

Plasminogen activator inhibitor type 1 – its structure, biological activity and role in tumorigenesis (Review).

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Abstract. Plasminogen activator inhibitor type-1 (PAI-1), is a unique member of serpin superfamily, the primary regulator of plasminogen activation and therefore essential factor regulating physiological thrombotic/fibrinolytic balance in vivo. Via interactions with integrins and extracellular matrix components it orchestrates as well cell adhesion and migration. Therefore, PAI-1 is considered one of the key regulators of tumor invasion and metastasis, as well as cancer-related angiogenesis. This review summarizes recent findings on the structure and functional activity of the plasminogen activator inhibitor type-1 as well as current opinions on its role in tumor genesis.

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1. Introduction

Plasminogen activator inhibitor type-1 (PAI-1), is a unique member of serpin (*serine proteinase inhibitors*) super family, the primary regulator of plasminogen activation and therefore essential factor regulating physiological thrombotic/fibrinolytic balance in vivo. PAI-1 is also considered one of the key regulators of tumor invasion and metastasis, as well as cancer-related angiogenesis. Abnormal expression of PAI-1 has also been reported in various types of human diseases, including atherosclerosis, coronary heart disease, sepsis, renal and lung fibrosis, obesity and insulin resistance (1). Although its role in many pathologies is commonly accepted, detailed mechanism of PAI-1 action in many disorders still needs further clarification. Its role in plasmin generation and fibrin-degrading activity is well known, however experimental data prove that in certain pathologies PAI-1's ability to act outside the plasminogen/plasmin system in a fashion not

related to its proteinase-inhibitor activity might be of equal importance. The aim of the present review is to analyze available literature data, experimental as well as clinical, to demonstrate broad spectrum of PAI-1 activities and recent progress in understanding the mechanism of its action.

2. PAI-1 structure

Human PAI-1 is a single chain glycoprotein. Cloning of cDNA from human endothelial cells showed molecule of 402 amino acids with molecular weight of 45 kDa (2). Most hypotheses and conclusions about PAI-1 structure and activity have been derived based on biochemical investigations probing site mutations and epitops responsible for intermolecular binding, and the anticipated structural homology to other serpins (3, 4). Serpins highly ordered tertiary structure was described as consisting of three β -sheets A, B and C, nine α -helices and a reactive site loop (P4-P10') at the carboxy terminal side of the protein. The reactive center P1-P1' peptide bond in PAI-1 has been identified (5) as Arg369-Met370 bond (numbering of all residues in this publication corresponds to a notation used for the human PAI-1 sequence in SwissProt deposit P05121). This is so called "bait" that mimicks the normal substrate of target proteinase and interacts with its active site. PAI-1 is synthesized as an active molecule that converts spontaneously to the latent conformation, with a half-life time of about 60 minutes at 37 °C. Inactive latent form can be partially reactivated by denaturants such as guanidinium chloride, sodium dodecyl sulfate or urea (6). It was only since the late 90's when the results of X-ray analyses were published that we gained an in depth knowledge about structural features of this molecule (7-11). Figures 1a and 1b present two forms of PAI-1: latent and active. In latent PAI-1 the amino-terminal part of the reactive site loop is inserted into β sheet A, from residue P15 to P4 forming central strand 4, between strands 3 and 5, while residues P3-P10' form a random coil at the side of the molecule.. As a result P1-P1' and secondary binding sites are not easily accessible for serine protease, which explains increased stability of PAI-1 latent form and the lack of its inhibitory activity In active form A3 and A5 strands are not separated and maintain the uniform β -sheet while the reactive site loop is exposed and ready for complexation with serine protease. The reaction with target proteinase follows the suicide substrate-like mechanism scenario.

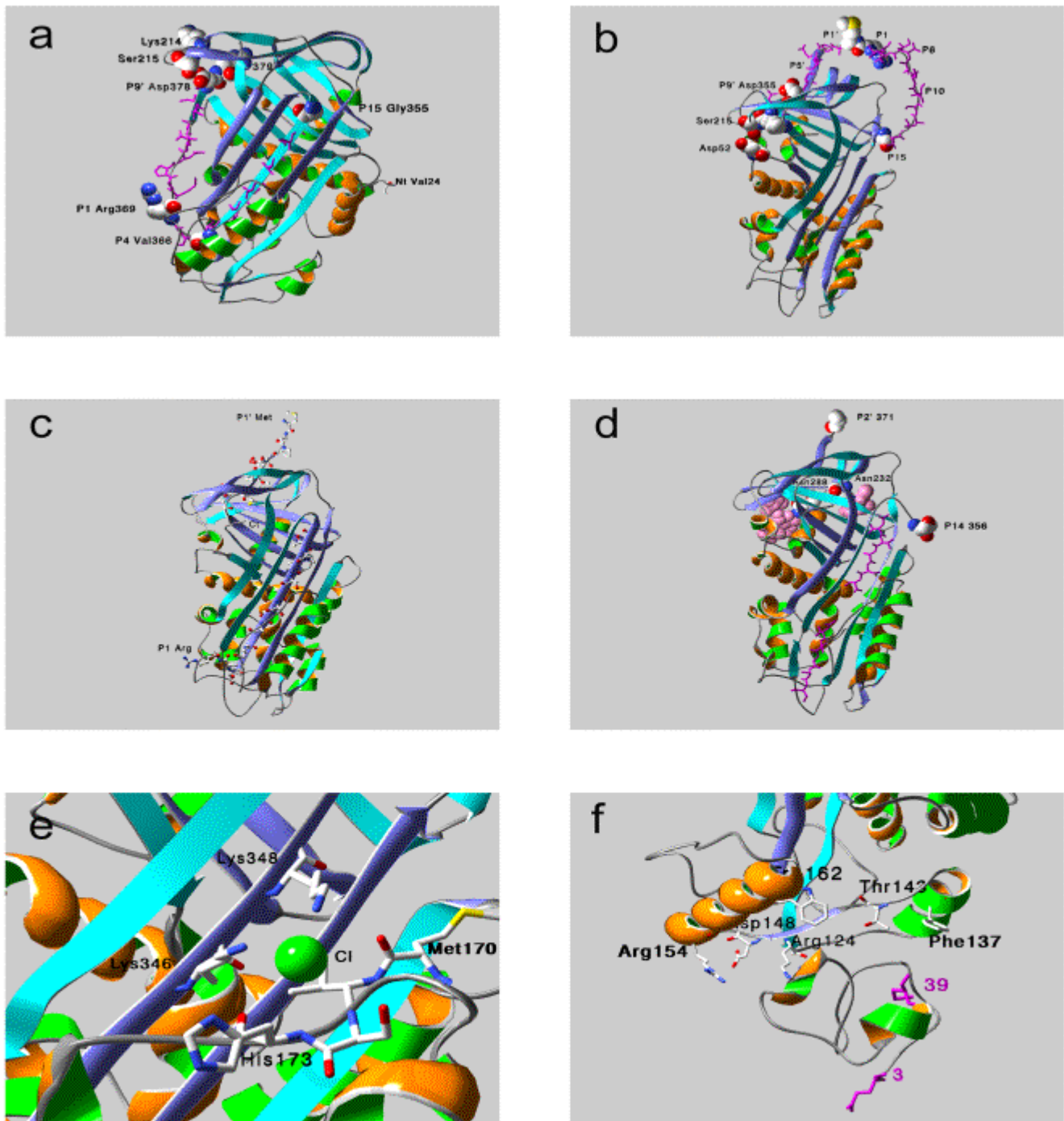


Fig. 1 All pictures have been made using program Deep View/Swiss-Pdb Viewer (1) and Povray (<http://www.povray.org/>) using atomic coordinates available in the Protein Data Bank, but with sequential numbering corresponding to SwissProt bank P05121, the same on all pictures. Nt, Ct marked the N- and C-terminus of PAI-1. (a) PAI-1 latent form, PDB entry 1C5G, reactive loop marked in magenta with selected residues, displayed as the space filling models, marking the scissile place (P1), A4 strand (P15-P4), a distal hinge P9'-P11', and plasmin cleavage site Lys214-Ser215. (b) PAI-1 active form, PDB entry 1B3K, selected residues marked to point out the features discussed in the text. (c) PAI-1 cleaved from, PDB entry 9PAI, P15-P1, P1'-P10 residues displayed as 'ball and sticks', P1 and P1' ends are separated by ~60Å. (d) Cleaved PAI-1 (missing residues 357-370), PDB entry 1A7C, showing two pentapeptide inhibitors N-Ac-TVASS-NH₂ mimicking the β -sheet strand A4. Pink space filling models represent sugars attached to Asn232 and Asn288. (e) PAI-1 chlorine binding site, PDB entry 1DVM molecule A, showing two lysines forming hydrogen bonds, and other residues ('stick' models) in the vicinity of the Cl⁻ ion (green sphere). (f) Vitronectin binding site of PAI-1, PDB entry 1OC0. Somatomedin B domain of vitronectin, residues 3-39 (magenta), selected residue of PAI-1 marked the segment of protein involved in binding.

Proteinase binds the reactive site of the exposed PAI-1's loop as a substrate, its active site initiates peptide bond hydrolysis comprising P1-P1' bond cleavage and new N- and C-termini formation, and a conformational rearrangement occurs yielding a thermodynamically stable new conformation (12). Structural analysis of the cleaved PAI-1 showed (7) a broken scissile bond and P1, P1' ends at the opposite pole of PAI-1 molecule, about 60Å apart (Fig. 1c), with P1' sticking out and P15-P1 slid in as in the latent form (Fig. 1c). Xue *et al.*, 1998, were probing that mechanism of a partial active loop insertion by using two molecules of the inhibitory reactive-centre loop peptide N-Ac-TVASS-NH₂ to form a complex with a cleaved PAI-1 lacking P13-P1 residues and 'frozen' in a pseudo-active form (9). Both pentapeptides were located bound within β sheet A between strands 3 and 5 mimicking P14-P10 and P6-P2 (Fig. 1d). It has been hypothesized that such polypeptide inhibitors may inactivate PAI-1 by hindering the conformational rearrangement leading to loop insertion and formation of the stable serpin-protease complex.

Indeed, according to some earlier studies, PAI-1 can also occur in a third conformation as a substrate form able to react with target proteinases (13). The bait P1-P1' region of such substrate form is accessible for proteinases and P1-P1' bond becomes cleaved following direct contact of both proteins. However, the reactive center loop insertion is delayed and stable covalent complex is not formed resulting in a regeneration of serine uPA or tPA functional activity (14). In wild-type molecule several different initial conformations exist that has been proven to predetermine the PAI-1 substrate-type behavior (15). This is in contrast to other serpins substrate-like activity that is believed to be consequence of the conformational changes induced during, not prior to the reaction with proteinase. Point mutations at P14 in the critical hinge region shows that the mechanism follows a branched pathway External factors such as temperature, pH, ionic strength and presence of cofactors might be also partially responsible for this uncommon among serpin-family behaviour (16). Presence of chlorine anions seems to stabilize PAI-1 active form by binding to its β -sheet A and preserving it in the five-stranded, compact form ((17), Fig. 1e). The experimental data show that described properties were exhibited by various PAI-1 mutants with charged residues introduced into the reactive site loop, like variants carrying mutations in the region P12-P8 and P15-P14 (18). As an explanation, the hypothesis of a branched pathway responsible for the formation of two possible reaction products – complexed or cleaved was proposed. It suggests that, the common intermediate formed by proteinase and PAI-1 could react either as an inhibitor, which forms a stable complex with target proteinase, or as a substrate that is cleaved by its proteinase, where formation of a stable inhibited complex with proteinase depends upon both the rate of active loop conformational change with P1-end insertion, and the rate of enzyme deacylation (19). This difference proves again the uniqueness of PAI-1 within the serpin family. PAI-1 rapidly and with high specificity reacts with urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA) with second-order association rate constants $\sim 2 \times 10^7 \text{ m}^{-1} \text{ s}^{-1}$ forming a 1:1 stoichiometric complex (20). Therefore, it is considered the main physiological inhibitor of both proteinases and indirectly,

principal regulator of plasminogen activation. Other proteinases that can cleaved and inactivate PAI-1 are plasmin, thrombin (18), human neutrophil elastase and Factor X_a with bound Ca⁺² (21). Plasmin can cut PAI-1 at peptide bond Lys214-Ser215 but only in the serpin's latent form (18) where OG from Ser215 is near P9' Asp378, and the scissile bond is exposed (Fig. 1a). In the active form the P9'-P11' fragment is in the opposite orientation and the loop where plasmin cuts is next to the turn after first helice with OG Ser 215 near Asp52 (Fig. 1b). As for other serpins, PAI-1 target specificity is mainly determined by the residues at the P1-P1' position. Experimental studies have shown that effective inhibition of uPA depends on the presence of a basic residue at the P1, while P1' might be substituted with any amino acid except proline (22). tPA down-regulation, on the other hand, is not affected even if neutral or hydrophobic amino acids are situated at this location (23). Inhibition of tPA by PAI-1 has been also proven to be more tolerant to the P2 and P3 positions diversity.

Gils and Declerc, 1997 have shown that although P1-P1' region is decisive for PAI-1 inhibitory specificity, it might be altered by mutations introduced in more distant parts of the molecule. Insertion of proline substitute at the position P6 (PAI-1P6(Val→Pro)) resulted in tPA target specificity, while the same substitute at the position 10 (PAI-1-P10 (Ser→Pro)) caused an increased uPA target specificity. PAI-1P18(Asn→Pro) variant was characterized by the selective inhibitory activity towards uPA (15). The distal hinge region P9'-P11', Asp378-Arg379-Pro380, is involved in maintaining the inhibitory conformation of PAI-1, while P5'Glu374 contributes into PAI-1 specificity toward uPA, plasmin and thrombin, but not for tPA (24). Apart from its anti-proteinase activity PAI-1 interacts also with the adhesive glycoprotein – vitronectin. Active PAI-1 is bound and stabilized by the vitronectin through the binding region comprising of α helix C, α helix E, β sheet 1A and localized on the pole opposite to the reactive loop. Although both functional sites of PAI-1 molecule are distant to each other, some studies suggest their close conformational linkage (25). In active PAI-1 conformation inhibitory and adhesive properties are present, while latent form lacks them both. Structural studies by X-ray analysis were done for the active PAI-1 complex with only somatomedin B domain of vitronectin (26). The region involved in binding have been identified (it is marked on Fig. 1f as 124-137,143-148,154-162), but the involvement of the active loop cannot be determined due to the absence of the rest of the vitronectin molecule and missing residues 361-371 in the active loop of PAI-1. Recently, it was observed that the PAI-1 bound to vitronectin prevents integrin binding independently from its proteinase-inhibitory functions and therefore down-regulates the integrin- and vitronectin-mediated cell activities. Apparently, the PAI-1- and the integrin-binding sites are localized in the same N-terminal region of vitronectin molecule, in a close proximity excluding simultaneous binding of both ligands. PAI-1 can also attach to fibrin localized mostly in the subendothelial extracellular matrix. The fact that interaction with both vitronectin and fibrin stabilizes the active form is of great importance for PAI-1 biological functions (25, 27, 28).

Like in other glycoproteins an impact of the large sugar moieties on the structural properties of PAI-

1 should not be neglected but it is difficult to evaluate, because of its disordered appearance in the electron density maps of the X-ray data. However, the work of Xue *et al.*, 1998, done at 1.95Å resolution, allowed to locate one D-mannose and three units of N-acetyl-D-galactosamine, confirmed their attachment to Asn232 and Asn288 (see Fig. 1d) and gave us a glimpse at the structural features of this region (9).

3. Regulation of PAI-1 gene expression, transcription and protein production

The PAI-1 gene has been cloned and sequenced. It is located at region q21.3-q22 of chromosome 7 in close proximity to loci for erythropoietin, paraoxonase, the metprotooncogene and cystic fibrosis (29). At the 5' regulatory end it contains several known consensus *cis* regulatory elements, which bind *trans*activating factors such as Sp1, activated protein-1 (AP-1), nuclear factor-κB (NF-κB), Smad3, Smad4 (30) and TFE3 (31). The PAI-1 gene transcriptional control is likely to be complex. It was shown that PAI-1 gene transcription is activated by inflammatory cytokines, especially IL-1β (32), TNF-α (33), TGF-β (34), FGF-2 and angiotensin-2 as well as glucocorticoids and nonspecific protein kinase C (PKC) activators such as phorbol myristate acetate (PMA) (35). Synthesis of the PAI-1 mRNA transcripts appears as well to be growth state regulated and increases significantly during the G(0)-G(1) transition in stimulated epithelial cells (36). Many cell types have been shown to produce PAI-1, such as endothelial cells, smooth muscle cells, hepatocytes, fibroblasts and inflammatory cells (28). Plasminogen activator inhibitor-1 is also present in the subendothelial extracellular matrix where it controls local proteolysis (27). However, in physiological conditions, the main source of circulating PAI-1 are the platelets, where it is stored and released upon activation (28). There are three different forms of circulating PAI-1 – the latent inactive form, the form complexed with tPA, and the free active form representing up to 80% of total pool.

The plasma level of PAI-1 is genetically controlled. The PAI-1 gene has been shown to contain several polymorphic loci, the 4G/5G insertion/deletion, at position 674 in the promoter region and the A-844G polymorphism. The 4G/5G polymorphism regulates the PAI-1 gene transcription via its impact on the binding of NF-κB to the *cis* regulatory region in -680 to -670. NF-κB-activated transcription is suppressed by the regulatory protein that binds to 5G allele but not to 4G sequence (27). As a result, PAI-1 plasma levels in subjects with 4G/4G genotype are approximately 25% higher than in individuals with the 5G/5G polymorphism (37). The 4G/5G polymorphism has been also shown to be associated with an increased risk of myocardial infarction, both 4G/5G and G-844A were found more frequently in patients with venous thromboembolism (38).

4. PAI-1 in the plasminogen/plasmin system

PAI-1 is the primary inhibitor of plasminogen activator in plasma and, as mentioned before, it has high affinity for both uPA and tPA. As a part of proteolytic cascade - the

complicated network consisting of plasminogen and its active form plasmin, plasminogen activators (uPA, tPA) and activator inhibitors (PAI-1, PAI-2 and nexin), it controls the plasminogen/plasmin system functional activity.

System up-regulation results in the generation of plasmin from its inactive precursor – plasminogen catalyzed by uPA or tPA. Plasmin is the serine proteinase known for its broad-spectrum specificity. It degrades fibrin into soluble degradation products, facilitating its removal from the circulation and securing in that way physiological suppression of thrombotic processes. Plasmin hydrolyzes as well many other proteins in the extracellular matrix (ECM) and basement membranes, including the most important – fibrinogen, laminin and type IV collagen. It catalyzes their degradation by either direct or indirect activation of several metalloproteinases precursors (pro-MMPs) for example: collagenase (MMP-1), stromelysin (MMP-3) and gelatinase B (MMP-9) (39). *In vivo* plasminogen and plasmin are found in blood (plasminogen concentration is about 2 μM), but might also be present in the extravascular compartment on the cell surface associated with the membranes via uPAR (40). tPA-mediated plasminogen activation is mainly involved in the dissolution of fibrin at the site of vascular injury, while uPA binds to the specific cellular receptor (uPAR) resulting in the enhanced activation of cell-bound plasminogen. Concentration of the proteolytic activity i.e. plasminogen and plasminogen activators on the cell surface provides milieu for plasmin generation and plays important role in maintaining of blood fluidity and non-thrombogenicity, is crucial as well for other biological processes including ovulation, embryogenesis, angiogenesis and tumor genesis (40).

It is believed that uPA initiates the cascade of pericellular proteolyses, facilitating disruption of the cell-extracellular matrix protein bonds and promoting cellular migration (41). Moreover, binding to uPAR induces accumulation of uPA on the cell surface, mostly at the specific sites of the cell – ECM contact points, particularly on the cell leading edge. uPA attachment induces uPAR cleavage exposing a chemotactic epitope that is localized in the exclusive proteinase-sensitive region of the receptor. Thus, uPA binding transforms uPAR into the cell-surface chemokine able to act in the autocrine or paracrine manner. Cell migration involves also its extension as well as binding of integrins and other adhesion receptors to their ECM ligands at the leading edge, together with dissociation of integrin-ligand complexes at the cell trailing edge. uPA stimulates cell migration by catalyzing all these processes. Additional contributing factors are the nonproteolytic stimulatory effects of uPA—uPAR binding. Stimulation of uPAR-integrin and uPAR- vitronectin interactions, as well as uPAR preference for the latter but not the other substratum element-fibronectin, simultaneously regulates cell adhesion at its both edges. As a result uPA-uPAR system possesses several chemokine-like qualities – induces cell adhesion, chemotaxis, receptor clustering and changes in the cell shape.

PAI-1 is a primary physiological inhibitor of uPA. It down-regulates its proteolytic activity and is responsible for half-life time shortening. PAI-1 promotes endocytosis and degradation of uPA- uPAR complex (41). This process requires additional α₂-macroglobulin receptor (low-density lipoprotein-related-receptor, LPR)

with binding site for uPA-PAI-1 complex. This transmembrane protein merges and is internalized together with uPAR-uPA-PAI-1 cluster. Afterwards PAI-1-uPA shifts into the lysosome while free uPAR is transported back to the cell surface, where it is available for new uPA molecule (42). Therefore, except of its main anti-migratory activity, PAI-1 indirectly supports the continuity of the adhesion-migration-adhesion cycle by internalizing and degrading uPA and parallelly recycling of the uPAR. Additionally, PAI-1 competes with uPAR for binding to vitronectin inhibiting uPA-dependent cell adhesion and migration.

5. PAI-1 and vitronectin interaction – role in cell migration

Apart from its proteolytic activity PAI-1 possesses another facet, crucial for the regulation of cell migration processes – ability to interact with a high specificity with one of the ECM components – vitronectin..

Vitronectin plays an important role in the process of cell attachment to the surrounding ECM and therefore participates in the regulation of cell differentiation, proliferation and morphogenesis (43). Vitronectin promotes cell adhesion, spreading and migration by interaction with integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_v\beta_6$, $\alpha_v\beta_8$). Moreover, as a bridging element between ECM and urokinase-dependent proteolysis it directs the localization of uPAR into the focal adhesions regulating strictly controlled and limited process of cleaving cell linkages to ECM and degrading basement membrane barrier promoting in that way cells displacement mechanism. The next step in cell movement is the adhesion facilitated by vitronectin via its interaction with integrins, as well as by direct interplay with uPAR. This receptor binds to vitronectin with high specificity, inhibiting cell association with fibronectin or fibrinogen. Moreover, there are some experimental data suggesting close interplay between vitronectin and growth factors signaling pathways (44). Their synergistic interaction, in addition to integrins involvement, provides supportive environment for cell proliferation.

As mentioned before, PAI-1 interacts with vitronectin with high affinity, competing for binding with uPAR and integrins. Therefore, PAI-1 coupled with vitronectin prevents its association to integrin and respectively down-regulates cell adhesion and migration. Similarly, by competing with uPAR for binding to vitronectin it inhibits uPA-dependent cell adhesion and migration. It should be pointed out as well that PAI-1 linked to vitronectin, in the contrast to the plasminogen activator-complexed PAI-1, is characterized by the extended proteinase-inhibitory activity since its spontaneous conversion into the latent form is delayed (26). Consequently, vitronectin bound PAI-1 demonstrates even stronger anti-migratory effect. In summary, the outcome of PAI-1 and uPAR-uPA interaction depends on the equilibrium in the complex network of intermolecular interactions susceptible to number of other external signals and stimuli. According to Blasi *et al.*, 1997, the plasmin-dependent pericellular proteolysis and activation of the chemotactic uPAR epitope provides the “GO” signal for the cell migration process (41). The free PAI-1, on the other hand, guarantees the negative regulation

of this process by inhibiting and disrupting uPA-uPAR interaction and preventing vitronectin-integrin and vitronectin-uPAR interplay suppressing therefore cell adhesion and movement (“STOP”). However, the PAI-1-dependent uPAR recycling may void the stop signal and allow therefore new u-PA-uPAR interactions, new adhesion and migration steps. Therefore, although the PAI-1 reveals significant anti-adhesive and anti-migratory effects, its interplay with uPA provides also the continuity of these processes.

Vitronectin has been found also to enhance moderation of PAI-1 activity by Factor X_a with bound Ca^{+2} ions, and thus has an impact on a total fibrinolytic activity (21).

6. PAI-1 and the tumorigenesis

As emphasized before, PAI-1 plays dual role in extravascular processes – via inhibiting plasminogen activators and via its ability to prevent cell adhesion. The exact contribution of both functions to the effects of PAI-1 on the tumour development, metastasis and angiogenesis remains not fully understood. Data form the literature suggesting altered expression of several genes including plasminogen/plasmin system constituents in highly metastatic cancer cells were not uniformly confirmed as far as PAI-1 was concerned (45, 46). It is however striking that increased expression of PAI-1 is associated with poor prognosis in many cancers (ovarian, breast, lung, kidney, gastric) (47-49). The clear explanation of this phenomena lays in the future, although one may speculate of PAI-1 role in anti-cancer defenses.

PAI-1 and the tumor-associated angiogenesis

Angiogenesis is the process of new vessels formation that occurs in healthy subjects during ovulation and wound healing while in cancer it is crucial for tumor growth and metastasis formation (40). The very first step of the neovascularization process comprises the local dissolution of the basement membrane by targeted proteolysis with resulting release of the endothelial cells, their reorientation, migration and proliferation. Subsequent movement and capillary extension also require active proteolysis. The plasminogen/plasmin system components provide not only efficient pericellular proteolysis, but also due to uPAR unique cellular localization may polarize expression of the protease to cell-cell contacts and cell-substratum contacts or to the leading edge of the migrating cells. Suppression of uPA/uPAR system by physiological or synthetic inhibitors effectively down-regulates its pro-angiogenic activity (50, 51). PAI-1's ability to bind and cause internalization of uPA complexes serves as a local barrier for uPA-mediated proteolysis protecting certain cells and matrices from excessive disruption. Experimental data suggest that physiologically PAI-1 is preferentially expressed in the vicinity of uPA- expressing new capillary structures proving functional interplay between both molecules and supporting the idea that PAI-1 protects neovascularized tissues from excessive proteolysis (52). PAI-1 was proven to act as a potent inhibitor of *in vitro* angiogenesis in the sprout formation assay (53, 54), as well as *in vivo* in the chicken chorioallantoic membrane assay (55), while Brodsky *et al.*, 2001 observed significantly

enhanced angiogenic potential of *ex vivo* cultured vessels obtained from PAI-1-deficient mice (56). This inhibitory effect is thought to be facilitated by two parallel mechanisms involving inhibition of proteinase activity and independently of PAI-1 anti-proteinase action by high affinity binding to vitronectin. However, in most malignant tumours the level of uPA is significantly higher than in the corresponding normal tissue or in benign tumours of the same tissue type (57). Similarly, PAI-1 content is considerably increased suggesting that the subtle physiological balance is missing in this environment (48). This might explain, to a certain extent, the contradictory results yielded by various studies regarding tumour-associated angiogenesis. Literature data demonstrate conflicting experimental outcomes as both inhibition (58) and stimulation (59), as well as lack of PAI-1 effect on this process (60). In PAI-1-deficient mice PAI-1 absence was, for example, associated with significant inhibition of new blood vessel formation (59). That might result from excessive proteolysis causing the loss of cell adhesion sites and angiogenesis suppression. On the other hand, PAI-1 over-expression could trigger the potent inhibition of proteolysis that in turn might also suppress angiogenesis by preventing angiogenic matrix fibryolysis and therefore endothelial cells migration, as it was suggested by Stefansson *et al.* 2001 (55). Regulatory role of PAI-1 was further proven by McMahon *et al.*, 2001, who demonstrated the diverse effects of high and low PAI-1 concentrations (61). While low amounts increased new blood vessels formation, PAI-1 excess inhibited this process almost completely. Apparently, angiogenesis proceeds efficiently only at the optimal PAI-1 level range. It has been recently suggested, that in this particular situation – when critical balance between proteinases and PAI-1 was achieved, PAI-1 might promote tumour angiogenesis by inhibiting the excessive proteolysis that prevents successful assembly of tumor vessels (62). In contrast, PAI-1 excess might block integrin adhesion by saturating vitronectin matrix, while its deficiency prevents uPA-uPAR-dependent polar localization of integrins. There are still some important questions to be answered concerning the functional redundancy between PAI-1 and other constituents of plasminogen/plasmin system. As mentioned before, *in vivo* studies with transgenic animals produced contradictory results. PAI-1 deficiency affected tumour-induced angiogenesis both negatively (62) and positively (58), as well as had no significant effect (63). Thus, the clinical significance of experimental data remains yet to be clarify. Current understanding of tumor neovascularization mechanisms provides however compelling evidence that PAI-1 is a potent regulator of angiogenesis and therefore tumor growth.

Tumor growth and metastasis formation

Since PAI-1 is a potent inhibitor of physiological cell migration and angiogenesis, its significant effect on tumor growth should be expected. Indeed, overexpression of PAI-1 inhibited PC-3 and LNCaP prostate carcinoma tumor growth (53, 58). In PAI-1-deficient mice both processes were significantly reduced (59, 64), while the growth of B16 murine melanoma and transgenic breast cancer tumors were unaffected in either PAI-1 null or PAI-1 overexpressing mice (60, 63). Just as for angiogenesis, McMahon *et al.*,

2001 observed close relationship between PAI-1 concentration and its effect on tumor growth (61). Low doses of PAI-1 increased tumor size, while higher dosage revealed strong inhibitory effect. That again might explain some discrepancies in literature data and put some light on the studies demonstrating the requirement of PAI-1 presence for tumor growth (59, 64). Hypothetically, only optimal range of PAI-1 levels exerts that effect, while its higher concentration blocks cell adhesion, angiogenesis and tumor growth.

Invasion of tumor cells is accomplished not only by cell movement on the ECM but also via active penetration into this milieu by means of plasminogen activation-mediated proteolysis. Therefore, mutually conflicting results concerning PAI-1 effects on cancer cells invasiveness might be determined by the expression of other components of uPA system, integrins, endocytosis receptors and the ECM components. PAI-1 was shown to inhibit uPA- and uPAR-dependent invasiveness of human lung carcinoma cells (65). Other authors observed its promigratory effects in melanoma and breast cancer cells populations (66, 67). In the last study however, phenotyping and functional analysis confirmed that different cancer cell subclones might use different strategies to migrate depending on both the environment and their expression of the uPA system. Correspondingly, Hjortland *et al.*, 2003, demonstrated that the cPAI-1 transfection of the glioma cells, either suppressed or revealed no effect on their motility and invasiveness, depending on different ECM and interstitial composition of the experimental model utilized in *in vitro* studies (68).

The proteins involved in cell invasiveness, growth and apoptosis regulate also process of metastasis formation via ECM adhesion disruption and ECM degradation. Many experiments with animal models have proven that uPA-catalyzed plasmin generation exerts decisive influence on the rate of the tumor metastasis formation (40). In contrast, no consistent picture exists concerning PAI-1 role in this process. Xenografts of human DU145 and LNCaP prostate carcinoma cells in *scid/scid* mice were inhibited by PAI-1 injections (69). Likewise, transduction of uveal melanoma cells with PAI-1 cDNA resulted in 50% reduction in the number of mice developing metastases and the sharp drop in the metastatic burden in animals with metastasis (70). However, mice engineered to overexpress human PAI-1 in multiple organs developed pulmonary metastases at the same rate as wild-type mice in a model of murine melanoma (60), while migration of malignant keratinocytes transplanted into the skin of PAI-1 null mice was severely impaired and subsequently restored by PAI-1 cDNA transduction (59).

Clinical studies have shown significantly increased levels of plasminogen/plasmin system components, including PAI-1 in tumor tissues. It was also shown repeatedly, that high tumor levels of PAI-1 predict poor prognosis (47-49). In some studies PAI-1 expression correlated with tumor size, lymph node involvement and differentiation. It seems to be an apparent contradiction to at least some studies showing anti-angiogenic, anti-migratory and anti-metastatic PAI-1 effects in tumorigenesis. Nevertheless, functional importance of PAI-1 expressed in a particular localization and biological situation cannot be deduced by simple detection of its presence. Important

message emerging from clinical studies concerns however different pattern of PAI-1 expression by certain types of tumours. Also its localization in cancer as well as stromal cells might be different proving that the type and location of primary tumor might be of crucial importance. However, the most significant conclusion rising from studies published so far, even contradictory ones, is the increasing understanding of the complicated system that PAI-1 functions in. Much evidence suggest that PAI-1 (24), as well as uPA-uPAR complex, possess also plasmin-independent functions (41).

7. Summary: PAI-1 is a multifunctional protein with distinct domains encoding its different various biological functions. Recent progress in structural and functional properties let us understand better the role that different amino acids and different molecular regions play in its

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