

Plasminogen activator inhibitor-I: The double-edged sword in apoptosis

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Summary

Plasminogen activator inhibitor type-I (PAI-I) is a multi-functional protein. It is a fast-acting inhibitor of plasminogen activators; urokinase-plasminogen activator and tissue type plasminogen activator, and also plays an important role in regulating cell proliferation, adhesion, migration, and signal transduction pathways. These biological events are important processes during angiogenesis and restenosis. PAI-I has been shown to regulate proliferation, migration, and apoptosis of vascular smooth muscle cells and endothelial cells. The ability of PAI-I to regulate cellular proliferation and migration has been attributed to its ability to control plasmin production, modify signaling pathways, and its inherent multifactorial ability to bind to vitronectin and lipoprotein receptor-related protein. However, the mechanism by

which PAI-I regulates the apoptotic pathway is not well understood. Evidence from the literature suggests that PAI-I or its deficiency alters key signalling pathways, such as the PI3-k/Akt and the Jak/STAT pathways, and is involved in maintaining endothelial cell integrity thereby regulating cell death. Other investigators have demonstrated that PAI-I directly binds to caspases as a mechanism of PAI-I-mediated cellular apoptosis. Moreover, results from studies assessing the role of PAI-I in apoptosis have suggested that PAI-I can exert pathogenic or protective effects, which may be related to the disease model or type of injury employed.

Keywords

Apoptosis, fibrinolysis inhibitors, plasminogen activator inhibitors

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Introduction

Programmable cell death or apoptosis of vascular cells is an important process that occurs during blood vessel remodeling under both physiological and pathological conditions and is an important determinant in the fate of tumor growth (1–3), as well as in the formation of an atherosclerotic plaque (4–5). In vascular smooth muscle cells (VSMC), both apoptosis and anoikis, which is detachment of the cell from the extracellular matrix (ECM), significantly affects the development of atherothrombosis, plaque rupture, and aneurysm formation (6, 7). Vascular cell apoptosis also occurs during neonatal vascular remodeling, where the VSMC and endothelial cells (EC) are subject to dramatic haemodynamic changes at birth (8). Components of the plasminogen-plasminogen activator system have been implicated in playing an important role in these processes by facilitating ECM remodeling. Both VSMC and EC exhibit considerable fibrinolytic activity, whereby inactive plasminogen is converted

to active plasmin by urokinase-plasminogen activator (uPA) and tissue type plasminogen activator (tPA). It has been demonstrated by studies *in vitro* and *ex vivo* utilizing VSMC, that tPA-mediated plasmin generation induced fibronectin fragmentation leading to cell detachment or anoikis (9). In an Alzheimer's disease model it was observed that activation of plasminogen by uPA or tPA was accompanied by increased viability of cerebrovascular smooth muscle cells due to degradation of the pathogenic amyloid-beta protein. Thus the plasmin-generating cascade serves a neuro-protective role. However, chronic expression of uPA and plasminogen activation led to significant cell detachment (10). It was observed that increased PAI-I expression in the hippocampus and amygdala regions of the brain specifically reduced tPA activity and clearance of the amyloid-beta protein (11). In certain acute neuronal insults, such as ischemia microglial activation, tPA synthesis increases triggering neuronal death. This effect could be prevented by the presence of PAI-I (12). It thus appears that not only tight control of plasmin gener-

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ation is important, but the neuroprotective effect of PAI-1 may surface during specific acute injuries, as in an ischemic attack. However, chronically increased levels of PAI-1 that inhibit plasmin generation promotes accumulation of the toxic amyloid-beta protein.

Apart from the perspicuous functional capability of uPA and its cognate receptor (uPAR) in playing a dominant role in matrix degradation, migration, proliferation, and cytoskeleton changes (13–15), all hallmark events in cellular transformation; an emerging role in apoptosis is evolving. Blocking interaction of uPA to uPAR, or down-regulation of both uPA and uPAR resulted in decreased tumor cell invasion, and increased apoptotic cell death in prostate cancer (16, 17) and breast cancer cell lines (18). Pre-exposure of nontransformed human retinal pigment epithelial cells to uPA diminished anoikis and UV-induced apoptosis, which were mechanistically attributed to up-regulation of the anti-apoptotic factor Bcl-xL via the MEK/ERK and PI3-k pathway. The protective anti-apoptotic effect was eliminated when uPA/uPAR expression was down-regulated by RNAi (19). Tumor necrosis factor (TNF)- α -induced apoptosis in monocytes could be inhibited by plasmin with concomitant reduction in the levels of active caspase-3, -8, and -9 (20). Although it has been established that tPA, uPA, uPAR, and plasmin are involved in regulating apoptosis, plasminogen activator inhibitor-1 (PAI-1), which is the primary inhibitor of the uPA/tPA-plasmin axis, is also known to regulate cellular apoptosis. This review focuses on the role of PAI-1 in apoptosis and a number of possible mechanisms are discussed herein.

The paradoxical PAI-1: To be apoptotic or not to be

PAI-1 is a rapid and most physiologically-relevant inhibitor of uPA and tPA. Several investigations have detailed a positive correlation between high levels of uPA and uPAR with poor prognosis and unfavorable clinical outcome in several types of cancers (2, 21–25). It would appear logical that PAI-1, by virtue of its ability to inhibit activation of plasminogen by uPA or tPA, would effectively inhibit tumor angiogenesis and growth. However, increased levels of PAI-1 are indicators of poor prognosis for cancer patient survival (26–30). The role of PAI-1 in angiogenesis is controversial, where it has been documented to be pro-angiogenic (31–35), anti-angiogenic (36–38), or indifferent (39). These observations were found to be dependent on the experimental setting, stage of cancer progression, and the origin of the cells. Furthermore, angiogenic effects of PAI-1 have also been found to be dose-dependent (3, 40). In these studies, physiological concentrations (nanomolar) of PAI-1 promoted angiogenesis through its anti-proteolytic activity, whereas high concentrations (micromolar) of PAI-1 were anti-angiogenic attributed to its vitronectin binding function (3). PAI-1 has been proposed to act as a molecular switch, and at high concentrations it dissociates EC uPAR from vitronectin promoting a migratory phenotype followed by correct presentation of integrins to matrix ligands that allows for EC adhesion (41, 42), thus promoting angiogenesis. Poor prognosis may be related to the source of PAI-1. Observations leading to poor prognosis correlated to high

PAI-1 levels in the surgical samples (43–48) or in the patient's plasma (49, 50). However, when tumor cell lines were transfected with PAI-1, the aggressiveness of tumor growth and metastasis was diminished in HT-1080 fibrosarcoma cells (51), murine melanoma (52), PC-3 prostate cancer cell lines (36), and in malignant keratinocytes (53). From these early studies emerged the tenet that PAI-1 may play a role in apoptosis. Subsequently other studies followed that corroborated the novel role of PAI-1 in regulating apoptosis.

PAI-1 and apoptosis in neovascularization

Tumor progression and dissemination are dependent on neovascularization or formation of new blood vessels, which involves tipping the pro-angiogenic and anti-angiogenic balance in favor of the former. Though this process involves a large number of proteases and extensive remodeling of the ECM promoting tumor invasiveness, investigations on mediating tumor cell apoptosis and its mechanism are significantly scrutinized as a means of controlling tumor growth. Neovascularization is also manifested during atherosclerotic plaque formation typically characterized by accumulation of smooth muscle cells and inflammatory cells in the media and subendothelial spaces. The role and mechanism of apoptosis in defined cell lineages, such as VSMC, EC, and macrophages during plaque formation would provide novel therapeutic insights for drug design in atherosclerosis. This section highlights the role of PAI-1 in apoptosis in pathophysiological settings of tumors and atherosclerosis.

When the human prostate cancer cell line PC-3, and the human promyelocytic leukemia cell line HL-60 were treated with recombinant wild-type (WT) PAI-1, induced and spontaneous apoptosis were diminished. This inhibition of apoptosis by PAI-1 was reversed by the presence of a neutralizing antibody that accelerated the conformational change of active PAI-1 to inactive PAI-1. Similarly, treatment of cells with latent PAI-1 did not inhibit apoptosis, thus suggesting that the reactive site loop may be required for the apoptosis inhibiting function. Furthermore, inhibition of apoptosis by PAI-1 is independent of the PAI-1:uPA complex, signalling through uPAR, or binding to vitronectin. Inhibition of apoptosis by an exogenous source of PAI-1 could very well mimic the tumor environment where PAI-1 is secreted in the tumor vasculature by surrounding stromal cells, and potentially increasing the aggressiveness of the tumor, thus giving credence to the reported observations of PAI-1 as a prognostic marker of poor overall survival (54). In contrast, tumor cells transfected to express high levels of PAI-1, acting as tumor-host PAI-1, behave differently, conferring anti-angiogenic properties (36, 51, 52). The differential effect of PAI-1 expressed by normal and cancerous cells on apoptosis has been documented by Lademann et al. (55), who have observed that fibrosarcomas obtained through spontaneous transformation in WT mice showed decreased cellular sensitivity to chemotherapy-induced apoptosis compared to fibrosarcomas obtained from PAI-1^{-/-} mice. Whereas, WT and PAI-1^{-/-} mice display similar sensitivity to etoposide exposure, clearly underlining that PAI-1 expressed by cancer cells behaves differently than PAI-1 expressed by normal cells on apoptosis, and that inhibiting PAI-1 may be beneficial for encouraging chemotherapy-induced apoptosis (55). Another study from the same group highlighting

the anti-apoptotic potential of PAI-1 demonstrated that fibrosarcoma cell lines established from PAI-1^{-/-} mice took significantly longer to grow and develop tumors when injected in WT or PAI-1^{-/-} mice compared to fibrosarcoma cell lines established in WT mice. Also, the PAI-1^{-/-} fibrosarcoma cells were more susceptible to TNF- α -induced apoptosis accompanied by increased levels of caspase-3 activity compared to the WT fibrosarcoma cells (34). These differences in PAI-1 expressing and PAI-1-deficient fibrosarcoma cells have been attributed to differences in cell proliferation, and that PAI-1 expressed by the tumor cells protects the tumors from apoptosis. Since exogenous PAI-1 could also inhibit apoptosis in non-tumor cells, such as HUVEC and the benign human breast epithelial MCF-10A cell lines, it is thought that regulation of apoptosis by PAI-1 could also have physiological implications (54).

The inconsistent nature of PAI-1 surfaced when rPAI-1 did not induce apoptotic death or control the growth and metastasis of implanted human colon cancer xenografts in nude mice. On the other hand, intraperitoneal infusion of rPAI-2 diminished the size of the primary tumor and enhanced the tumor apoptotic index (56). A similar anti-cancer activity of PAI-2 was observed due to enhanced apoptosis in metastatic prostate cancer cells (57). However, stable transfection of the human prostate cancer PC-3 cells that conditionally expressed active PAI-1 regulated by doxycycline resulted in dramatic inhibition of angiogenesis as observed by CD31 staining, when these cells were injected in nude mice. The PAI-1-induced diminished angiogenesis was ascribed to an early wave of apoptosis in tumor EC with a concomitant decrease in cell proliferation. The induction of EC apoptosis by PAI-1 was found to be vitronectin-dependent, which served as an adhesion matrix during angiogenesis (58). PAI-1 induces EC apoptosis by preventing the interaction of EC integrins, $\alpha_v\beta_3$, to the RGD-binding site of vitronectin resulting in disruption of integrin-mediated signalling and hence triggering anoikis (59). At the time of a pathological condition, such as cancer progression, PAI-1 levels are dramatically increased. Studies initiated with different cell lines, such as PC-3 or malignant PDVA keratinocytes stably transfected with PAI-1 cDNAs express approximately nine- to 18-fold elevated levels of secreted PAI-1 antigen (36, 53, 58). Tumorigenicity and angiogenic phenotype of these transfected cell lines were observed when injected in either WT or PAI-1-deficient mice to allow investigation on the effect of PAI-1 on newly established tumor vasculature in early stages of tumor progression. It is possible that these high levels of PAI-1 present during pathological conditions are sufficient to compete and displace the binding of EC integrins $\alpha_v\beta_3$, to the RGD-binding sites of vitronectin. Furthermore, recapitulation of PAI-1 inducing EC apoptosis, which is dependent on vitronectin, was observed *in vitro* when microvascular EC co-cultured with PC-3 cells, stably transfected to express PAI-1 exclusively, underwent apoptosis when plated in the presence of vitronectin (58). Similarly, PAI-1 could inhibit EC tube formation in the presence of vitronectin but not in the presence of fibronectin (60). These *in vitro* experiments were performed utilizing rPAI-1 at high concentrations of 40–100 nM. However, it should be noted that this mechanism of PAI-1-mediated tumor apoptosis by a vitronectin-dependent mechanism might be possible in an environment where the tumor is established and angiogenesis is still active. In

such a scenario the EC detach and launch to re-adhere to vitronectin, which is inhibited due to the presence of high levels of PAI-1 in the tumor microenvironment. Since tumor growth is dependent on neovascularization, this would result in an inability of the tumor to sustain further growth.

A similar anti-adhesive property of PAI-1, the result of a two- to three-fold increase in apoptosis, was documented in EC and VSMC, and found to be vitronectin-dependent. Induction of apoptosis was mediated by the caspase-3 pathway and the apoptotic cells were associated with foam cells as observed in atherosclerotic vessel sections, thus re-enforcing the importance of apoptotic and anti-adhesive mechanism in tissue remodeling during neointima formation (61). In contrast PAI-1 did not induce apoptosis of human brain microvascular EC even though it inhibited $\alpha_v\beta_3$ integrin-mediated adhesions to vitronectin. Instead it stimulated migration of EC from vitronectin towards fibronectin, promoting cell migration away from the vitronectin-rich perivascular spaces towards fibronectin-rich tumor tissues. Thus, a lack of adhesion alone is not sufficient to induce apoptosis by PAI-1 suggesting that other mechanisms may be involved (60).

Tumor growth of T241 fibrosarcoma cells was significantly suppressed in PAI-1^{-/-} mice accompanied by decreased proliferation and increased apoptosis in tumors from these deficient mice compared to WT mice (32). In this case, lack of host PAI-1 did not sustain tumor growth and survival potentially due to a lack of the ability to disrupt EC adhesion from the surrounding vitronectin and promote motility, thereby preventing neovascularization. EC isolated from aortas of PAI-1^{-/-} mice exhibit increased proliferative phenotype associated with hyperactivation of the Akt molecule required to maintain cell survival and integrity. The increased levels of Akt(P-Ser473) were responsible for increased levels of inactive caspase-9(P-Thr163) and hence lower levels of active caspase-3 in the PAI-1^{-/-} cells compared to WT cells. Spontaneous apoptosis was also decreased in PAI-1^{-/-} EC relative to WT cells. Treatment of PAI-1^{-/-} EC with recombinant PAI-1 induced a WT proliferative phenotype, and also increased the levels and activity of caspase-3 which increased spontaneous apoptosis, establishing a novel role for PAI-1 in regulating apoptosis via the Akt signalling pathway. It was determined that interaction of PAI-1 with the endocytic receptor lipoprotein receptor-related protein (LRP) was essential for PAI-1 to negatively regulate cell proliferation, since the mutant R76E[PAI-1], which has diminished ability to bind to LRP, was unable to regulate cell proliferation of PAI-1^{-/-} cells (62, 63). It appears from the studies of Balsara et al. (63) and Chen et al. (58) that either an exogenous source of PAI-1, in case of *in vitro* EC studies or tumor cells overexpressing PAI-1 is effectively able to induce apoptosis and control growth of tumor vasculature. Conversely, a complete deficiency of host PAI-1 also does not support tumor growth by induction of apoptosis as observed when PAI-1^{-/-} mice were injected with the T241 fibrosarcoma cells (32), and these observations could reflect that the source of PAI-1 is an important determinant for deciding the fate of a tumor.

Increased proliferation and decreased apoptosis of VSMC is a distinguishing hallmark of restenosis (64–66) accompanied by increased synthesis of PAI-1 in patients with type 1 diabetes

(67–69), suggesting that increased PAI-1 expression could affect VSMC apoptosis. It has been observed by Chen et al. (70) that VSMC from SM22-PAI-1⁺ mice, which overexpress PAI-1 by two-fold, were approximately 20% less apoptotic than control WT cells when stimulated with tissue necrotic factor and/or phorbol myristate acetate. These findings are in concordance with the fibrosarcoma studies of Romer et al. described earlier (34). Decreased apoptosis was due to a decrease in caspase-3 activity, and when exogenous PAI-1 was added to VSMC lysates or directly to recombinant caspase-3, inhibition of caspase-3 activity was observed. Solid phase binding assays demonstrated that PAI-1 could bind to caspase-3 with an apparent K_d of ~3 nM. The proteolytically inactive PAI-1 (H190L-H191L) could neither bind to caspase-3 nor inhibit caspase-3 activity suggesting that the inhibitory activity of PAI-1 is essential for its anti-apoptotic activity (70). The high-affinity interaction, *in vitro*, of PAI-1 with caspase-3 to form a complex is not considered stable as the 1:1 stoichiometric complex formed between PAI-1 and uPA or tPA. The PAI-1/caspase-3 complex is dissociable under reducing conditions. Analogous interactions between other inhibitory proteinases of the serpin superfamily and caspases have been documented typically described as “cross-class” interaction, in which a serpin inhibits a non-serpin proteinase (71, 72). The serpin CrmA (Cytokine Response Modifier A) binds to caspase-1 modulating host inflammatory responses (73), and the PI9 (Proteinase Inhibitor 9) serpin interacts with caspases-1, -4, and -8 (72). While binding of CrmA to caspase-1 is rapid with a second-order rate constant of $2 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ (71), the second-order rate constant for PI9 and caspase-1 is $700 \text{ M}^{-1}\text{s}^{-1}$ (72). This could have significant physiological implications where CrmA could be required to completely inhibit an inflammatory response. In contrast, inhibition of caspase activity in a more controlled manner would be beneficial. Though not much is known about inhibition of caspase-3 activity by interaction with PAI-1 it is possible that intracellular PAI-1 can bind to caspase-3 or pro-caspase-3 attenuating apoptosis. It is also possible that internalization of secreted PAI-1 by the uPA/uPAR/PAI-1 tri-complex could bind and inhibit caspase-3, thereby serving as an anti-apoptotic agent.

Further consequences of increased PAI-1 levels in the SM22-PAI-1⁺ VSMC resulted in increased proliferation due to increased expression and activity of FLICE-like inhibitory protein (FLIP) which in turn induces nuclear factor kappa-B (NFκ-B) and ERK signalling to promote proliferation. Additionally, the levels of dichotomous cleaved caspase-8 that can cleave caspase-3 or cleave FLIP to generate p43 that promotes proliferation, was increased in the SM22-PAI-1⁺ VSMC favoring proliferation compared to littermate control VSMC. Inhibition of NFκ-B and ERK signalling effectively diminished cell proliferation of SM22-PAI-1⁺ VSMC indicating that PAI-1-mediated cell proliferation in these cells is through both of these pathways (74). Conversely, plasminogen-induced apoptotic index was high in VSMC from PAI-1^{-/-} mice compared to VSMC from WT, uPA^{-/-}, and tPA^{-/-} mice. The pro-apoptotic index paralleled the generation of plasmin activity, which was higher in PAI-1^{-/-} VSMC, and the PAI-1-deficient cells showed morphological alterations reminiscent of apoptosis, such as cell shrinkage and nuclear fragmentation (65). In fact, the apoptotic index of VSMC in

atherosclerotic aortas from ApoE^{-/-}:PAI-1^{-/-} mice was higher accompanied by enhanced *in-vivo* levels of plasmin, and active caspase-3, consequently leading to a decrease in VSMC density relative to the ApoE^{-/-} littermates (76).

Fibroblasts and myofibroblasts are key cells involved in wound closure and reepithelialization and undergo physiologic apoptosis during wound repair, the absence of which could cause tissue fibrosis (67, 78). Plasminogen-induced apoptosis of fibroblasts, which is accompanied with fibronectin proteolysis and anoikis of fibroblasts, could be inhibited by transcriptional growth factor (TGF)β-1 due to TGFβ-1-mediated upregulation of PAI-1, and subsequent inhibition of plasminogen activation. Furthermore, TGFβ-1 was unable to protect the cells from apoptosis when lung fibroblasts from PAI-1^{-/-} mice were treated with plasminogen. PAI-1^{-/-} fibroblasts demonstrated increased plasminogen-induced apoptosis, which was reversed when the deficient cells were reconstituted with exogenous PAI-1 (79). High levels of PAI-1 prevented extracellular proteolysis resulting in accumulation of extracellular matrix components, such as fibronectin, thereby promoting progressive tissue fibrosis. Although the precise mechanism as to how PAI-1 regulates apoptosis in fibroblast cells is unknown, it has been shown to be associated with pericellular proteolysis of fibronectin. Therefore, PAI-1 plays a central role in protecting cells against plasminogen-induced apoptosis.

There is strong evidence suggesting a role for PAI-1, and in general the plasminogen/plasmin system, in VSMC apoptosis which is a major process that determines atherosclerotic plaque vulnerability (4, 5). The significance of these findings allows for mechanistic insights regarding the pivotal role of PAI-1 in vascular remodeling, whereby increased expression of PAI-1 in diabetic patients may promote increased accumulation of VSMC in response to a vessel wall injury. However, neointima formation was attenuated following an arterial copper cuff injury (80) or carotid artery ligation (81) in PAI-1^{-/-} mice compared to control mice with no differences observed in proliferation of PAI-1^{-/-} VSMC. This highlights the conflicting effects of PAI-1, which may be a reflection of variation in experimental scenarios that may be dependent on cell types and the challenge models that are employed.

PAI-1 and apoptosis in the central nervous system

In the central nervous system the tPA-plasmin system has been known to play a significant role in synaptic plasticity, remodeling (82, 83) and in regulation of neuronal survival in response to excitotoxicity (84, 85). However, PAI-1 although secreted by astrocytes does not behave as the prototypic inhibitor of the neural protease cascade but rather functions as a neuroprotective agent (86, 87), and high levels of PAI-1 in cerebrospinal fluids (88, 89) and the central nervous system could also serve as an index of neurological diseases (90–92). There is some evidence of a role for PAI-1 in regulating neuronal apoptosis. When PC-12 neurons were grown in medium containing PAI-1 obtained from astrocyte-derived conditioned medium, the neurons maintained their morphology and survived. However, when the neurons were cultured in medium deficient of PAI-1, neuron survival decreased by 50%, and the cells exhibited typical apoptotic characteristics of a rounded morphology and DNA fragmentation. The mechanism by which a

PAI-1 deficiency promotes apoptosis is through stimulating the release of mitochondrial cytochrome c, and decreasing mRNA levels of anti-apoptotic Bcl-2 and Bcl-X_L genes with a concomitant increase in pro-apoptotic Bcl-X_S and Bax mRNA (93). Eventually, the formation of the apoptosome composed of cytochrome c, Apaf-1, and procaspase-9 results in upregulation of caspase-3 activity. Similar results were observed when an anti-PAI-1 neutralizing antibody was added to the neuronal medium. Anti-apoptotic characteristics were restored to the neurons when recombinant PAI-1 was added to the culture medium. Interestingly, neural plasminogen activation activity was not affected by the presence or absence of PAI-1. Thus reinforcing that PAI-1 is not the primary inhibitor of the neural plasminogen system, and that it plays an important role in regulating survival of neurons (93). The anti-apoptotic or pro-survival function of PAI-1 on neurons was demonstrated in PC-12 cells where PAI-1 promoted neurite outgrowth and survival. These phenotypic observations were reversed in the absence of PAI-1 when the PC-12 cells exhibited a typical apoptotic/necrotic phenotype. The pro-survival function of PAI-1 was found to be due to activation of c-jun and ERK pathways, which was preceded by the activation of the nerve growth factor receptor, Trk A (94). Table 1 summarizes the pro- and anti-apoptotic activities of PAI-1 in different cell types.

In other studies of N-methyl-D-aspartate (NMDA)-induced excitotoxicity of co-cultures of neurons and astrocytes, TGF- α and - β -mediated neuroprotection against apoptotic cell death was attributed to increased expression of PAI-1 by astrocytes via two different signalling pathways. TGF- α -1-mediated increase in PAI-1 expression was through activation of the ERK pathway, whereas TGF- β -1-mediated increase in PAI-1 expression was through the Smad-3-dependent signalling pathway. This neuroprotection was not observed when neurons were co-cultured with PAI-1^{-/-} astrocytes, thus highlighting the role of PAI-1 in controlling excitotoxic neuronal death stimulated by the glutamatergic agonist NMDA (95, 96). This is in consonance with earlier studies where the cerebral ischemic infarct size was significantly enlarged in PAI-1^{-/-} mice compared to WT mice and this was reversed when the PAI-1^{-/-} mice were injected with recombinant adenovirus encoding PAI-1 protein (97). However, in primary cortical neurons, blocking of the serine protease HtrA1 (high temperature responsive antigen 1), which is known to regulate several pathologies, such as Alzheimer's disease, macular degeneration, and osteoarthritis, led to overexpression of neuronal PAI-1 mediated by TGF- β -1 signalling and eventually to neuronal death (98). This observation is in contrast to the neuroprotective effect of PAI-1 against NMDA-induced excitotoxicity (95, 96). However it is possible that HtrA1-mediated TGF- β -1 signalling effect on PAI-1 expression could play a central role in neuronal maturation during brain development.

Conclusion

It is evident that PAI-1 is a functionally promiscuous protein, not only acting as a primary inhibitor of uPA and tPA, but also involved in modulating cell proliferation, migration, and apoptosis (58, 60, 62, 93, 99). PAI-1 expression is tightly regulated, and under normal conditions PAI-1 is present as a trace protein in plasma. However, during a pathological condition, such as

cancer, atherosclerosis, diabetes, and severe obesity PAI-1 levels are dramatically elevated (100).

The involvement of PAI-1 in angiogenesis is controversial and not fully understood. It regulates this process through binding of PAI-1 to vitronectin (60) or through inhibition of uPA and tPA where excess plasmin activity is abrogated leading to vessel stabilization (33, 101). Components of the plasminogen activators-plasmin system are also known to regulate apoptosis; however, PAI-1 can be pro-apoptotic (61, 63, 87) and anti-apoptotic (70, 93, 94, 96). Increased levels of PAI-1 in tumor pathologies prove to be beneficial to the tumor as it down-regulates apoptosis promoting a more aggressive phenotype and supporting the paradox that elevated PAI-1 levels correlate with poor patient prognosis. Indeed, addition of exogenous PAI-1 to HL-60 and PC-3 cells could inhibit apoptosis that required the inhibitory activity of PAI-1, but was not necessarily via the uPA/uPAR-signalling axis (54). Another advantage for cancer cells producing high levels of endogenous PAI-1 may be that they are less sensitive to chemotherapy treatment. Church et al. (102) demonstrated that MDA-MB-435 breast cancer cells expressing WT PAI-1 had an increased recovery compared to MDA-MB-435 cells expressing inactive PAI-1 after treatment with paclitaxel. Hence, up-regulation of PAI-1 in cancer cells foster tumor growth and spread. Furthermore, by cDNA microarray analysis it was demonstrated that MDA-MB-435 cells expressing WT PAI-1 down-regulated genes, such as follistatin, which is an inhibitor of anti-proliferation (102). Similarly, fibrosarcoma fibroblasts established from PAI-1^{-/-} mice were more sensitive to apoptotic stimuli and had a longer lag-phase before they could establish tumors when injected in mice (34). From the above investigations it appears that down-regulation of PAI-1 would be beneficial for killing tumorigenic growth.

Absence of PAI-1 in VSMC, an important component of an atherosclerotic plaque, increases the apoptotic index, which may

Table 1: Effect of PAI-1 on apoptosis on some select cell lines. PAI-1, whether indigenously present (P) or absent (A) in the cells or added as recombinant protein (r-PAI-1) can affect apoptosis differently. This anomalous property of PAI-1 may depend on the origin of PAI-1, its ability to interact with vitronectin, or on the cell type and experimental setting.

Cell type	PAI-1: present (P)/absent (A)/r-PAI-1	Apoptosis increased (↑) or decreased (↓)
PC-3	r-PAI-1	↓
HL-60	r-PAI-1	↓
Spontaneous fibrosarcoma	P	↑
Colon cancer xenografts	r-PAI-1	No difference
VSMC (SM22PAI-1+)	P	↓
Primary PAI-1 ^{-/-} EC	A	↓
PAI-1 ^{-/-} VSMC	A	↑
APO E ^{-/-} :PAI-1 ^{-/-} VSMC	A	↑
PC12 neurons	r-PAI-1	↓

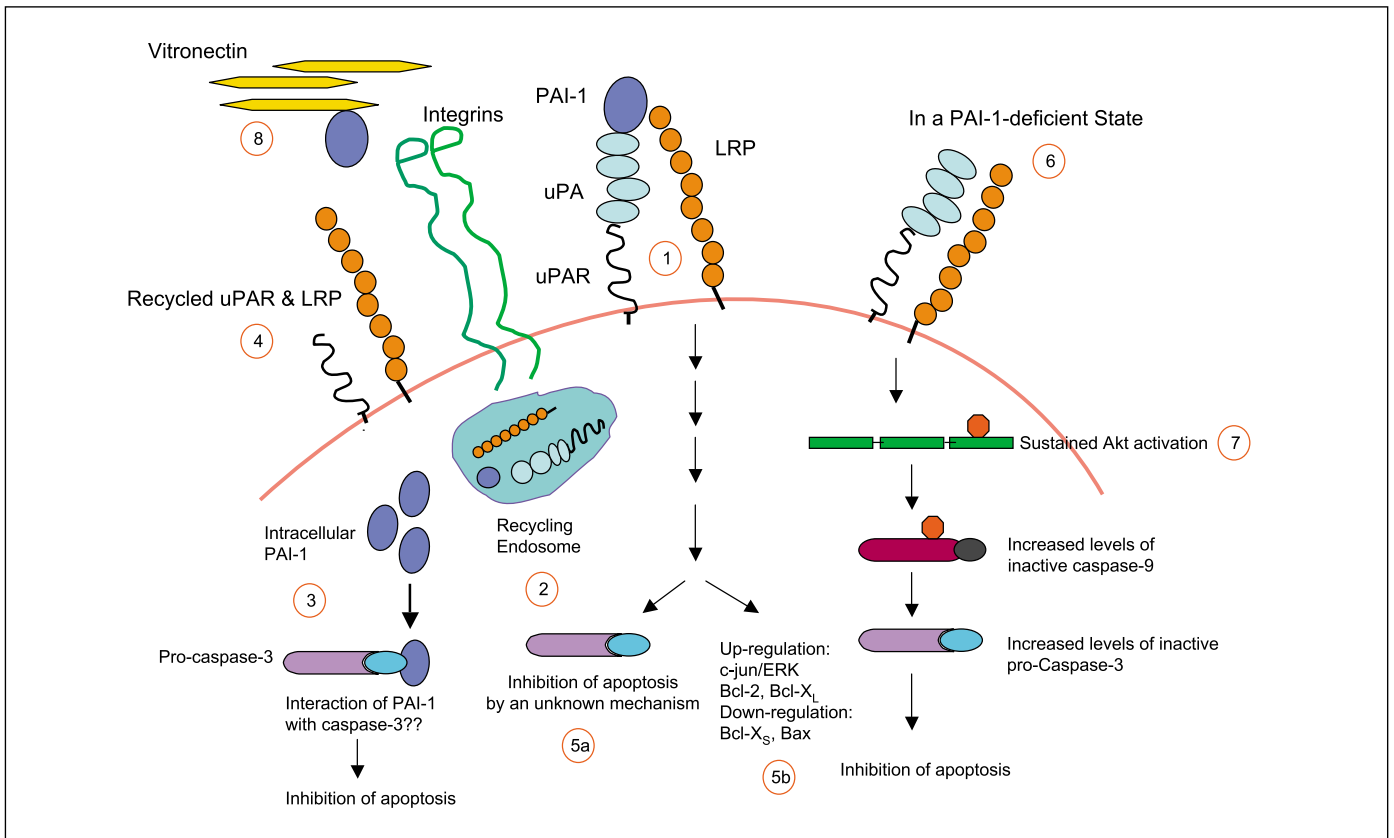


Figure 1: PAI-1-mediated apoptosis. The exact mechanism of PAI-1-regulated apoptosis is not clear, and several different scenarios are possible. The uPA/uPAR/PAI-1 complex (1) in the presence of LRP is endocytosed (2). Intracellular PAI-1 can complex with caspase-3 leading to its inactivation promoting an anti-apoptotic effect (3), while uPAR and LRP are recycled to the cell surface (4). Alternatively, PAI-1 due to its interaction with LRP can transduce signalling, intermediate inhibiting apoptosis (5a) by an unknown mechanism or promote survival by up-regulation of the c-jun/ERK pathway and anti-apoptotic proteins Bcl-2, Bcl-X_L (5b). In a PAI-1-deficient state (6) there is sustained Akt activation resulting in increased levels of phosphorylated caspase-9 and increased levels of pro-caspase-3 promoting an anti-apoptotic state (7). Alternatively, PAI-1 can bind to vitronectin (8) competing with EC integrins and preventing binding of the cell to the matrix causing anoikis.

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be beneficial in preventing atherosclerotic plaque formation or restenosis. Increased expression of PAI-1 or addition of exogenous PAI-1 controlled VSMC apoptosis by inhibiting caspase-3 (70). Addition of plasminogen to PAI-1^{-/-} VSMC induced an increase in apoptosis compared to WT, tPA^{-/-}, or uPA^{-/-} VSMC, indicating that plasminogen-mediated apoptosis is dependent on plasmin generated by tPA or uPA and is dampened by PAI-1 (75, 76). These data provide mechanistic insights in terms of the pathogenic or protective role of PAI-1 in vascular remodeling. Figure 1 schematically portrays the possible mechanisms by which PAI-1 could be regulating apoptosis. Although PAI-1 is a secreted protein, any intracellular events occurring after PAI-1 is internalized and is able to interact with caspases or other apoptotic proteins should not be discounted. Besides, interaction of PAI-1 with LRP may elicit recruitment of signalling intermediates that could regulate apoptosis via an unknown mechanism. There is a high probability that such a mechanism is possible when PAI-1 is added exogenously. We have demonstrated that r-PAI-1 could effectively diminish the hyperactivation of Akt in PAI-1^{-/-} EC with downstream consequences of increasing levels of active caspase-3 thereby increasing spontaneous apoptosis (63). Excess PAI-1 synthesized in a diseased state is known to compete for

binding with vitronectin causing detachment of EC. It is also possible that PAI-1 could be controlling apoptosis by two different mechanisms simultaneously.

Another mechanism by which PAI-1 can control proliferation is by controlling key cell-cycle-progression proteins. It has been reported that mouse embryonic fibroblasts (MEFs) from PAI-1^{-/-} mice display an uncontrolled proliferative phenotype compared to PAI-1 expressing MEFs mediated by a senescence bypass mechanism. As observed in primary murine aortic PAI-1^{-/-} EC (63), the PAI-1^{-/-} MEFs also exhibited sustained activation of Akt(P-Ser473) accompanied by nuclear retention of cyclin D1 and increased inactivation of GSK3-β was sufficient to bypass senescence (103). Thus PAI-1 acts as an inhibitor of proliferation.

In pathological conditions where PAI-1 overexpression promotes accumulation of VSMC, as in the increased incidence of restenosis after percutaneous coronary interventions, or renders tumor cells resistant to chemotherapeutic agents, decreasing PAI-1 levels would be beneficial. Therefore, in-depth mechanistic studies on the mode of action of PAI-1 in apoptosis would be therapeutically beneficial in enabling the design of drugs that diminish levels or selectively target specific functional domains of PAI-1.

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