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Review Article

Plasminogen activator inhibitor type 1 (PAI-1) in cancer: a potential new target for antiinvasive and antimetastatic therapy

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Introduction

Cancer cells must degrade extracellular matrix proteins in order to invade and subsequently metastasize. There is today sufficient experimental and clinical evidence for a critical role of proteolytic enzymes in the invasive process (1-5). The proteolytic activity seen in cancer is the result of a concerted action of several enzyme systems which act in a complicated interplay. The involved enzyme systems include metalloproteinases (6), the serine protease plasmin generated through the urokinase pathway of plasminogen activation (1), cysteine proteases (7) and other extracellular enzymes (6, 8). Not only do these different enzyme systems interact with regard to activation of the pro forms of the enzymes (9-12), but also some of the enzymes share common substrates (*i.e.*, both plasmin and stromelysin-1 degrades fibrin) (13). Extracellular tissue degradation is not a unique characteristic of cancer, since these degradative enzyme systems are also operative in a number of nonmalignant tissue remodeling and repair processes such as wound healing (14, 15), mammary gland involution (16) and trophoblast invasion (17, 18). The difference between the malignant and nonmalignant tissue remodeling appears to be a lack of normal regulation of this process in cancer (1, 6, 19, 20), resulting in a continuous and self-perpetuating tissue destruction.

The basic knowledge on proteases and cancer invasion obtained over the last decade has generated experiments aimed at clinical application. These studies can be divided into prognostic studies, where the tissue or blood levels of a protease, its receptors or natural inhibitors have been correlated with patient survival (21, 22), and studies aimed at developing inhibitors of protease function, thereby inhibiting the spread of cancer cells (invasion and metastasis) (23-27).

Among the various enzymes, receptors and inhibitors involved in the invasive process, plasminogen activator inhibitor type 1 (PAI-1) has attracted specific attention. This paper will review the literature concerning PAI-1 and cancer, as well as discuss hypotheses for the possible roles of PAI-1 in tumor progression.

Properties of PAI-1

PAI-1 is a strong and fast-acting inhibitor of the plasminogen activation system (PA system) which is a complex proteolytic system able to produce relatively large amounts of plasmin from its precursor, plasma plasminogen (Fig. 1). Plasmin is, either directly or through its ability to activate pro-metalloproteinases, able to degrade most components of the extracellular matrix (1, 28-31). In addition, plasmin activates various growth factor systems (32, 33) and may through these systems modulate tumor neoangiogenesis and migration of both normal and malignant cells (34-37). Activation of plasminogen is catalyzed by two different plasminogen activators, urokinase-type (uPA) and tissue-type (tPA). While uPA plays a central role in tissue remodeling, including cancer invasion (1), tPA plays its major role in thrombolysis through its dissolution of fibrin in the circulation (38). uPA is synthesized as a virtually inactive single chain proenzyme (pro-uPA) requiring proteolytic activation to form active two-chain uPA before it can activate plasminogen (39). Pro-uPA activation can be catalyzed by plasmin, thus leading to a strong amplification of the overall reaction (1). Pro-uPA and uPA bind to a specific glycolipid-anchored cell surface receptor (uPAR) (40-44). Pro-uPA can be activated when it is receptor bound, and receptor-bound uPA is catalytically active (41, 45). Concomitant cell-surface binding of pro-uPA and plasminogen enhances as well as focalizes plasmin generation (45. 46).

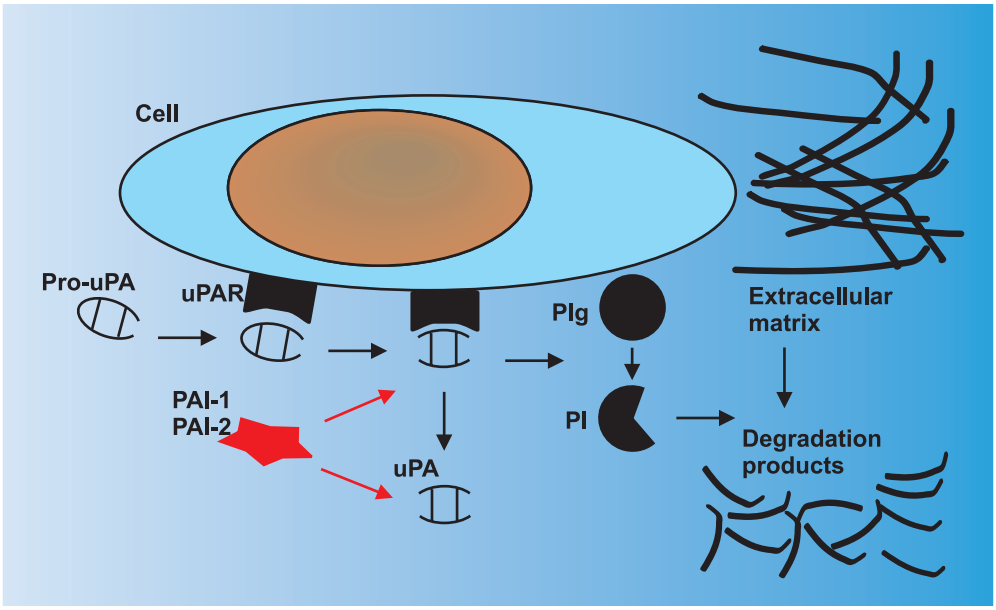

Fig. 1. Drawing of the uPA-catalyzed plasminogen activation and degradation of extracellular matrix. Plg = plasminogen; PI = plasmin; uPA = urokinase plasminogen activator; Pro-uPA = pro-urokinase plasminogen activator; uPAR = uPA receptor; PAI-1, PAI-2 = plasminogen activator inhibitor, type 1, 2.

uPA (and tPA) activity is primarily regulated by PAI-1 and PAI-2 (plasminogen activator inhibitor types 1 and 2), but PAI-3 (protein C inhibitor) and protease-nexin also have an inhibitory effect, although with much lower affinity (47). PAI-1, which is a single-chain glycoprotein consisting of 379 amino acids (48-51), is the principal physiological inhibitor of both tPA and uPA in humans (47). PAI-1 is a member of the serpin (serine protease inhibitor) superfamily, which comprises more than 60 single-chain proteins (52). As found in other serpins, PAI-1 has a reactive center loop, close to the C-terminal end, consisting of approximately 20 amino acids (53). PAI-1 is synthesized as an active molecule, but has a metastable conformation, that can exist in three interconvertible forms (active, latent and substrate) (54-57). During inhibition of either tPA or uPA, the protease and active PAI-1 form 1:1 stoichiometric, SDS-stable complexes. The PAs recognize the 'bait' sequence P_1-P'_1 (Arg^{346}-Met^{347}), exposed on the PAI-1 reactive center loop, that mimics the bond of plasminogen which is cleaved by uPA or tPA during conversion of plasminogen to the active enzyme plasmin (58). Active PAI-1 in free solution converts spontaneously to a latent conformation with a $t_{1/2}$ of only 2 hours at 37 °C. This inactivation is delayed by binding of PAI-1 to vitronectin (see below) (59-62). Latent PAI-1 has no inhibitory activity, but can be reactivated by denaturation with SDS or chaotropic agents such as guanidinium chloride (54). *In vivo*, latent PAI-1 may be reactivated by negatively charged phospholipids, present in both platelets and at sites of thrombosis (63, 64). PAI-1 inhibits receptor-bound uPA nearly as efficiently as uPA in solution (65). The entire complex of PAI-1, uPA and uPAR binds to the α_2-macroglobulin receptor/low density lipoprotein receptor-related protein (α_2MR/LRP) and glycoprotein 330 and is internalized through these. The individual components are degraded, except for uPAR that may recycle (66). The internalization occurs only on cells expressing these so-called endocytosis receptors (α_2-MR/LRP and gp330) (67, 68).

The X-ray crystal structure of latent PAI-1 shows that the uncleaved reactive center loop is completely inaccessible to the protease and withdrawn by insertion into β-sheet A (69). PAI-1 can also exist in a nonfunctional so-called substrate form, which is cleaved as a substrate by the target protease and is thus not able to inactivate the protease (55).

PAI-1 is synthesized by a variety of cell types, including endothelial cells, adipocytes, hepatocytes, megakaryocytes (70-75), and is found throughout the body in varying concentrations, the highest being in the liver and spleen (76). Plasma levels are highly variable, ranging from 5-85 ng/ml, with the major PAI-1 reservoir being platelets (77, 78). PAI-1 expression is affected by numerous cytokines, hormones, growth factors and endotoxins (78-81). In blood as well as in the extracellular matrix (ECM), active PAI-1 is stabilized by binding to the multifunctional protein vitronectin (VN) (82-84).

PAI-1 inhibition of uPA or tPA is neutralized in the ECM by activated protein C (APC) and limited proteolysis by elastase (81, 85, 86). Furthermore PAI-1 inhibits thrombin in the presence of VN, by forming PAI-1:VN:thrombin complexes, and the association rate with thrombin is enhanced by binding of heparin to PAI-1 (87-91). As PAI-1 inhibits thrombin, PAI-1 undergoes substantial proteolytic cleavage (88). Finally, active PAI-1 may also bind to fibrin, although with a 1000-fold lower affinity than to VN (92, 93).

PAI-1 deficiency in mice and humans

The disruption of the PAI-1 gene in mice proved to have no significant influence on viability, fertility or development (94). This was rather surprising since PAI-1 deficiency (partial or complete) in humans is very rare, and at the time the first PAI-1 knockout mouse was produced, only 5 patients with PAI-1 deficiency were known worldwide, all of whom had a hemorrhagic tendency with delayed rebleeding after trauma or surgery (95-99). Recently, 7 more individuals with complete PAI-1 deficiency have been identified and they all suffer from abnormal bleeding patterns (*e.g.*, intracranial and joint bleeding after mild trauma, delayed surgical bleeding, severe menstrual bleeding and frequent bruising) (100).

PAI-1 deficient mice have so far been investigated primarily in the context of fibrinolysis/thrombosis. PAI-1 -/- mice were found to be mildly hyperfibrinolytic and to have a greater resistance to venous thrombosis, but with no impairment of hemostasis (101). It was shown that they had accelerated neointima formation after injury of the arterial vessel wall with an electric current (102). Furthermore, these mice were found to be protected from bleomycin-induced pulmonary fibrosis (103). PAI-1 deficient mice have also been found to be protected against development of venous thrombosis following injection of endotoxins in the footpad (101).

PAI-1 and cancer

The role of PAI-1 in cancer is thus far not well understood, possibly because it plays different roles in different cancer types. Based on the consistent finding of an association between high tumor tissue levels of PAI-1 and short survival in a variety of cancer forms (5, 104-113), 4 different hypotheses have been raised as to the role of PAI-1 in cancer progression: 1) PAI-1 protects the tumor stroma from autodegradation by uPA catalyzed plasmin formation (2, 114) (Fig. 2); 2) PAI-1 reduces cell adhesion by displacing uPAR from VN, thus facilitating the dissemination of cancer cells to distant tissue sites (115) (Fig. 3); 3) PAI-1 is expressed in budding capillaries and is simply a marker of the intensity of neovascularization going on in the tumor (116); and 4) PAI-1 has been suggested as an acute phase reactant and could therefore be elevated in cancer according to the severity of the disease (117-119).

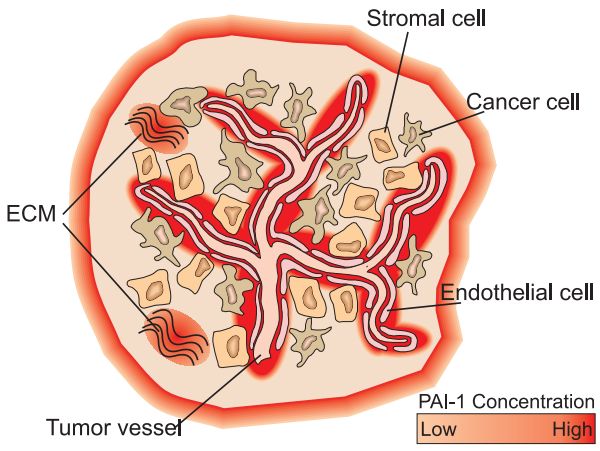

Fig. 2. A drawing of the possible role of PAI-1 protecting the angiogenesis/endothelial cells as well as extracellular matrix against proteolytic degradation through uPA. The so-called gas mask hypothesis, with PAI-1 being the gas mask and uPA the gas.

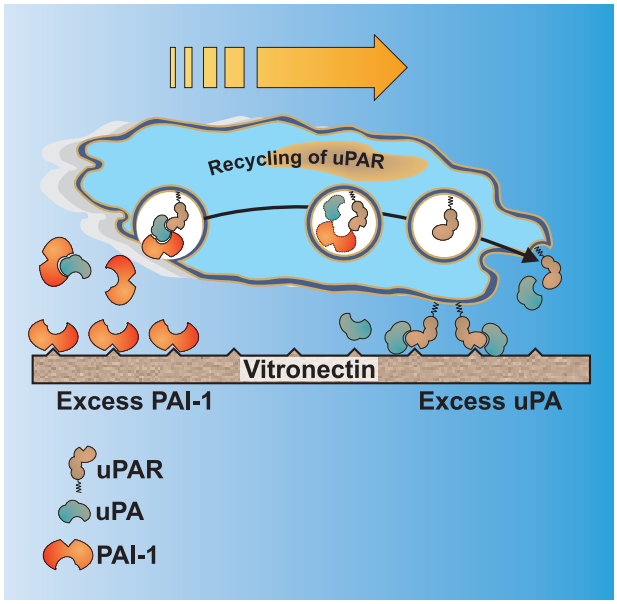

Fig. 3. A cartoon of the possible role of PAI-1/uPAR interaction in the migrating cell. uPAR is presented at the protruding leading edge with a surplus of uPA as compared to PAI-1. At the same time PAI-1 is presented in surplus as compared to uPA at the trailing edge, leading to release of the trailing edge with subsequent internalization of the formed PAI-1:uPA: uPAR complexes. uPAR is thought to recirculate.

Following is a discussion of each of the 4 hypotheses.

1) Localization studies in cancer tissue have revealed a highly complicated interplay between uPA, its receptor (uPAR) and PAI-1, as it has been shown that some of these molecules are expressed by the epithelial carcinoma cells, while others are expressed by the tumor infiltrating stromal cells, *e.g.*, fibroblasts, macrophages and endothelial cells (120, 121).

Many different tumors have been investigated as to the localization of PAI-1, using both immunohistochemistry and *in situ* hybridization (Table I). In many of these studies PAI-1 was shown to be produced by endothelial cells or macrophages (2, 122-125). However, as shown in Table I, the data on cellular localization of PAI-1 in cancer tissue are somewhat contradictory. One important piece of information to keep in mind when studying the immunohistochemical data is that uPA:PAI-1 complexes are internalized upon binding to uPAR and may thus be found in cancer cells without these being the source of the PAI-1. Despite the discrepancies between the different studies,

Table I: Localization studies of PAI-1 in turmors.

Tumor type	Ref.	Cancer cells		Endothelial cells		Other stromal cells	
		mRNA	Protein	mRNA	Protein	mRNA	Protein
Murine Lewis lung carcinoma	160		+				
Colon adenocarcinoma	2	-		+			
Colon adenocarcinoma	161		+		+		
Colon adenocarcinoma	162		-		+		+
Colon adenocarcinoma	163	-			+		
Breast carcinoma	Pyke et al. unpublished	(+) <5%	(+) <5%	+	+	+	+
Breast carcinoma	123		+		+		
Breast carcinoma	164		+		+		+
Breast carcinoma	165		+		+		+
Breast carcinoma	166		+				
Astrocytomas	125	+		+			
Gliomas	124		-		+		
Gliomas	167		+		-		
Giant cell bone tumors	168	+		+			
Cutaneous squamous cell carcinoma	126	+					
Malignant melanoma	127	+					
Malignant melanoma	128	+					

+Detected; -not detected.

there are some important, rather consistent findings. The endothelial cells are the primary location of PAI-1 for several cancer forms, such as colon cancer (2, 122), breast cancer (123) and brain tumors (124, 125). Often other stromal cells, such as macrophages and fibroblasts, also express PAI-1. For skin cancer (except basal cell carcinomas) and malignant melanoma, however, it seems that the cancer cells are the primary source of PAI-1 (126-128).

The results of the localization studies, together with the clinical data showing shorter survival of cancer patients with high tumor levels of PAI-1 in breast cancer (5, 21, 104-113) (Fig. 4), gastric cancer (5, 105), lung cancer (106, 107), colon cancer (108, 109), renal cell carcinoma and ovarian cancer (112, 113), have led to the hypothesis that PAI-1 serves to protect the tumor stroma, *e.g.*, the endothelial cells against the proteolytic degradation which the tumor imposes upon the tumor stroma (2, 114). We have named this hypothesis the gas mask hypothesis, with PAI-1 being the gas mask protecting the extracellular matrix and the endothelial cells, and uPA being the gas (Fig. 2). It is interesting to speculate that the inhibitors of metalloproteases, the TIMPs, may serve a similar role (129-132).

2) Another possible explanation for the association between high tumor tissue levels of PAI-1 and progression of cancer is that PAI-1 plays a role in tumor cell migration (115, 133, 134). In support of this hypothesis are recent experimental findings indicating that PAI-1 may be a modulator of migration through its interaction with VN (115, 133, 134). Although the migratory function of PAI-1 is not necessarily related to its protease inhibitory function, PAI-1-induced changes in cell migration could be relevant to the progression of cancer. It is well known that active PAI-1 binds to VN and is thereby stabilized in the active conformation and still able to bind to and inactivate uPA (81-84, 131). The UPA:PAI-1 complex thus formed has very low affinity for VN and is rapidly released (79). uPAR has also been shown to bind to VN with high affinity, and this affinity is increased 8-fold through the binding of uPA to uPAR (136-138). Both uPAR and PAI-1 bind to the somatomedin B domain of VN, thus competing for this binding site (115), although PAI-1 has a 30-fold higher affinity than uPAR for VN (61, 139). The interaction between uPAR and VN can thus be regulated through the

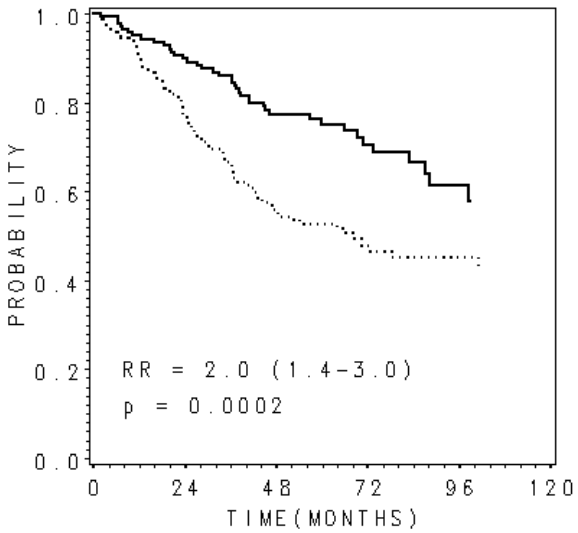

Fig. 4. Impact of PAI-1 level in cytosolic tumor extracts on relapse-free survival in 295 low-risk breast cancer patients. Patients were divided into two groups with PAI-1 levels below (—) and above (- - -) the median value. The *p* value was calculated by the log-rank test and the relative risk (RR) was calculated by the Cox regression model.

balance of uPA *versus* PAI-1. A surplus of PAI-1 releases uPAR from VN, whereas a surplus of uPA releases the rapidly formed uPA:PAI-1 complex from VN and hence facilitates the binding of uPAR to VN (115). In this way local overexpression of PAI-1 can mediate release of cells bound to VN through uPAR, and thereby possibly promote migration which in turn could lead to cancer cell dissemination (115). In this context it should be mentioned that some studies have shown PAI-1 to inhibit cell migration through its interaction with cell surface bound uPA:uPAR, integrins and VN (133, 134, 140), while others have shown PAI-1 to stimulate cell migration on VN (141). None of these studies take into account the importance of the pericellular localization of PAI-1 in the intact organism. One could hypothesize that a surplus of PAI-1 at the trailing edge of the tumor cell would facilitate release of this end from VN, whereas a surplus of cell surface bound uPA:uPAR complexes at the migratory leading edge (142) would facilitate new adhesion sites and thus enhance migration (143). This hypothesis is explained graphically in Figure 4.

3) Several studies have shown that PAI-1 is localized primarily to the tumor vessel (*i.e.*, budding capillaries) (2, 116). This observation has led to the hypothesis that the amount of PAI-1 in a tumor is directly related to the amount of angiogenesis. Since a high degree of angiogenesis in a tumor is closely related to short patient survival (144), the relation between PAI-1 and angiogenesis could explain the observed relation between PAI-1 and patient outcome and therefore one could imagine a simple relationship between the size of the tumor (*i.e.*, size of sprouting vascular bed) and the amount of PAI-1.

4) PAI-1 has for many years been proposed as an acute phase reactant (117, 118). Thus, the observed elevation of PAI-1 in blood from cancer patients (145) and in highly vascularized tumors might be a simple reflection of the severity of the disease. However, in a recent study by von Tempelhoff *et al.*, a lack of correlation between plasma PAI-1 levels in breast cancer patients and C-reactive protein or fibrinogen, two known acute phase reactants, was found (146). We had a similar observation when comparing plasma PAI-1 and C-reactive protein in preoperatively obtained blood samples from 591 patients with colorectal cancer (Nielsen and Brünner, unpublished data).

Conclusions

With the basic mechanisms and actions of the urokinase plasminogen activation system in mind, it seems logical that the initial speculations as to the role of PAI-1 in cancer were true, that indeed PAI-1 might inhibit cancer invasion and/or cancer growth. Accordingly, several studies in the early 90s showed PAI-1 to be inhibitory in different model systems (147-149). At the same time, however, other reports suggested a far more complicated role for PAI-1 in cancer invasion and metastasis, with one report on the necessity of PAI-1 for the metastatic process of human melanoma cells in nude mice (150).

Since then, studies using both *in vitro* and *in vivo* model systems for invasion/metastasis have been conflicting. Some studies have shown reduced invasion in Matrigel assays with increasing expression of PAI-1 (151), and also inhibition of primary tumor growth and metastasis by PAI-1 expression in athymic mice (152). Other groups, on the other hand, have reported that coexpression of uPA, uPAR and PAI-1 is needed for optimum invasiveness through Matrigel (153). In addition, an experimental *in vivo* study has given some indications as to the importance of PAI-1 in cancer progression. In this study suppression of metastases of HT1080 human fibrosarcoma cells to the lungs in athymic mice was accomplished by the addition of antibodies against human PAI-1 (154).

Although the experimental studies appear to be conflicting, they may not necessarily be mutually exclusive. A manyfold surplus of PAI-1, as compared to uPA, should be expected, both *in vivo* and *in vitro*, to give some inhibition of uPA mediated plasmin generation and proteolysis and thereby inhibit invasion and also metastases. This certainly does not rule out the possibility that a fine balance between proteolysis and protease inhibition exists at the cellular level in the cancer tissue.

In recent years several compounds have been found which are able to inhibit the function or production of PAI-1. These compounds, including new butadiene derivatives, diketopiperazines, as well as compounds based on a 14-mer peptide corresponding to the PAI-1 reactive center loop, have mainly been developed in an attempt to promote fibrinolysis in the treatment of thromboembolic disease (155-159). Future studies using these specific PAI-1 inhibitors will hopefully lead to a clarification of the role of PAI-1 in cancer progression.

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