

Theme Issue Article

The effect of plasminogen activator inhibitor type I on apoptosis

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Summary

Plasminogen activator inhibitor type-I (PAI-1), an inhibitor of plasminogen activators, inhibits formation of plasmin and plasmin-mediated proteolysis. Apoptosis, or programmed cell death, is a potentially important phenomenon in mediating overall cell death. This review focuses on the influence of PAI-1 on apoptosis. Greater expression of PAI-1 has been associated with increased survival of cells and resistance to apoptosis. PAI-1 ap-

pears to influence apoptosis by decreasing cell adhesion (anoikis) as well as its effect on intracellular signaling. Mechanisms by which PAI-1 may render a cell resistant to apoptosis include its ability to inhibit generation of plasmin, its ability to inhibit caspase 3, and its ability to inhibit cell adhesion mediated by vitronectin. Inhibition of caspase 3 by PAI-1 may divert intracellular signalling from induction of apoptosis to induction of proliferation.

Keywords

Plasminogen activator inhibitor, apoptosis, vascular remodelling, cancer

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Introduction

Plasminogen activator inhibitor type-1 (PAI-1) is a serine protease inhibitor (serpin) and the primary physiologic inhibitor of plasminogen activators (urokinase type, u-PA, or tissue type, t-PA). This single chain glycoprotein contains a reactive center that is accessible when the molecule is in a strained loop (active) conformation. The strained loop serves as a pseudo-substrate or bait for plasminogen activators. When PAI-1 is in its active conformation, the reactive center is exposed on the surface. When PAI-1 is in its latent conformation, the active center is not available. PAI-1 is expressed by multiple tissues including the heart, lung, aorta, muscle, adipose, and liver. A complete review of the molecular characteristics, distribution, and properties of PAI has been published recently (1).

Inhibition of plasminogen activators by PAI-1 decreases generation of plasmin and thereby inhibits fibrinolysis. Proteolytic effects of plasmin are not confined to fibrinolysis. Plasmin degrades matrix proteins, activates proteases such as matrix metalloproteases, and activates growth factors. Because PAI-1 attenuates generation of plasmin and thereby degradation of matrix, it influences cell migration. Cell adhesion (i.e. reflecting attachment and detachment) is influenced also by PAI-1. PAI-1 binds to vitronectin on the surface of cells. Integrins as well as the u-PA receptor bind to vitronectin (2). Competition between PAI-1 and integrins can influence cell adhesion. In addition to the extracellular effects it exerts, PAI-1 can modulate intracellular signaling.

For example, PAI-1 binds to and inhibits caspase-3, an aspartate-specific cysteinyl protease that mediates apoptosis (programmed cell death). PAI-1 appears to influence apoptosis by virtue of its extracellular effects on generation of plasmin and cell adhesion as well as its intracellular effect of inhibition of caspase-3.

Apoptosis is an important mechanism mediating cell death. It can be initiated by diverse factors including the absence of cell adhesion or tethering (anoikis). PAI-1 can limit cell detachment and hence apoptosis by inhibiting plasmin (3) but can also promote cell detachment and apoptosis through its binding to vitronectin (2). In some clinical states (such as those characterized by neoplastic cells) apoptosis may be desirable; in others it may be deleterious (such as exacerbation of myocardial infarction). Attenuation of apoptosis is a potentially attractive target for protecting the heart and other organs and tissues under circumstances in which they are jeopardized by ischemia. Conversely, promotion of apoptosis may improve prognosis in patients with cancer. This review focuses on the effects of PAI-1 on apoptosis.

The influence of PAI-1 on the response to injury

Expression of PAI-1 is increased in the vessel wall in patients with diabetes, patients who are particularly prone to exhibit restenosis after percutaneous coronary intervention (4–6). In mice and other laboratory animals, increased arterial wall expression of PAI-1 promotes greater overall neointimal cellularity after ex-

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ogenous vascular injury (7, 8). Conversely, we have shown that increased expression of PAI-1 in vessel walls diminishes the contribution of vascular smooth muscle cells (VSMC) to evolving atherosclerotic plaques thereby rendering them prone to develop features associated with plaque rupture (9, 10). These apparently opposite effects are likely to be mediated by the influence of PAI-1 on two separate processes. Restenosis and a proliferative response to vascular injury are likely secondary to the effect of PAI-1 on cell proliferation that is stimulated by an exogenous injury such as balloon dilatation. The contribution of PAI-1 to the generation of vulnerable atherosclerotic plaques appears to reflect its inhibition of cell surface plasminogen activation-dependent migration of VSMC into the neointima (11). Thus, greater expression of PAI-1 appears to promote both a proliferative response to vessel injury that contributes to restenosis after coronary intervention (by promoting proliferation) as well as the genesis of vulnerable atherosclerotic plaques (by inhibiting cell migration).

The cellular response to exogenous injury is significantly decreased after insults affecting arterial walls in PAI-1-deficient mice (7, 12). In addition, VSMC cellularity is diminished, and the prevalence of apoptosis is greater in mice with combined deficiency of PAI-1 and apolipoprotein E compared with both in mice deficient only in apolipoprotein E (13). Thus, expression of PAI-1 appears to influence neointimal cellularity by affecting apoptosis as well as migration of VSMC. Greater expression of PAI-1 appears to promote the neointimal cellular response to exogenous vascular injury, and the absence of PAI-1 is associated with less neointimal cellularity.

Downregulation of expression of PAI-1 in the heart after myocardial infarction has been reported to be associated with a marked (70%) reduction of apoptosis of cardiomyocytes two weeks after induction of infarction (14). Intramyocardial injection of a sequence-specific catalytic DNA enzyme at the time of coronary artery ligation that suppresses expression of PAI-1 mRNA has been reported to decrease apoptosis. We have found that PAI-1 knockout mice have dense infarcts and a high incidence of cardiac rupture early after coronary artery ligation (15); however, the extent of apoptosis is limited early after infarction (16). Thus, the exacerbation of infarction may reflect unleashing of the influx of inflammatory cells secondary to lack of inhibition of their cell surface plasminogen activators. Although apoptosis is limited early after infarction, increased expression of PAI-1 may influence apoptosis of cardiomyocytes later and thereby tend to preserve cardiac function.

PAI-1 and apoptosis

A pivotal characteristic of cells that undergo neoplastic transformation is their ability to evade apoptosis (17). Increased expression of PAI-1 in tumor cells has been associated with an adverse prognosis (18). Greater expression of PAI-1 by breast cancer cells has been associated with greater survival of malignant cells suggesting a lower incidence of apoptosis (19). Survival of other types of neoplastic cells is greater when expression of PAI-1 is increased (20, 21). These associations suggest that PAI-1 may influence prognosis in patients with cancer by attenuating apoptosis thereby influencing the survival of malignant cells.

The addition of wild-type PAI-1 or a variant that maintains functional activity in malignant cells decreases both spontaneous and induced apoptosis (22). This effect has been seen with a prostate cancer cell line as well as a promyelocytic leukemia cell line and was not apparent when inactivated or latent forms of PAI-1 were added to cultures. Overexpression of PAI-1 by Chinese hamster ovary cells attenuates apoptosis by decreasing plasmin mediated cell detachment (23). The anti-apoptotic effects of PAI-1 appear to be mediated by the active conformer of PAI-1 and presumably by its active site.

Implantation of fibrosarcoma cells into knockout mice deficient in PAI-1 is associated with a greater incidence of apoptosis (21). Fibroblasts obtained from wild-type and PAI-1 knockout mice undergo spontaneous malignant transformation and proliferate *in vitro* (24). PAI-1-deficient cells are more sensitive to apoptotic stimuli *in vitro* and exhibit a prolonged lag time before growth *in vivo* (24). Whereas spontaneous transformation of cells to a malignant phenotype renders them sensitive to chemotherapy-mediated apoptosis, deficiency of PAI-1 abrogates this effect (25). Thus, malignant cells deficient in PAI-1 are more prone to apoptosis compared with those that are not deficient.

Expression of PAI-1 has been linked to the survival of non-malignant cells in culture and their risk of undergoing apoptosis. Human umbilical vein endothelial cells (HUVEC) that become apoptotic after exposure to serum starvation *in vitro* express less PAI-1 than do surviving cells (26). By contrast, expression of u-PA is similar in cells exhibiting apoptosis compared with those surviving serum starvation. We have found that VSMC from transgenic mice that overexpress PAI-1 are resistant to apoptosis (27). Apoptosis of VSMC in the neointima is greater in mice deficient in both PAI-1 and apolipoprotein E (9). The release of PAI-1 by astrocytes decreases apoptosis of neuronal cells in culture (28). Accordingly, consistent with results seen with malignant cells, greater expression of PAI-1 by non-malignant cells in culture renders those cells less likely to undergo apoptosis.

Mechanisms by which PAI-1 influences apoptosis and proliferation

PAI-1 influences apoptosis by inhibiting generation of plasmin (13, 29–31). Activation of plasminogen to form plasmin induces apoptosis (32–36) and inhibition of plasmin generation by PAI-1 attenuates apoptosis. VSMC from PAI-1-deficient mice are more prone to apoptosis than those from wild-type mice. In the absence of plasminogen, differences are not apparent. The extent of apoptosis correlates positively with the generation of plasmin (29). Consistent with this observation, activation of plasminogen by fibroblasts is associated with apoptosis (30). Both are inhibited by PAI-1. Effects are apparent with endothelial cells, VSMC, fibroblasts, cells of neuronal origin, and neoplastic cell lines (13, 29–36).

A second mechanism by which PAI-1 appears to influence apoptosis is by inhibiting cell adhesion mediated by vitronectin (37). PAI-1 binds to vitronectin on the cell surface, a phenomenon that limits cell adhesion. Induction of apoptosis correlates strongly with the anti-adhesive effect of PAI-1 seen with HUVEC and VSMC in culture. Immunostaining of athero-

sclerotic vessel sections demonstrates congruent distribution of vitronectin and PAI-1 with apoptotic cells lining foam cell lesions (38). Accordingly, PAI-1 appears to promote apoptosis by anoikis (decreased tethering or adherence of cells).

A third mechanism by which PAI-1 appears to influence apoptosis is through inhibition of caspase 3 (26). PAI-1 has been shown to exhibit direct inhibitory effects on the activity of caspase-3, and this effect appears to be mediated by the active site of PAI-1 (26). PAI-1-deficient endothelial cells exhibit hyperactivity of the signaling protein Akt, increased content of inactivated caspase-9, decreased content of cleaved caspase-3 and resistance to apoptosis (39). These results suggest that PAI-1 may influence caspase signaling and hence apoptosis by exerting effects on Akt.

We have found that VSMC that over express PAI-1 are resistant to apoptosis and exhibit increased proliferation (26, 40). These effects may be linked. PAI-1 inhibits directly the activity of caspase-3 but not caspase-8 (26). Thus, inhibition of caspase-3 should increase expression and/or cleavage of FLIP (FLICE-like inhibitory protein). FLIP diverts Fas-mediated signals from induction of death to induction of proliferation in lymphocytes (41, 42). Accordingly, inhibition of caspase-3 by PAI-1 prevents apoptosis, and inhibition of caspase-3 appears to

promote activation of FLIP that, in turn, promotes proliferation through induction of nuclear factor κ B and activation of extracellular signal-regulated kinase (ERK) signaling.

Conclusions

Increased expression of PAI-1 has been associated with increased survival of cells *in vivo* and *in vitro*. PAI-1 appears to increase the survival of cells, at least in part, by rendering them resistant to apoptosis. PAI-1 appears to influence apoptosis by inhibiting generation of plasmin, inhibiting caspase-3, and limiting cell adhesion. Although effects of PAI-1 on plasmin generation increase apoptosis, they may also limit apoptosis by decreasing cell detachment. Direct effects of PAI-1 on caspase-3 may divert signaling favoring apoptosis to signaling favoring proliferation by increasing cleavage and activity of FLIP. Whereas apoptosis of malignant cells may be beneficial, apoptosis of non-malignant cells such as cardiomyocytes and vascular wall cells may contribute to progression of ischemic injury and atherosclerotic disease. Accordingly, full elucidation of the mechanisms by which PAI-1 influences apoptosis may identify novel therapeutic targets designed to either promote or retard cell death.

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