Plasminogen Activator Inhibitor-1 in Cancer: Rationale and Insight for Future Therapeutic Testing

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Abstract

Despite its function as an inhibitor of urokinase and tissue-type plasminogen activator (PA), PA inhibitor-1 (PAI-1) has a paradoxical pro-tumorigenic role in cancer promoting angiogenesis and tumor cell survival. In this review we summarize pre-clinical evidence in support of the pro-tumorigenic function of PAI-1 that has led to the testing of small molecule PAI-1 inhibitors, initially developed as anti-thrombotic agents, in animal models of cancer. The review discusses the challenges and the opportunities that lay ahead to the development of efficacious and non-toxic PAI-1 inhibitors as anti-cancer agents.

Keywords

Plasminogen activator inhibitor-1 (PAI-1); PAI-1 inhibitor; pre-clinical

Introduction

Plasminogen Activator Inhibitor-1 (PAI-1) is a serine protease inhibitor (serpin) and the main regulator of the plasminogen activation system. It acts by inhibiting tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) (1). PAI-1 has a pleiotropic biological function which stems from its complex structure. Three specific protein-binding domains in the molecule have been well characterized (Figure 1) (1): a domain in the reactive center loop (RCL) that binds to PA, a domain in the flexible joint region that binds to vitronectin comprised of helix D (hD), helix E (hE), and helix F (hF), and a domain within hD and hE that binds to low-density lipoprotein receptor-related protein (LRP1). Upon binding to the RCL, PA cleaves PAI-1 at P1-P1′ inducing a dynamic conformational change that inserts the RCL as a β-strand (4A) into the core of the protein. The binding of PAI-1 to PA forms a catalytically inactive complex, which is internalized

when combined with the receptor for uPA (uPAR) and LRP1 (2). Premature insertion of the RCL into the β-sheet is the basis for the conversion of active PAI-1 into its latent form. Binding of PAI-1 to LRP1 also promotes cell migration and signaling (3). Binding of the flexible joint region of PAI-1 to vitronectin has multiple consequences: it masks the adjacent RGD binding site for αv integrins and inhibits cell attachment to vitronectin (2) and stabilizes PAI-1 inhibiting its conversion to latency and increasing its anti-uPA ability by 200 fold (4). Adding to the complexity of the function of PAI-1 in cancer is the fact that it can have paracrine and autocrine effects as it is produced by tumor cells and non-malignant cells including endothelial cells (EC), macrophage cells or adipocytes in the tumor microenvironment.

On the basis of the pro-tumorigenic role of uPA in angiogenesis, tumor invasion, and metastasis, it was anticipated that PAI-1 would have an anti-tumorigenic function. Surprisingly, several studies revealed a paradoxical association between elevated levels of PAI-1 in blood and tissue samples of cancer patients and an unfavorable clinical outcome and poor response to therapy (1,5). The observation that the production of PAI-1 by EC, fibroblasts, adipocytes, smooth muscle cells, and macrophage cells in the tumor microenvironment was stimulated by pro-tumorigenic factors such as transforming growth factor (TGF)-β interleukin (IL)-6, and tumor necrosis factor (TNF)-α added further evidence supporting a pro-tumorigenic role (6,7). In this article, we review the mechanisms and evidence in mouse models supporting a pro-tumorigenic function for PAI-1. We then present recent pharmacological approaches and pre-clinical studies aimed at targeting PAI-1 in cancer and discuss the lessons learned from these studies.

PAI-1 is pro-angiogenic

A first clue explaining the paradoxical association of PAI-1 with more aggressive forms of cancer came from the observation by several laboratories, including our own, that PAI-1 has a pro-angiogenic activity through its anti-protease and vitronectin-binding functions. The activity of PAI-1 on angiogenesis is, however, dose-dependent with a promoting activity at physiological concentrations (8,9) and an inhibitory activity at pharmacological concentrations (10). This is explained by the fact that at physiological concentrations, PAI-1 inhibition of uPA limits the cleavage by pericellular plasmin of membrane associated Fas-L at R144-K145 and the release of a soluble Fas-L fragment that induces Fas-mediated apoptosis in EC (11). PAI-1 also stimulates angiogenesis through its vitronectin-binding function promoting the detachment of EC from vitronectin and their migration toward fibronectin rich tissues (9).

PAI-1 inhibits spontaneous apoptosis in cancer cells

PAI-1 protects tumor cells from apoptosis through multiple mechanisms. Similar to EC, it inhibits Fas-mediated apoptosis in several human cancer cells including brain metastasis through its control over pericellular plasmin-activity (12,13). PAI-1 also affects intrinsic apoptosis, as the absence of extracellular PAI-1 in tumor cells results in higher levels of activated caspase 9 (14). Intracellular PAI-1 also promotes cell survival through its ability to inhibit caspase 3 protecting tumor cells from chemotherapy-induced apoptosis (15).

What have we learned from mouse tumor models?

Observations in PAI-1 deficient mice have provided additional and important clues on the function of PAI-1 in tumorigenesis and angiogenesis. The vast majority of experiments using PAI-1 deficient murine tumor cells syngeneically implanted into WT or PAI-1 deficient mice consistently demonstrated the contributory role of host and tumor-derived PAI-1 to tumor growth, angiogenesis and metastasis (Table 1) (10,16–21). Consistently, maximum anti-tumor effect was shown upon PAI-1 suppression in both host and tumor cells pointing to a combined role for extracellular PAI-1. Similar observations were made when PAI-1 deficient human tumor cells were xenotransplanted in immunodeficient PAI-1 null mice. These studies typically demonstrated that suppression of PAI-1 expression in human tumor cells and in mouse host cells resulted in poor tumor take and slower tumor growth (11,12,22,23). However in some situations, such as in skin carcinoma, the effects observed depended on the tumor grade with minimal effects observed in high grade tumors (23).

In contrast to the above data, experiments using genetically engineered mice (GEM) prone to develop cancer crossed with PAI-1 null mice failed to demonstrate any effect of PAI-1 suppression on tumor initiation, growth, or metastasis. For example, when FVB-PymT mice prone to develop mammary tumors were crossed with PAI-1 null mice, there was no effect on the development of mammary tumors, metastasis, or survival (24). Similarly, genetic ablation of PAI-1 had no effect on tumor development in Apc/Apc1638N mice prone to develop colon cancer (25), in TRP-1SV40 Tag/PAI-1$^{-/-}$ mice prone to develop ocular tumors (26), and in K14-HPV16/PAI-1$^{-/-}$ mice prone to develop skin cancer (27). An explanation for this difference of effect between transplanted and GEM mice is the possible presence of compensatory serpins including PAI-2, protein C inhibitor, protease nexin-1, or maspin (28–31), whose overexpression in transformed cells may have compensated for a lack of PAI-1 (27–31).

Pharmacological inhibition of PAI-1

Over the last two decades, several laboratories and pharmaceutical companies have developed a variety of small molecule PAI-1 inhibitors using high throughput screening (32). The vast majority of these inhibitors are molecules interfering with the molecular interactions of PAI-1 to inhibit tPA and uPA by binding to the reactive center loop and by inducing a conformational change that promotes an irreversible conversion of PAI-1 into its latent form. To our knowledge, no inhibitors specifically interacting with the vitronectin-binding domain of PAI-1 have been reported yet. The main focus of investigations on the activity of these inhibitors in biological processes has been in cardiovascular diseases (thrombus repermeabilization), lung fibrosis, Alzheimers disease and to a lesser degree in cancer. A first family of PAI-1 inhibitors developed consisted of diketopiperazine inhibitors that interfere with uPA binding to the protease binding domain of the RCL. These inhibitors (XR334, XR1853, XR5082, and XR5118) block tPA inhibition by PAI-1 *in vitro* (33,34). However, their *in vivo* efficacy is limited by their low solubility, poor oral availability, or high IC$_{50}$ values (in the 5 μM to > 1000 μM range) that are unachievable in the blood upon administration in animals. Other menthol-based, benzothiophene, and butadiene small molecules with lower IC$_{50}$ values (644 nM to 44 μM) *in vitro* were developed (35–37),

however despite their good anti-PAI-1 activity *in vitro,* they were either not tested or not further pursued in animal experiments for reasons not reported.

One indole oxoacetic acid PAI-1 inhibitor, PAI-039 (tiplaxtinin) (38), was extensively and successfully tested for its anti-thrombotic activity in pre-clinical rat and canine models of acute arterial thrombosis (38,39). Other inhibitors derived from the structure of this inhibitor (PAI-749 and PAZ-417) then underwent human phase I clinical trials in healthy volunteers and in patients with Alzheimer disease (40–42). To date, the results of these studies have not been reported.

As an alternative approach, using computer simulation models mimicking the RCL insertion within the strands of β-sheet A, conformation disrupting agents were designed. Such compounds include a family of dimeric 2-acylamino-3-thiophenecarboxylic acid derivatives (TM5001 and TM5007) developed with the anticipation that by binding to the s4A position of the A β-sheet, they would induce the conversion of PAI-1 into its latent and inactive form (43). These inhibitors were found active against vascular thrombosis in a rat model and against lung fibrosis in murine models. Their pharmacokinetic properties, however, were sub-optimal due to their relatively high lipophilicity [calculated octanol/water partition coefficient (ClogP) value of 5.79, above the ideal 2–3 value]. A second generation molecule (TM5275) with better oral pharmacokinetic properties and lower ClogP of 3.37 was then developed (44). TM5275 showed activity in rat and mouse models of acute arterial thrombosis and lung fibrosis. Importantly, this inhibitor had no systemic toxicity as it did not prolong bleeding time in non-human primates (45).

A limiting element in the activity of these inhibitors so far, has been their lack of activity against the stable form of PAI-1 bound to vitronectin (46). Thus, the recent focus has been on the development of inhibitors active against vitronectin-bound PAI-1. These efforts, however, have been limited by a lack of crystal structure information on vitronectin-bound PAI-1 (47). Polyphenolic inhibitors of PAI-1 (CDE-066 and 096) synthesized on the basis of the structure of small molecules identified by a high throughput screen were found to bind to the sB/sC pocket of PAI-1 with an IC_{50} of 32 μM and 25 nM, respectively (Figure 1) (48,49). CDE-096 induces allosteric conformational changes affecting the flexibility of PAI-1 and preventing it from binding to PA and decreasing vitronectin binding (49). CDE-096 was still able to interact with vitronectin-bound PAI-1, although the magnitude of polarization was decreased 7.3-fold compared to binding free PAI-1. The *in vivo* pharmacokinetic profile of these inhibitors is unknown.

Pharmacological inhibition of PAI-1 in cancer therapy

Pharmacologic inhibition of PAI-1 in cardiovascular diseases has been the primary goal with the objective to prevent inhibition of intravascular fibrinolysis and subsequently promote thrombus repermeabilization in an acute setting. In contrast, pre-clinical evidence supporting the therapeutic efficacy of small PAI-1 inhibitors in cancer has been limited (Table 2). In cancer, the objective is to prevent inhibition of peri-cellular activation of plasminogen and interfere with PAI-1-vitronectin interactions. Whereas in cardiovascular and thrombotic

disease the desired inhibition is short-term; in cancer (or other chronic conditions) it is long-term requiring a pharmacological profile suitable for chronic administration (32).

Insofar, four small molecule PAI-1 inhibitors have been tested in pre-clinical models of cancer. Although limited, these studies have shown anti-tumor activity. SK-116 and SK-216 inhibitors administered orally for 9 weeks to Min mice that spontaneously developed intestinal polyps caused an almost 2-fold reduction in the number of small intestinal polyps (50). When administered to PAI-1 producing Lewis lung carcinoma and PAI-1-non-producing B16 melanoma tumor-bearing mice, SK-216 caused a 2-fold reduction in subcutaneous primary tumor size and inhibited angiogenesis and metastases (51). The oral administration of PAI-039 to mice xenotransplanted with human T24 bladder and HeLa cervical cancer cells resulted in a 2-fold reduction of tumor volume after 14 days associated with a decrease in tumor cell proliferation and vascularization and an increase in apoptosis (52). The TM5275 inhibitor was recently shown to increase apoptosis *in vitro* in ovarian cancer cell lines (53). The *in vivo* activity of these inhibitors in cancer models have not been reported with the exception of TM5275 and TM5441 in a most recent study (54).

Challenges and opportunities

Despite the fact that much is known on the structure of PAI-1, its complex biological function, and its pro-tumorigenic role in cancer progression, its potential as a target for therapeutic intervention in cancer has only been recently considered and explored. These studies provide important insight on the need to develop better inhibitors for cancer. The need for chronic administration and thus a pharmacological profile with a prolonged plasma half-life of orally available inhibitors is an important consideration. Although much work has been done on the development of orally available compounds, the half-life of these inhibitors *in vivo* is 2–3 hours and therefore would be impractical in long term cancer therapy. The fact that most inhibitors developed until now are active *in vitro* at concentrations in the μM range represents another limitation. Such concentrations are typically difficult to reach in blood and more importantly in tumor tissues for an extensive period of time. The lack of activity of most inhibitors against the stable vitronectin-bound form of PAI-1 is a third limitation, since this is the predominant form of PAI-1 in vitronectin-rich tumor tissues (55). A fourth limitation is the potential systemic effect that the chronic administration of PAI-1 inhibitors may have on hemostasis. By suppressing the control that PAI-1 exerts on plasmin-mediated fibrinolysis, small molecule PAI-1 inhibitors may impair hemostasis and cause excessive spontaneous bleeding (56). Although studies in non-human primates have shown an absence of side effects on systemic hemostasis following the chronic administration of some PAI-1 inhibitors (44,45), this aspect deserves more extensive investigation.

In summary, targeting PAI-1 in cancer therapy remains an attractive but also challenging approach that will require the design and development of inhibitors that address some of the current limitations outlined above. As new inhibitors with better pharmacological profiles are developed, they should continue to be tested in pre-clinical models of cancer.

Acknowledgments

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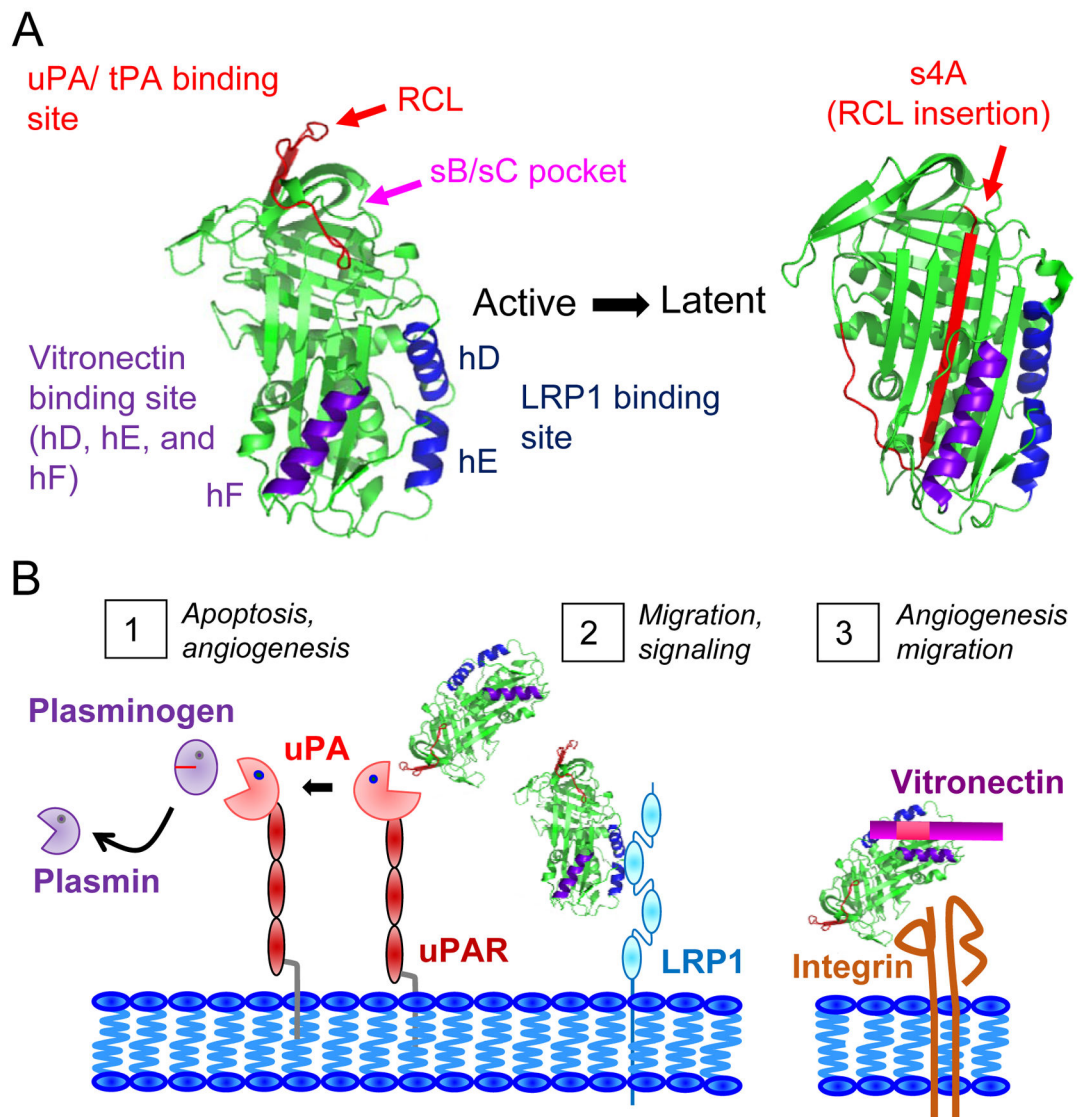
Figure 1. Structure of PAI-1 and inhibitor targeting sites

A) Structure of PAI-1 and targeted binding sites. The reactive center loop (RCL) is shown in red, which inserts itself to become s4A in the latent form of PAI-1. The sB/sC pocket is indicated by the arrow (pink). The vitronectin (hD, hE, and hF) and LRP1 (hD and hE) binding regions are shown in purple and blue, respectively. Molecular modeling was carried out using Pymol software version 1.2r1 and the PDB files 1B3K for active PAI-1 (57) and 1DVN for latent PAI-1 (58). **B**) Roles of PAI-1 in tumorigenesis: 1) the inhibitory role of PAI-1 on uPA action to prevent the cleavage of plasminogen into plasmin and subsequent substrates on the cell surface leading to angiogenesis or intracellular apoptotic signaling, 2) the ability of PAI-1 to bind to LRP1 directly leading to intracellular signaling and cell migration, and 3) the ability of PAI-1 to bind to vitronectin in the extracellular matrix to promote endothelial cell migration and angiogenesis.

Table 1

Outcome of the genetic ablation of PAI-1 in the host and/or implanted tumor cells in murine models of cancer

GEMM: genetically engineered mouse model. $^{+/+}$ indicates transgenic overexpressing PAI-1 mice, $^{-/-}$ indicates PAI-1 deficient mice. n/e indicates that the experimental parameter was not evaluated.

Model	PAI-1 Host Mouse	Implanted Cells	Tumor Growth	Angiogenesis	Metastasis	Reference
	+/+	B16 melanoma	No difference	n/e	No difference	21
	−/−	B16 melanoma	No difference	n/e	No difference	21
	−/−	Malignant keratinocytes	Decreased	Decreased	n/e	17
	−/−	T241 fibrosarcoma	Decreased	Decreased	Decreased	16
Syngeneic Implantation	+/+	Malignant keratinocytes	Decreased	Decreased	n/e	10
	−/−	Malignant keratinocytes	Decreased	Decreased	n/e	10
	−/−	Spontaneously transformed primary lung fibroblasts	Decreased	n/e	n/e	18
	−/−	Spontaneously transformed primary fibrosarcoma	Decreased	n/e	n/e	19
	−/−	Spontaneously transformed primary fibrosarcoma	Decreased	n/e	n/e	20
	−/−	Human neuroblastoma	Decreased	Decreased	n/e	11
	+/+	Human M21 melanoma	Increased	Increased	n/e	22
Xenograft	−/−	Human M21 melanoma	Decreased	Decreased	n/e	22
	−/−	Human HaCaTII-4 and HaCaT A5-RT3	Decreased	Decreased	n/e	23
	−/−	Human PAI-1 KD HT1080 fibrosarcoma, colon cancer HCT116, breast cancer MDA-MB-231, lung cancer A549	Decreased	Decreased	n/e	12
	MMTV-PymT/PAI-1$^{-/-}$		No difference	No difference	No difference	24
GEMM	Apc/Apc1638N/PAI-1$^{-/-}$		No difference	n/e	n/e	25
	TRP-1/SV40 Tag/PAI-1$^{-/-}$		No difference	No difference	Decreased	26
	K14-HPV16/PAI-1$^{-/-}$		No difference	No difference	n/e	27

Table 2

Outcome of pharmacologic inhibition of PAI-1 in pre-clinical murine models of cancer

n/e indicates that the experimental parameter was not evaluated.

Compound	Inhibitor Class	IC50 (uM)	Half-life (hours)	Cancer Model		Efficacy in cancer models		
					in vitro	Tumor growth	Angiogenesis	Metastasis
PAI-039	Indole oxoacetic acid	2.7	2.95 – 3.73	Bladder and cervical cancer	Yes	Yes	Yes	n/e
TM5275	N-acylanthranilic acid	6.9	2.5	Ovarian cancer	Yes	n/e	n/e	n/e
SK-116, SK-216	not stated	35, 44	n/e	ApcMin/+ GEMM	Yes	Yes	n/e	n/e
SK-216	not stated	44	n/e	Lung cancer and melanoma	Yes	Yes	Yes	Yes