

Plasminogen Activator Inhibitor 1: Physiological and Pathophysiological Roles

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Plasminogen activator inhibitor 1 (PAI-1) inhibits plasminogen activators (u-PA and t-PA) by forming stable complexes endocytosed via a low-density lipoprotein receptor superfamily member-dependent mechanism. PAI-1 circulates actively in plasma and latently in platelets but is also secreted and deposited into the matrix by several cells, where it participates in tissue repair processes.

Plasminogen activator inhibitor 1 (PAI-1) belongs to the family of serine protease inhibitors (SERPINs). At the time of its discovery, the circulating PAI was termed PAI-1; PAI-2 was the intracellular PAI, contained mainly in leukocytes, the placenta, and the plasma of pregnant women, and PAI-3 was the protein C inhibitor. Now a new nomenclature has been proposed (19) on the basis of their phylogenetic relationships. PAI-1 is now called Serpin E1, PAI-2 Serpin B2, and PAI-3 Serpin A5. Other members of this family include antithrombin III, the major inhibitor of blood clotting; α_2 -antiplasmin; α_1 -protease inhibitor, the major inhibitor of neutrophil elastase; and protein C inhibitor, a major inhibitor of several proteases present in the reproductive tract. Deficiencies of these inhibitors were elucidated by gene deficiency models in mice or pathologies in patients. They lead to thrombotic tendency in the case of antithrombin III deficiency, bleeding disorders in the case of α_2 -antiplasmin deficiency, pulmonary emphysema in the case of α_1 -protease inhibitor deficiency, and male infertility in the case of protein C inhibitor deficiency.

PAI-1 is the major inhibitor of plasminogen activation by tissue-type (t-) and urokinase-type (u-) plasminogen activator (PA). t-PA is generally thought to be responsible for intravascular plasminogen activation, and its activity is regulated by the presence of fibrin. u-PA is the major PA on migrating cells, and its activity is regulated by the presence of the U-PA receptor (u-PAR) on different cells. PAI-1 therefore is capable of inhibiting not only intravascular fibrinolysis but also cell-associated proteolysis. In addition to its ability to bind and to inactivate PAs, PAI-1 also has other ligands that might mediate functions of PAI-1. Those ligands include glucosaminoglycans, matrix proteins such as vitronectin, and scavenger receptors such as members of the low-density lipoprotein receptor (LDLR) superfamily, specifically the lipoprotein receptor-associated protein (LRP).

The activity of PAI-1 is tightly regulated on the transcriptional level. The major regulator of PAI-1 expression and in turn of local PAI-1 activity is the cytokine transforming growth factor (TGF)- β . TGF- β activity is, on the other hand, itself regulated by PAI-1, because the u-PA plasminogen activation system is capable of activating latent TGF- β to its active form and

TGF- β in turn induces PAI-1, thereby turning off its own activation mechanism; the TGF- β autoregulatory loop links PAI-1 to wound healing.

Several other transcription factor consensus sequences have been identified in the PAI-1 promoter, for instance the peroxisomal proliferator regulators (PPARs), mediating effects of lipids on PAI-1 expression and several other consensus binding sites. None of these, however, seems to mediate an inflammatory response of PAI-1 transcription, which has been seen in several in vivo and cell culture studies.

In addition to transcriptional regulation, PAI-1 activity is also controlled by the environment: in its free, unbound form, the half-life of active PAI-1 is short and PAI-1 is converted into its inactive latent form after only a few minutes; when bound, however, to the matrix protein vitronectin, its half-life as an active protein is prolonged >10 times, making the matrix resistant to proteolysis originating from invading cells.

Under physiological conditions, PAI-1 is released into the circulation and the extracellular space by only a few cells: liver cells, smooth muscle cells (SMC), adipocytes, and platelets are the major sources of PAI-1. This results in plasma levels of only 5–20 ng/ml of active PAI-1, sufficient to control fibrinolysis and extracellular proteolysis.

Under pathological conditions, however, several other tissues secrete quite large amounts of PAI-1: tumor cells, endothelial cells in response to inflammatory cytokines, and other inflammation-activated cells. High PAI-1 plasma levels are consistently found in patients with severe sepsis but also with other acute or chronic inflammatory diseases such as atherosclerosis. PAI-1 is upregulated by inflammatory cytokines and may therefore be regarded as a marker for an ongoing inflammatory process. It is of major importance, however, that no classic inflammatory response element was found in the PAI-1 promoter region, and it is still unclear via which mechanism PAI-1 expression is upregulated during inflammation.

In addition to the question of PAI-1 regulation, it is furthermore unclear what the exact physiological or pathophysiological function of PAI-1 is in humans. It is understandable that PAI-1 deficiency causes a bleeding phenotype in genetically manipulated mice as well as in patients with a PAI-1 deficiency

and a thrombotic tendency in mice overexpressing PAI-1. It is, however, unclear what symptoms moderately increased levels of PAI-1 might cause and whether such increased PAI-1 levels found in patients are harmful and contribute to the disease or reflect an ongoing repair process. There is no clear-cut correlation between increased PAI-1 levels and a thrombotic tendency in humans, and it could be speculated that PAI-1 is involved in repair processes leading to a modulation of the local proteolytic potential, thereby governing cell migration. In this context it is of interest that high PAI-1 expression in tumor cells correlates with the malignancy of these cells, that PAI-1-deficient mice are prone to accelerated atherosclerosis and restenosis, and that PAI-1 deficiency is somehow linked to a defect in local angiogenesis in a tumor transplantation model. All of this indicates additional functions of PAI-1 not easily attributable to the mere inhibition of PAs by PAI-1

The following questions arise: 1) What is the mode of action of PAI-1, and are there additional PAI-1 activities that might explain clinical and experimental findings? 2) How is PAI-1 regulated, and can we explain PAI-1 levels found under clinical and experimental conditions by the known regulatory mechanism of PAI-1? 3) What are the distinct pathologies found under experimental conditions or in humans that might specifically correlate with increased levels of PAI-1?

PAI-1 structure

PAI-1 is a single-chain glycoprotein with a molecular weight of ~50,000. The mature, secreted form of this inhibitor consists of 379 amino acids and contains ~13% carbohydrate. It lacks cysteine residues but contains multiple methionine residues, which may account for its susceptibility to irreversible inactivation by oxidizing agents. The lack of cysteine residues and hence disulfide bonds may in turn account for its instability in solution. The reactive center of the inhibitor (Arg³⁴⁶-Met³⁴⁷) is contained within the exposed "strained loop region" at the carboxy terminus of the molecule and serves as a pseudosubstrate for the target serine protease (Fig. 1).

PAI-1 is the unique, rapid, and specific inhibitor of both t-PA and u-PA, with second-order rate constants ranging between $0.45 \times 10^7 \text{ M}^{-1}\cdot\text{s}^{-1}$ and $2.7 \times 10^7 \text{ M}^{-1}\cdot\text{s}^{-1}$ (15) and serves as the primary regulator of plasminogen activation in vivo. In addition, PAI-1 is known to inhibit plasmin and trypsin, with second-order rate constants of $\sim 10^6 \text{ M}^{-1}\cdot\text{s}^{-1}$ (10), as well as thrombin and activated protein C, obviously in less efficient reactions, ranging from 10^3 to $10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$.

PAI-1 conformational states. PAI-1 exists in several conformations (17). Besides the active and inactive forms, latent stages have also been identified. The native form of PAI-1 is shown as a ribbon structure in Fig. 1. PAI-1 is produced by different cell types, as described below, and is secreted. It accumulates in the culture medium over time in inactive, active, or latent form. The active form spontaneously converts to the latent with a half-life of ~1 h (10). The latent form can be converted into the active form by treatment with denaturants, negatively charged phospholipids, or vitronectin, although this third reaction is very slow. Latent PAI-1 can also be reactivated in vivo, although the mechanism is unknown. PAI-1 is the only

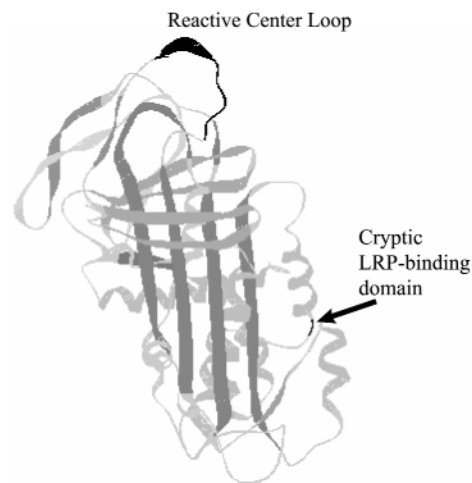


FIGURE 1. Three-dimensional protein structure of plasminogen activator inhibitor-1 (PAI-1) molecule in native conformation presented as ribbons. The spatial orientation of the reactive center loop (Ser³³³-Lys³⁴⁶) defines the activity status of the molecule, thereby modulating its binding abilities. On complex formation with plasminogen activators [e.g., urokinase (u-) or tissue (t-) PA], the cryptic binding domain (Lys⁶⁹) is accessible for binding to lipoprotein receptor-associated protein (LRP). Image processed with Swiss Protein Data Base (PDB) Viewer 3.7. PDB ID: IDB2 (<http://rcsp.org/pdb>).

SERPIN that can reversibly switch between the active and latent conformational states.

Recently, the three-dimensional structure of the latent form of PAI-1 was also resolved. In this structure, the entire amino terminal side of the reactive center loop is inserted as the central strand into β -sheet A. This accounts for the increased stability of latent PAI-1 as well as the lack of inhibitory activity. In addition to the latent form of PAI-1, a second inactive form has also been identified that results from oxidation of one or more critical methionine residues within active PAI-1 (14). Analysis of oxidized PAI-1 indicates that inactivation is correlated with rapid conformational change to a structure that is distinct from both active and latent PAI-1. Oxidative inactivation of PAI-1 may be an important mechanism for the regulation of the PA system. Oxygen radicals produced locally by neutrophils or other cells could inactivate PAI-1 and thus allow the generation of plasmin activity at sites of infection or in areas of tissue remodeling.

As a result of the unique labile structure of PAI-1, immunological methods for determining PAI-1 concentrations can vary by >10-fold, depending on the specific mix of conformations present and the specificity of the indicator antibodies. Thus PAI-1 antigen measurements should be interpreted with caution and a functional "bioimmunoassay" is to be preferred.

PAI-1 interaction with vitronectin and heparin. PAI-1 in plasma or in the extracellular matrix is stabilized by vitronectin. In solution, vitronectin-bound PAI-1 is approximately twice as stable as unbound PAI-1, and on extracellular matrix the half-life is reported to be >24 h. Very small amounts of PAI-1 can be demonstrated in normal fresh plasma, and most of the PAI-1 in whole blood, which appears to be latent, is contained in platelets. However, platelets contain vitronectin, which can potentially function to reactivate latent platelet PAI-1. Results

from clot lysis studies suggest that platelet PAI-1 may be a major factor in the resistance of platelet-rich thrombi to thrombolysis.

PAI-1 also binds to heparin with high affinity. This glucosaminoglycan-binding property of PAI-1 serves as an additional mechanism to anchor PAI-1 to the matrix (5). Heparin, however, does not influence functional activity of PAI-1.

PAI-1 and endocytosis of PA complexes. PAI-1 can complex with u-PA that is specifically bound to u-PAR, and the inhibitor-proteinase-receptor complex can be removed from the cell surface via members of the LDLR family e.g., LRP (identical with the α_2 -macroglobulin receptor), whereby PAI-1 builds the bridging element between the PA-receptor complex and LRP. The heparin-binding domain of PAI-1 contains a high-affinity cryptic binding site (Lys⁶⁹) for LRP involving basic residues of the inhibitor, which is exposed and made accessible for LRP binding when PAI-1 is complexed with proteases. The PA-inhibitor complex then undergoes lysosomal degradation, whereas the u-PAR is recirculated and reappears on the cell surface (18). PAI-1 also plays a role in the clearance of t-PA, whereby the endocytosed t-PA is transported to the lysosomes and degraded.

The interaction of the PAI-1 with LRP can be antagonized with a high-affinity endogenous ligand, the 39-kDa receptor-associated protein that is used in several studies to prove the involvement of members of the LDLR family in the process of cellular uptake.

The process of removal of PAs by first forming complexes with PAI-1 and then binding to LRP not only serves as a clearance mechanism for proteolytic activity but also to redistribute u-PAR to the leading edge of migrating and invading cells. Furthermore, via endocytosis of the complex, intracellular signaling events are likely to occur that might be linked with hitherto unexplained effects of PAI-1 on cell adhesion (Fig. 2).

PAI-1 gene and its regulatory regions

The human PAI-1 gene is ~12.2 kb long, comprising nine exons and eight introns. The gene is located on the long arm of chromosome 7. Two distinct transcripts result in mRNA of different lengths in their untranslated regions that appear to be formed by alternative polyadenylation. The 3' end of the larger transcript contains an AT-rich sequence (lacking in the shorter transcript) homologous to sequences implicated in the control of mRNA stability that might also be significant for differential regulation of PAI-1 protein.

The 5' region has been extensively characterized. The transcription initiation site was identified as well as a consensus TATAA sequence for RNA polymerase binding. Sequence comparisons revealed consensus sequences for several known regulatory elements, which led to the conclusion that this region can be considered to be the PAI-1 promoter (Fig. 3).

Two types of repetitive DNA elements are located within the PAI-1 structural gene and flanking DNAs. First, there are 12 Alu

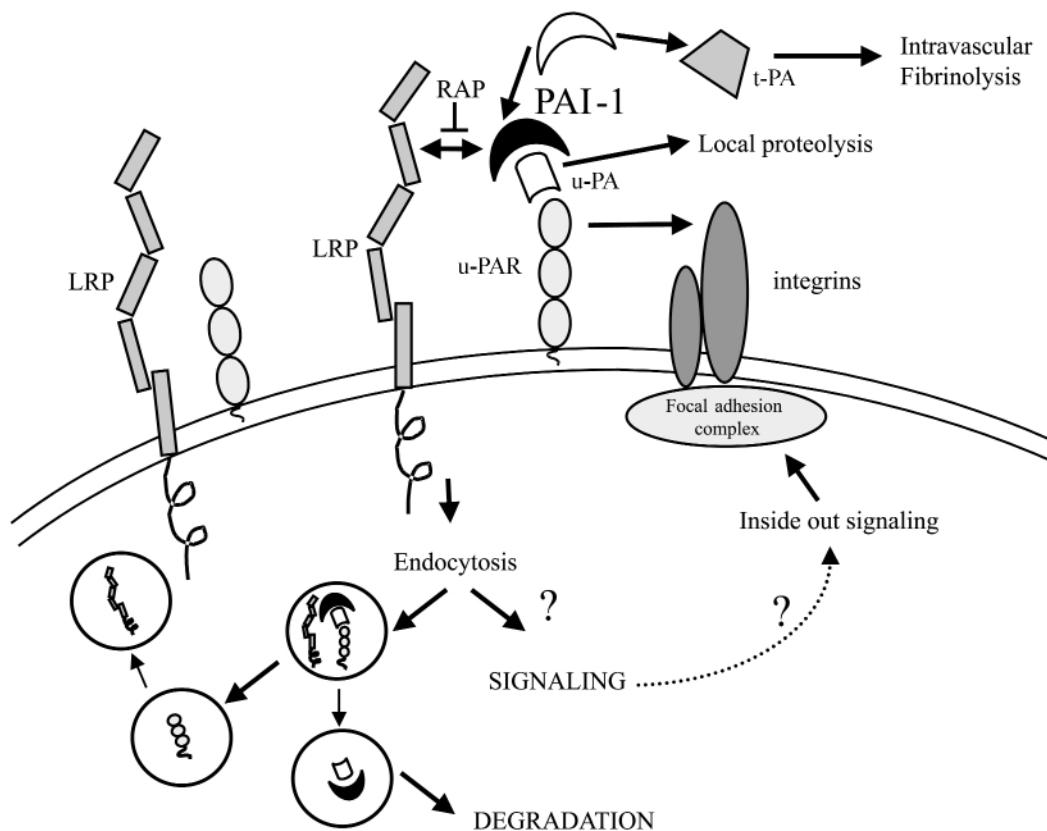


FIGURE 2. Schematic representation of PAI-1 action. PAI-1 not only inhibits local proteolysis and fibrinolysis but also induces turnover of PA-PAI complexes with or without receptors and also induces signaling events. RAP, receptor-associated protein; u-PAR, u-PA receptor.

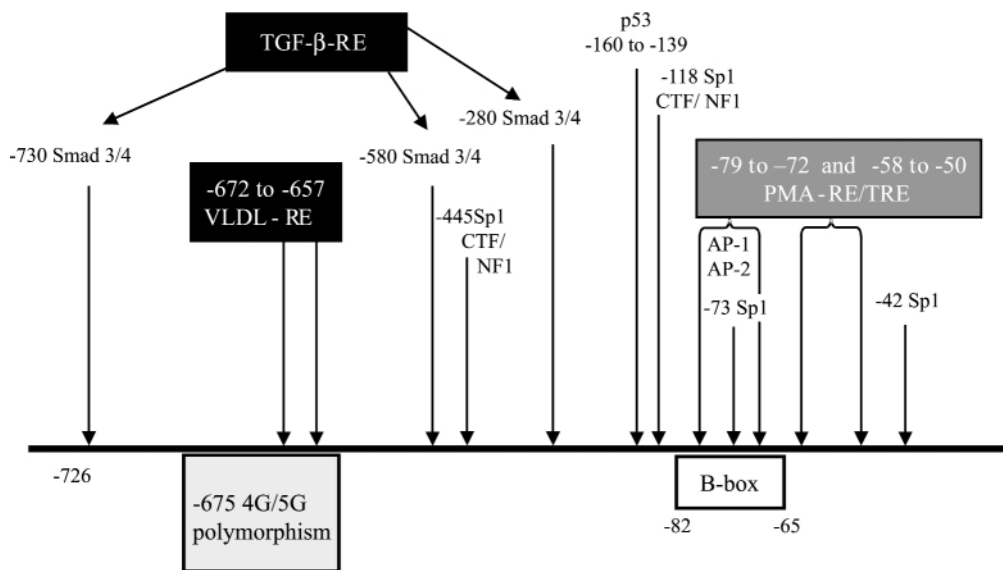


FIGURE 3. The PAI-1 promoter and consensus sequences. TGF, transforming growth factor; VLDL, very-low-density lipoprotein; CTF, CCAAT box binding transcription factor; NF1, nuclear factor 1; PMA, phorbol 12-myristate 13-acetate; AP, activator protein; -RE, responsive element; TRE, tetradecanoyl phorbol acetate response element.

elements and 5 repeats of a long poly (Pur) element. These Alu-Pur elements may represent a subset of the more abundant Alu family of repetitive sequence elements. In addition, there also exists a 4G/5G polymorphism and a G/A polymorphism in the promoter; however, data on the clinical relevance of these polymorphisms are controversial (8).

Regulation of PAI-1 biosynthesis in vitro

In vitro studies indicate that PAI-1 is synthesized by variety of cells and that its biosynthesis can be induced by growth factors, cytokines, hormones, and other compounds (12). The high expression of PAI-1 in cultured endothelial cells suggests that these cells substantially contribute to plasma PAI-1. However, the lack of demonstration of endothelial PAI-1 by immunohistochemistry or in situ hybridization and the presence of PAI-1 in various other cell types (e.g., hepatocytes, smooth muscle cells, adipocytes) points toward the possibility that plasma PAI-1 may originate from a variety of tissues. PAI-1 is also contained in platelets.

Inflammatory mediators. Lipopolysaccharide (LPS) induces elevated PAI-1 levels in the plasma of patients with Gram-negative septicemia. Analysis of the effects of LPS on PAI-1 gene expression in cultured cells indicates that PAI-1 synthesis by human and bovine endothelial cells is stimulated by LPS (20). It seems that plasma PAI-1 levels observed in Gram-negative sepsis result, at least in part, from increased biosynthesis of PAI-1 by endothelial cells of the vessel wall where the increase in PAI-1 antigen was preceded by an increase in the steady-state level of PAI-1 mRNA.

The effects of LPS are mediated in part by tumor necrosis factor- α (TNF- α). It stimulates PAI-1 synthesis by endothelial cells and HT-1080 fibrosarcoma cells and decreases t-PA production by these cells. Thus the antifibrinolytic effects of this molecule result from changes at the level of both PAI-1 and t-

PA. Another cytokine, interleukin-1 (IL-1), also stimulates the production of PAI-1 synthesis by endothelial cells and suppresses t-PA production. Both TNF- α and IL-1 induce tissue factor activity in endothelial cells. The decreased fibrinolytic activity and increased procoagulant activity of endothelial cells exposed to these cytokines may therefore promote both the formation and maintenance of fibrin. The induction of tissue factor activity by inflammatory mediators like TNF- α and IL-1 ultimately leads to increased generation of thrombin, which has also been reported to increase PAI-1 activity in the medium of umbilical vein endothelial cells. This regulatory loop therefore favors fibrin deposition.

TGF- β 1. TGF- β 1 is abundant in the α -granules of platelets, from where it can be released after vascular injury and can stimulate local PAI-1 expression. This can lead to insensitivity of thrombi to become lysed, leading in turn to increased fibrin deposits and progression of atherosclerosis. TGF- β 1 was one of the very first cytokines that was described to regulate PAI-1 expression on a transcriptional level, and since then substantial progress has been made in understanding the processes that mediate this regulatory mechanism. Parts of the PAI-1 promoter are now common tools in studies dealing with TGF- β 1 signal transduction. TGF- β 1 response elements in the PAI-1 promoter concentrated on binding of nuclear proteins include activator protein-1 binding sites but predominately binding sites for SMA/Mothers against decapentaplegic (SMAD) proteins, which play the key role in TGF- β 1-mediated activation of PAI-1 transcription (4). Smad3/Smad4-binding sequences, termed CAGA boxes, were identified within the promoter and are essential and sufficient for the induction by TGF- β 1. The protein complex that modulates the transcriptional control seems to contain several more players, like transcription factor for immunoglobulin heavy chain enhancer 3 (TFE3), the oncoproteins c-Ski and c-Sno, as well as the nuclear hormone receptor coactivator Ski-interacting protein (SKIP).

Triglycerides and free fatty acids. A very-low-density lipoprotein (VLDL)-responsive element was reported in the PAI-1 promoter covering the region from -672 to -657. This element could be responsible for the effect of plasma triglycerides on PAI-1 expression. Here also a possible connection to the 4G/5G polymorphism that is located in its proximity could exist. Free fatty acids (FFA) are increased in individuals with diabetes mellitus, and FFA increased the expression of PAI-1 in

“PAI-1 is the major inhibitor of plasminogen activation by t-PA and u-PA.”

the human hepatoma cell line Hep G2. FFAs activate a transcription factor that binds to the sequence 5'-TG(G/C)1-2CTG-3' that is repeated four times in the PAI-1 promoter between bases -528 and -599. This sequence is nearly identical to the DNA-binding site for the gene regulatory protein Sp1. Also, the transcription factor PPAR γ might be involved in PAI-1 regulation and PPARs might participate in vascular diseases (16).

Phorbol esters. The transcription factors c-Jun/c-Fos are also important in the induction of PAI-1 transcriptional activation. Phorbol 12-myristate 13-acetate (PMA) induction leads first to an increase in c-Jun/c-Fos mRNA levels and only secondarily to transcriptional activation of PAI-1. Two PMA-responsive elements (at -79 to -72 and -58 to -50) were identified with two distinct binding complexes that both contain c-Jun. The helix-like transcription factor that binds to the so-called B-box (-82 to -65) seems not to be involved in the PMA-induced but rather in the basal PAI-1 transcriptional activation via interaction with Sp1 and Sp3.

p53 and the cell cycle. It is disputed whether PAI-1 transcriptional regulation in cancer and metastasis can be negatively correlated to the regulation of u-PA and t-PA. A binding site for p53 in the region (-160 to -139) of the PAI-1 promoter was identified (13) that is responsible for stimulation of the PAI-1 transcription by binding of p53. The same transcription factor can repress u-PA and t-PA transcription, which would in turn lead to active regulation of several factors that are responsible for proteolytic degradation of the extracellular matrix and thereby promote local invasiveness and metastasis. Also, a cell cycle-dependent regulation of PAI-1 seems to exist. PAI-1 is known to be cell cycle regulated with increased expression during growth activation (G_0 to G_1 transition), and PAI-1 could therefore resemble an indirect proliferation marker.

PAI-1 in disease states

PAI-1 in vascular diseases. Increased plasma levels of PAI-1 are positively correlated with the risk of developing coronary artery disease as well as the extent of coronary sclerosis, restenosis, myocardial infarction, and deep vein thrombosis. Increased local expression of PAI-1 is observed in restenosis and in atherosclerotic plaques. Therefore PAI-1 seems to be a key molecule in thrombotic vascular diseases (12). PAI-1 expression in atherosclerosis is correlated with the cellular replicative senescence of vascular SMC in vitro (3). Late pas-

sage SMC express high PAI-1 levels and upregulate PAI-1 synthesis in human umbilical vein endothelial cells through a secreted soluble factor, thereby leading to a feedforward mechanism rendering the vessel wall antifibrinolytic. This PAI-1 overexpression might resemble a teleologically useful mechanism to limit cell proliferation by, for example, inhibiting proteolytic activation of latent TGF- β . Early passages of SMC and fibroblasts, however, downregulate endothelial PAI-1 synthesis instead. This negative feedback mechanism that limits and localizes cell proliferation and migration is in agreement with published data on PAI-1 knockout mice, which exhibit an increase of neointima formation in response to injury (2). Increased levels of PAI-1, seen by several groups (12) during the progression of atherosclerosis and restenosis, might represent an indirect marker for an ongoing proliferative tissue repair process, possibly indicating progression of atherosclerosis and development of restenosis after percutaneous coronary interventions.

PAI-1 in metabolic disorders. Plasma PAI-1 is closely correlated to serum triacylglycerol concentrations and insulin resistance. Therefore, PAI-1 might represent a link between insulin resistance and coronary artery disease. FFAs, triacylglycerol, and insulin, the plasma levels of which are all elevated in insulin resistance and diabetes mellitus type 2 (11), could be the mediators of PAI-1 elevation. (Pro)insulin, VLDL-triacylglycerol, and FFA stimulate PAI-1 production in hepatocytes, whereas VLDL and FFA enhance PAI-1 expression in cultured human endothelial cells. Although insulin infusion induces an increase in PAI-1 in rabbits, no elevation of plasma PAI-1 was observed on infusion of insulin in humans. In a recent study, combined hyperinsulinemia, hyperglycemia, and hypertriglyceridemia resulted in a rise of plasma PAI-1 in healthy humans.

PAI-1 and other disease states. To examine the importance of the balance between the PAs on the one hand and PAI-1 on the other, several lines of genetically altered mice have been generated that either overexpress or completely lack PAI-1. In contrast to patients with low or absent PAI-1 levels, PAI-1-deficient mice did not reveal spontaneous or delayed rebleeding, even after trauma (1). On the other hand, transgenic mice with a high PAI-1 level in blood due to PAI-1 expression under the control of the murine metallothionein promoter developed venous thrombi and lesions in the tail, hindquarters, and feet within the first week after birth (6).

Besides hemostatic disorders, a high PAI-1 level in blood also correlates with obesity. Clinical studies demonstrated that weight loss due to surgical treatment or diet significantly reduced blood PAI-1 levels in obese humans. PAI-1 mRNA can be found in adipose tissues from human subjects.

PAI-1 also has an important role in acute and chronic inflammatory lung disorders. In adult respiratory distress syndrome, idiopathic pulmonary fibrosis, hyperoxide lung injury, and bronchopulmonary dysplasia, we find intra-alveolar fibrin disposition and pulmonary fibrosis. In fact, all of these disease states are characterized by high levels of PAI-1 found in the bronchoalveolar lavage fluids.

PAI-1 also seems to play a role in renal diseases. It has been reported that elevated PAI-1 levels are associated with nephritic syndrome and the hemolytic uremic syndrome. In

patients with hemolytic uremic syndrome, glomerular fibrin deposition is found. Clinical studies suggest that PAI-1 is the circulating inhibitor of fibrinolysis in this syndrome and that the normalization of elevated PAI-1 activity correlates with improvement in renal function.

PAI-1 also seems to play an important role in several malignant processes, such as tumor cell invasion, metastasis, and neovascularization. It has been shown that high PAI-1 levels are associated with a poor prognosis in several different tumors (7). Tumors in PAI-1 knockout mice displayed lower proliferative and higher apoptotic indices and exhibited different neovascular morphology compared with wild-type mice. These results are consistent with the decreased growth rates of tumor in these PAI-1 knockout mice (9).

Conclusion

The SERPIN PAI-1 is the major plasminogen activator inhibitor in humans. Its physiological function is, however, not only inhibition of intravascular fibrinolysis but it is also involved in regulation of cell adhesion, migration, and invasion. PAI-1 activity itself is regulated on the transcriptional, mRNA, and protein stability levels; conversion between an active and a latent state of PAI-1 is unique for that SERPIN. Pathologies related to variations in PAI-1 levels include vascular occlusive diseases, fibrin deposition in lungs and kidneys, and malignancies; PAI-1 plasma levels are also influenced by metabolic and inflammatory diseases. Intervention with PAI-1 function might be an important future tool for additional therapeutic strategies in such diseases.

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References

1. Carmeliet P, Kieckens L, Schoonjans L, Ream B, van Nuffelen A, Prendergast G, Cole M, Bronson R, Collen D, and Mulligan RC. Plasminogen activator inhibitor-1 gene-deficient mice. I. Generation by homologous recombination and characterization. *J Clin Invest* 92: 2746–2755, 1993.
2. Carmeliet P, Moons L, Lijnen R, Janssens S, Lupu F, Collen D, and Gerard RD. Inhibitory role of plasminogen activator inhibitor-1 in arterial wound healing and neointima formation: a gene targeting and gene transfer study in mice. *Circulation* 96: 3180–3191, 1997.
3. Christ G, Hufnagl P, Kaun C, Mundigler G, Laufer G, Huber K, Wojta J, and Binder BR. Antifibrinolytic properties of the vascular wall. Dependence on the history of smooth muscle cell doublings in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 17: 723–730, 1997.
4. Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, and Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene.

- EMBO J* 17: 3091–3100, 1998.
5. Ehrlich HJ, Gebbink RK, Preissner KT, Keijer J, Esmon NL, Mertens K, and Pannekoek H. Thrombin neutralizes plasminogen activator inhibitor 1 (PAI-1) that is complexed with vitronectin in the endothelial cell matrix. *J Cell Biol* 115: 1773–1781, 1991.
6. Erickson LA, Fici GJ, Lund JE, Boyle TP, Polites HG, and Marotti KR. Development of venous occlusions in mice transgenic for the plasminogen activator inhibitor-1 gene. *Nature* 346: 74–76, 1990.
7. Foekens JA, Peters HA, Look MP, Portengen H, Schmitt M, Kramer MD, Brunner N, Janicke F, Meijer-van Gelder ME, Henzen-Logmans SC, van Putten WL, and Klijn JG. The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Res* 60: 636–643, 2000.
8. Grubic N, Stegnar M, Peternel P, Kaider A, and Binder BR. A novel G/A and the 4G/5G polymorphism within the promoter of the plasminogen activator inhibitor-1 gene in patients with deep vein thrombosis. *Thromb Res* 84: 431–443, 1996.
9. Gutierrez LS, Schulman A, Brito-Robinson T, Noria F, Ploplis VA, and Castellino FJ. Tumor development is retarded in mice lacking the gene for urokinase-type plasminogen activator or its inhibitor, plasminogen activator inhibitor-1. *Cancer Res* 60: 5839–5847, 2000.
10. Hekman CM and Loskutoff DJ. Kinetic analysis of the interactions between plasminogen activator inhibitor 1 and both urokinase and tissue plasminogen activator. *Arch Biochem Biophys* 262: 199–210, 1988.
11. Juhan-Vague I and Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. *Thromb Haemost* 78: 656–660, 1997.
12. Kohler HP and Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med* 342: 1792–1801, 2000.
13. Kunz C, Pebler S, Otte J, and von der Ahe D. Differential regulation of plasminogen activator and inhibitor gene transcription by the tumor suppressor p53. *Nucleic Acids Res* 23: 3710–3717, 1995.
14. Lawrence DA and Loskutoff DJ. Inactivation of plasminogen activator inhibitor by oxidants. *Biochemistry* 25: 6351–6355, 1986.
15. Lawrence D, Strandberg L, Grundstrom T, and Ny T. Purification of active human plasminogen activator inhibitor 1 from *Escherichia coli*. Comparison with natural and recombinant forms purified from eucaryotic cells. *Eur J Biochem* 186: 523–533, 1989.
16. Marx N, Bourcier T, Sukhova GK, Libby P, and Plutzky J. PPARgamma activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARgamma as a potential mediator in vascular disease. *Arterioscler Thromb Vasc Biol* 19: 546–551, 1999.
17. Nar H, Bauer M, Stassen JM, Lang D, Gils A, and Declerck PJ. Plasminogen activator inhibitor-1. Structure of the native serpin, comparison to its other conformers and implications for serpin inactivation. *J Mol Biol* 297: 683–695, 2000.
18. Nykjaer A, Conese M, Christensen EI, Olson D, Cremona O, Gliemann J, and Blasi F. Recycling of the urokinase receptor upon internalization of the uPA:serpin complexes. *EMBO J* 16: 2610–2620, 1997.
19. Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PGW, Irving JA, Lomas DA, Luke CJ, Moyer RW, Pemberton PA, Remold-O'Donnell ER, Salvesen GS, Travis J, and Whisstock JC. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins: evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem* 276: 33293–33296, 2001. First published 2 July 2001; 10.1074/jbc.R100016200.
20. Zhang WJ, Wojta J, and Binder BR. Notoginsenoside R1 counteracts endotoxin-induced activation of endothelial cells in vitro and endotoxin-induced lethality in mice in vivo. *Arterioscler Thromb Vasc Biol* 17: 465–474, 1997.