). The ability of excess Lp(a) to interfere with plasminogen binding, and hence plasmin generation, may link a subclinical thrombotic tendency and the development of atherosclerosis.

Plasminogen Activators

In addition to binding plasminogen, the endothelial cell also expresses cell surface binding sites for plasminogen activators, allowing enzyme and substrate to assemble on the cell surface while also preserving the catalytic activity of the activator.

Urokinase. Urokinase is an M_r 55,000 serine protease synthesized and secreted as the single-chain proenzyme (scu-PA). On the cell surface, scu-PA is converted to a two-chain enzyme also known as high molecular weight urokinase (49). This form can be further cleaved by plasmin to yield a low molecular weight species and an amino-terminal fragment (ATF) that contains the epidermal growth factor (EGF) and kringle domains (50).

Urokinase has been shown to bind a variety of cell types, including monocytoid U937 cells (51–54), A431 epidermoid carcinoma cells (55, 56), fibroblasts (57–59), mouse spermatozoa (60), Friend erythroleukemia

cells (61), and bovine corneal endothelial cells (62). In addition, u-PA has recently been observed to bind human umbilical vein endothelial cells (42, 50, 63). In binding assays conducted on endothelial cell monolayers, ^{125}I-labeled scu-PA bound the cell monolayer in a saturable, reversible, and specific manner with K_d 2.8 nM and B_{max} 2.2 × 10^5 sites/cell (50). Binding of labeled scu-PA was inhibited by the unlabeled ligand, by ATF, and by a peptide fragment mimicking the EGF domain. It appears from these data that a portion of the EGF domain is required for binding of scu-PA to endothelial cells. The EGF domain has also been implicated in u-PA binding to the U937 monocytoid cell line (64). In fact, all cells that have receptors for u-PA on the cell surface appear to specifically bind u-PA via this ATF sequence (64). Thus, the ATF-deficient low molecular weight u-PA is unable to bind the endothelial cell while the ATF-containing scu-PA and two-chain enzyme retain high affinity.

Recently, cDNA encoding a urokinase receptor was cloned and sequenced from a human fibroblast cDNA library (65). The full-length cDNA predicted a protein of 313 amino acids with a 21-residue signal peptide. Expression of the protein in murine LB6 cells conferred the ability to bind urokinase as judged by a caseinolytic assay. Further analysis of the purified protein revealed a single M_r 55,000–60,000 band by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, which was reduced to M_r 35,000 upon enzymatic deglycosylation (63, 66). Urokinase associated with this receptor appeared to maintain its susceptibility to the physiologic inhibitor, plasminogen activator inhibitor 1 (67, 68), which may promote its clearance by monocytoid cells (69). A similar u-PA-binding protein has been identified by ligand blotting in extracts of a plasma membrane preparation from cultured human umbilical vein endothelial cells (42).

Tissue Plasminogen Activator. Another plasminogen activator that possesses high affinity for the endothelial cell surface is tissue plasminogen activator (70). t-PA has previously been shown to function efficiently on fibrin, thrombospondin, or histidine-rich glycoprotein (71). However, recent evidence has shown that t-PA can also bind through a variety of mechanisms to many types of cells, including human aortic endothelial cells (72), human fibroblasts (70, 73), rat and human hepatoma cells (74–76), bovine alveolar macrophages (77), neuroblastoma cells (78), and human smooth muscle cells (70), in addition to cultured human umbilical vein endothelial cells (70, 79, 80).

t-PA binding to cultured human umbilical vein endothelial cells has been studied in some detail. The binding isotherm described a specific, largely reversible, high affinity interaction consisting of two classes of saturable sites (K_{d1} 29 pM, B_{max1} 3,700 sites/cell and K_{d2} 18 nM, B_{max2} 815,000 sites/cell) (70). Unlike plasminogen, t-PA interaction with the major binding site was essentially lysine-binding site independent. Once bound to the endothelial cell surface, t-PA appeared to be protected from its physiologic inhibitor, plasminogen activator inhibitor, type 1 (70). Further evidence suggests that the high affinity t-PA binding site associated with endothelial cell monolayers represents PAI-1, which is primarily matrix associated (80).

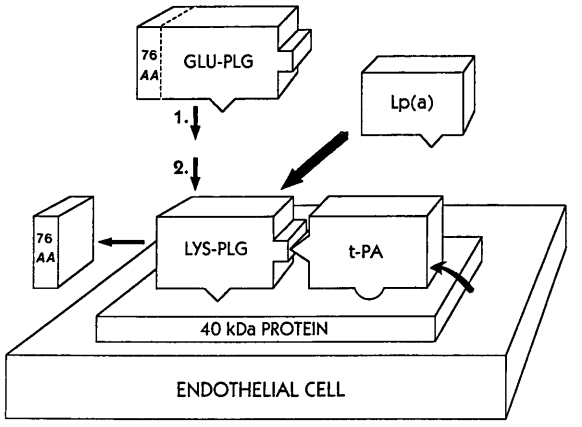

Figure 1. Hypothetical model of plasminogen and tissue plasminogen activator assembly on the endothelial cell surface. Upon binding to the endothelial cell surface, circulating N-terminal glutamic acid plasminogen is converted to its truncated, noncirculating form, N-terminal lysine plasminogen, through the proteolytic release of a 76-amino acid preactivation peptide (76 AA). Lys-PLG binds with high affinity to a 40-kDa cell surface associated protein. Tissue plasminogen activator, synthesized and secreted by the endothelial cell, can bind to the same protein at a separate domain. Assembly of plasminogen and t-PA in complex with the 40-kDa protein on the cell surface would foster efficient generation of plasmin. Lipoprotein(a), in sufficient concentration, would compete with plasminogen for its binding site on the endothelial cell, thereby dampening production of the active protease.

To study membrane-associated binding sites on cultured endothelial cells in the absence of matrix, cell membrane preparations were isolated and studied. A plasma membrane fraction was found to bind ^{125}I-t-PA at a single saturable site (K_d 9.1 nM, B_{max} 3.1 pmol/mg membrane protein) (42). Ligand-blotting experiments revealed an M_r 40,000 membrane protein present in detergent extracts of isolated membranes that bound both single and double chain t-PA (42). The interaction was reversible, cell-specific, and sensitive to nanomolar concentrations of trypsin. The relevant binding protein was not found in the subendothelial matrix, failed to react with antibodies against PAI-1, and interacted with t-PA in an active site-independent manner (42). The isolated t-PA binding site was resistant to reduction and preserved its capacity for plasmin generation (42). These studies clearly distinguished the t-PA binding protein from the u-PA receptor and also from PAI-1. Further studies of this receptor have revealed a separate binding domain for plasminogen and t-PA (41), raising the possibility that it may facilitate the assembly of both

plasminogen and t-PA, thereby promoting generation of plasmin (Fig. 1).

Summary

Through assembly of plasminogen and its activators, the endothelial cell surface may provide a favorable environment for constitutive generation of plasmin. This system may be regulated at multiple levels. Abundant expression of a 40-kDa protein with dual ligand-binding capacity may promote cell surface plasmin formation by colocalizing t-PA and plasminogen in a catalytically favorable configuration. Conversion of Glu-PLG to the preactivated form Lys-PLG, in the vicinity of the cell surface, may also precede plasmin formation. Physiologic concentrations of Lp(a), furthermore, may serve to modulate plasminogen activation at the cell surface by competing for binding sites, whereas elevated levels of Lp(a) might suppress this mechanism and lead to a subclinical prothrombotic state. Finally, cell surface binding sites for both plasmin and t-PA appear to protect these molecules from their physiologic antagonists, α_2-plasmin inhibitor and plasminogen activator inhibitor, type-1, respectively. Plasmin formation may contribute to the nonthrombogenicity of the blood vessel wall.

This work was supported by the NIH (HL 42493, HL 46403, HL 18828), the American Heart Association, with funds contributed by the New York affiliate, and the Council for Tobacco Research-USA, Inc. Dr. Hajjar is a Syntex Scholar and an Established Investigator of the American Heart Association.

1. Rosenberg RD. Biochemistry of heparin antithrombin interactions, and the physiologic role of this natural anticoagulant mechanism. Am J Med **87**:2S–9S, 1989.
2. Stern DM, Nawroth P, Marcum J, Handley D, Kisiel W, Rosenberg R, Stern K. Interaction of antithrombin III with bovine aortic segments. Role of heparin in binding and enhanced anticoagulant activity. J Clin Invest **75**:272–279, 1985.
3. Ishii H, Salem HH, Bell CE, Laposata EA, Majerus PW. Thrombomodulin, an endothelial anticoagulant protein, is absent from the human brain. Blood **67**:362–365, 1986.
4. Maruyama I, Bell CE, Majerus PW. Thrombomodulin is found on endothelium of arteries, veins, capillaries and lymphatics, and on syncytiotrophoblast of human placenta. J Cell Biol **101**:363–371, 1985.
5. Johnson AE, Esmon NL, Laue TM, Esmon CT. Structural changes required for activation of protein C are induced by Ca^{++} binding to a high affinity site that does not contain gamma-carboxyglutamic acid. J Biol Chem **258**:5554–5560, 1983.
6. Owen WG, Esmon CT. Functional properties of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. J Biol Chem **256**:5532–5535, 1981.
7. Harris KW, Esmon CT. Protein S is required for bovine platelets to support activated protein C binding and activity. J Biol Chem **260**:2007–2010, 1985.
8. Stern DM, Nawroth PP, Harris K, Esmon CT. Cultured bovine aortic endothelial cells promote activated protein C-protein S-mediated inactivation of factor Va. J Biol Chem **261**:713–718, 1986.
9. Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. J Clin Invest **66**:979–986, 1980.
10. Weksler BB, Ley CW, Jaffe EA. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin and the ionophore A 23187. J Clin Invest **62**:923–930, 1978.
11. Myers P, Guerra R Jr, Harrison D. Release of NO and EDRF from cultured bovine aortic endothelial cells. Am J Physiol **256**:H1030–H1037, 1989.
12. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature **327**:524–526, 1987.
13. Radomski MW, Palmer RMJ, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. Br J Pharmacol **92**:181–187, 1987.
14. Glasgow JG, Schade R, Pitlick FA. Evidence that ADP hydrolysis by human cells is related to thrombogenic potential. Thromb Res **13**:255–266, 1978.
15. Marcus AJ, Safier LB, Hajjar KA, Ullman HL, Islam N, Broekman MJ, Eiroa AM. Inhibition of platelet function by an aspirin-insensitive endothelial cell ADPase. Thromboregulation by endothelial cells. J Clin Invest **88**:1690–1696, 1991.
16. Saksela O. Plasminogen activation and regulation of pericellular proteolysis. Biochim Biophys Acta **823**:35–65, 1985.
17. Castellino FJ. Biochemistry of human plasminogen. Semin Thromb Hemost **10**:18–23, 1984.
18. Hajjar, KA. Assembly of the fibrinolytic system on endothelial cells. In: Braunwald E, Haber E, Eds. Thrombolysis: Basic Contributions and Clinical Progress. St. Louis, CV Mosby, pp27–32, 1990.
19. Dichek D, Quertermous T. Thrombin regulation of mRNA levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in cultured human umbilical vein endothelial cells. Blood **74**:222–228, 1989.
20. Levin EG, Marotti K, Santell L. Protein kinase C and the stimulation of tissue plasminogen activator release from human endothelial cells. Dependence on the elevation of messenger RNA. J Biol Chem **264**:16030–16036, 1989.
21. Kooistra T, van den Berg J, Tons A. Butyrate stimulates tissue-type plasminogen activator synthesis in cultured human endothelial cells. Biochem J **247**:605–612, 1987.
22. Diamond SL, Eskin SG, McIntire LV. Fluid flow stimulates tissue plasminogen activator secretion by cultured human endothelial cells. Science **243**:1483–1485, 1989.
23. Medina R, Socher SH, Han JH, Friedman PA. Interleukin-1, endotoxin or tumor necrosis factor/cachectin enhance the level of plasminogen activator inhibitor messenger RNA in bovine aortic endothelial cells. Thromb Res **54**:41–52, 1989.
24. Sawdey M, Podor TJ, Loskutoff DJ. Regulation of type-1 plasminogen activator inhibitor gene expression in cultured bovine aortic endothelial cells. Induction by transforming growth factor-beta, lipopolysaccharide, and tumor necrosis factor-alpha. J Biol Chem **264**:10396–10401, 1989.
25. Van den Berg EA, Sprengers ED, Jaye M, Burgess W, Maciag T, van Hinsbergh VWM. Regulation of plasminogen activator inhibitor-1 mRNA in human endothelial cells. Thromb Haemost **60**:63–67, 1988.
26. Van Hinsbergh VWM, Kooistra T, van den Berg EA, Princen HMG, Fiers W, Emeis J. Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. Blood **72**:1467–1473, 1988.
27. Etingen OR, Hajjar DP, Hajjar KA, Harpel PC, Nachman RL. Lipoprotein(a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. A potential mechanism in thrombogenesis. J Biol Chem **266**:2459–2465, 1990.
28. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the

activation of plasminogen by human tissue plasminogen activator. J Biol Chem **257**:2912–2919, 1982.
29. Markus G, Evers JL, Hobika GH. Comparison of some properties of native (Glu) and modified (Lys) human plasminogen. J Biol Chem **253**:733–739, 1978.
30. Markus G, Priore RL, Wissler FC. The binding of tranexamic acid to native (Glu) and modified (Lys) human plasminogen and its effect on conformation. J Biol Chem **254**:1211–1216, 1979.
31. Tate KM, Higgins DL, Holmes WE, Winkler ME, Heyneker HL, Vehar GA. Functional role of proteolytic cleavage at arginine-275 of human tissue plasminogen activator as assessed by site-directed mutagenesis. Biochemistry **26**:338–343, 1987.
32. Ichinose A, Kisiel W, Fujikawa K. Proteolytic activation of tissue plasminogen activation by plasma and tissue enzymes. FEBS Lett **175**:412–418, 1984.
33. Kasai S, Arimura H, Nishida M, Suyama T. Primary structure of single-chain pro-urokinase. J Biol Chem **260**:12382–12389, 1985.
34. Hajjar KA, Nachman RL. Endothelial cell-mediated conversion of Glu-plasminogen to Lys-plasminogen. Further evidence for assembly of the fibrinolytic system on the endothelial cell surface. J Clin Invest **82**:1769–1778, 1988.
35. Schafer AI, Rodriguez R, Loscalzo J, Gimbrone MA Jr. Inhibition of vascular endothelial cell prostacyclin synthesis by plasmin. Blood **74**:1015–1020, 1989.
36. Hajjar KA, Harpel PC, Jaffe EA, Nachman RL. Binding of plasminogen to cultured endothelial cells. J Biol Chem **261**:11656–11662, 1986.
37. Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor-bound urokinase: A kinetic study with both cell-associated and isolated receptor. J Biol Chem **266**:12752–12758, 1991.
38. Miles LA, Dahlberg CM, Plescia J, Felez J, Kato K, Plow EF. Role of cell-surface lysines in plasminogen binding to cells: Identification of α-enolase as a candidate plasminogen receptor. Biochemistry **30**:1682–1691, 1991.
39. Kanalas JJ, Makker SP. Identification of the rat Heymann nephritis autoantigen (GP330) as a receptor site for plasminogen. J Biol Chem **266**:10825–10829, 1991.
40. Broder CC, Lottenberg R, Von Mering GO, Johnston KH, Boyle MDP. Isolation of a prokaryote plasmin receptor: Relationship to a plasminogen activator produced by the same organism. J Biol Chem **266**:4922–4928, 1991.
41. Hajjar KA. The endothelial cell tissue plasminogen activator receptor: Specific interactions with plasminogen. J Biol Chem **266**:21962–21970, 1991.
42. Hajjar KA, Hamel NH. Identification and characterization of human endothelial cell membrane binding sites for tissue plasminogen activator and urokinase. J Biol Chem **265**:2908–2916, 1990.
43. Scanu AM, Fless GM. Lipoprotein(a): Heterogeneity and biological relevance. J Clin Invest **85**:1709–1715, 1990.
44. Uterman G. The mysteries of lipoprotein(a). Science **246**:904–910, 1989.
45. Loscalzo J. Lipoprotein(a): A unique risk factor for atherothrombotic disease. Arteriosclerosis **10**:672–679, 1990.
46. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu Q-T, Miller CG, Lawn RM, Scanu AM. Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. Proc Natl Acad Sci USA **84**:3224–3228, 1987.
47. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature **330**:132–137, 1987.
48. Weitkamp LR, Guttormsen SA, Schultz JS. Linkage between the loci for the Lp(a) lipoprotein (Lp) and plasminogen (PLG). Hum Genet **79**:80–82, 1988.
49. Hajjar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature **339**:303–305, 1989.
50. Barnathan ES, Kuo A, Rosenfeld L, Kariko K, Leski M, Robbati F, Nolli ML, Henkin J, Cines DB. Interaction of single-chain urokinase-type plasminogen activator with human endothelial cells. J Biol Chem **265**:2865–2872, 1990.
51. Appella E, Robinson EA, Ullrich SJ, Stoppelli MP, Corti A, Cassani G, Blasi F. The receptor-binding sequence of urokinase. J Biol Chem **262**:4437–4440, 1987.
52. Cubellis MV, Nolli ML, Cassani G, Blasi F. Binding of single-chain prourokinase to the urokinase receptor of human U937 cells. J Biol Chem **261**:15819–15822, 1986.
53. Stoppelli MP, Corti A, Soffientini A, Cassani G, Blasi F, Assoian RK. Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA **82**:4939–4943, 1985.
54. Vassalli JD, Baccino D, Belin D. A cellular binding site for the M_r 55,000 form of the human plasminogen activator, urokinase. J Cell Biol **100**:86–92, 1985.
55. Fibbi G, Dini G, Pasquali F, Pucci M, Del Rosso M. The M_r 17,500 region of the A chain of urokinase is required for interaction with a specific receptor in A431 cells. Biochim Biophys Acta **885**:301–308, 1986.
56. Stoppelli MP, Tacchetti C, Cubellis MV, Corti A, Hearing V, Cassani G, Appella E, Blasi F. Autocrine saturation of prourokinase receptors on human A431 cells. Cell **45**:675–684, 1986.
57. Bajpai A, Baker JB. Cryptic urokinase binding site on human foreskin fibroblasts. Biochem Biophys Res Commun **133**:475–482, 1985.
58. Bajpai A, Baker JB. Urokinase binding sites on human foreskin cells. Evidence for occupancy with endogenous urokinase. Biochem Biophys Res Commun **133**:994–1000, 1985.
59. Del Rosso M, Dini G, Fibbi G. Receptors for plasminogen activator, urokinase, in normal and Rous sarcoma virus-transformed mouse fibroblasts. Cancer Res **45**:630–636, 1985.
60. Huarte J, Belin D, Bosco D, Sappino AP, Vassalli JD. Plasminogen activator and mouse spermatozoa: Urokinase synthesis in the male genital tract and binding of the enzyme to the sperm cell surface. J Cell Biol **104**:1281–1289, 1987.
61. Del Rosso M, Pucci M, Fibbi G, Dini G. Interaction of urokinase with specific receptors abolishes the time of commitment to terminal differentiation of murine erythroleukemia (Friend) cells. Br J Haematol **66**:289–294, 1987.
62. Shuman MA, Merkel CH. Urokinase binding to bovine corneal endothelial cells. Exp Eye Res **41**:371–382, 1985.
63. Barnathan ES, Kuo A, Kariko K, Rosenfeld L, Murray S, Behrendt N, Ronne E, Weiner D, Henkin J, Cines DB. Characterization of human endothelial cell urokinase-type plasminogen activator receptor protein and messenger RNA. Blood **76**:1795–1806, 1990.
64. Blasi F, Stoppelli MP, Cubellis MV. The receptor for urokinase-plasminogen activator. J Cell Biochem **32**:179–186, 1986.
65. Roldan AL, Cubellis MV, Masucci MT, Behrendt N, Lund LR, Dano K, Appella E, Blasi F. 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, 1990.
66. Behrendt N, Ronne E, Ploug M, Petri T, Lober D, Nielsen LS, Schleuning W-D, Blasi F, Appella E, Dano K. The human receptor for urokinase plasminogen activator. NH_2 terminal amino acid sequence and glycosylation variants. J Biol Chem **265**:6453–6460, 1990.
67. Ellis V, Wun TC, Behrendt N, Ronne E, Dano K. Inhibition of receptor-bound urokinase by plasminogen-activator inhibitors. J Biol Chem **265**:9904–9908, 1990.
68. Cubellis MV, Anderson P, Ragno P, Mayer M, Dano K, Blasi F.

Accessibility of receptor bound urokinase to type-1 plasminogen activator inhibitor. Proc Natl Acad Sci USA **86**:4828–4832, 1989.
69. Cubellis MV, Wun TC, Blasi F. Receptor-mediated internalization and degradation of urokinase is caused by its specific inhibitor PAI-1. EMBO J **9**:1079–1085, 1990.
70. Hajjar KA, Hamel NM, Harpel PC, Nachman RL. Binding of tissue plasminogen activator to cultured human endothelial cells. J Clin Invest **80**:1712–1719, 1987.
71. Silverstein RL, Nachman RL, Leung LLK, Harpel PC. Activation of immobilized plasminogen by tissue activator. J Biol Chem **260**:10346–10352, 1985.
72. Sanzo MA, Howard SC, Wittwer AJ, Cochrane HM. Binding of tissue plasminogen activator to human aortic endothelial cells. Biochem J **269**:475–482, 1990.
73. Reilly TM, Whitfield MD, Taylor DS, Timmermans PB. Binding of tissue plasminogen activator to cultured human fibroblasts. Thromb Haemost **61**:454–458, 1989.
74. Morton PA, Owensby DA, Wun TC, Billadello JJ, Schwartz AL. Identification of determinants involved in binding of tissue-type plasminogen activator-plasminogen activator inhibitor type 1 complexes to HepG2 cells. J Biol Chem **265**:14093–14099, 1990.
75. Bu G, Williams S, Strickland DK, Schwartz AL. Low density lipoprotein receptor-related protein/α_2-macroglobulin receptor is an hepatic receptor for tissue type plasminogen activator. Proc Natl Acad Sci USA **89**:7427–7431, 1992.
76. Nguyen G, Self SJ, Camani C, Kruithof EKO. Demonstration of a specific clearance receptor for tissue-type plasminogen activator on rat Novikoff cells. J Biol Chem **267**:6249–6256, 1992.
77. Otter M, Barret-Bergshoeff MM, Rijken DC. Binding of tissue-type plasminogen activator by the mannose receptor. J Biol Chem **266**:13931–13935, 1991.
78. Parkkinen J, Rauvala H. Interactions of plasminogen and tissue plasminogen activator (t-PA) with amphoterin: Enhancement of t-PA-catalyzed plasminogen activation by amphoterin. J Biol Chem **266**:16730–16735, 1991.
79. Beebe DF. Binding of tissue plasminogen activator to human umbilical vein endothelial cells. Thromb Res **46**:241–254, 1987.
80. Barnathan ES, Kuo A, Van der Keyl H, McCrae KR, Larsen GR, Cines DB. Tissue-type plasminogen activator binding to human endothelial cells. Evidence for two distinct sites. J Biol Chem **263**:7792–7799, 1988.