

# Plasminogen activator inhibitor-I (PAI-I): A molecule at the crossroads to cell survival or cell death

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Plasmin and its precursor plasminogen are largely known due to their role in fibrinolysis, the removal of a haemostatic blood clot. Thereby, the inactive zymogen plasminogen needs to be converted into the active serine protease plasmin. This intravascular conversion is mainly catalyzed by the action of tissue-type plasminogen activator (tPA), whilst urokinase-type plasminogen activator (uPA) catalyzes plasmin formation predominantly in the extravascular space. Plasmin thereby dissolves the fibrin of a blood clot, but due to its broad substrate specificity it also degrades proteins in basement membranes and extracellular matrix (ECM). Herewith it controls biological processes such as cell migration, cell invasion, organ involution, tissue remodelling, tissue destruction and tumor metastasis.

Such a multiplicity of biological functions requires precise regulation of enzyme action which can be achieved at various levels. First, plasminogen is synthesised by the liver and distributed throughout the organism via the circulation, while plasminogen activators (PA) are only secreted by specific cell types. Thus, plasmin generation is spatially restricted. Second, cellular expression of uPA and tPA is regulated by a variety of hormones, cytokines, and growth factors. Thus, plasmin generation is stimulus-controlled. Third, uPA is secreted from cells as a zymogen with a very low activity and uPA activation requires another proteolytic step. Thus, plasmin generation depends on the proteolytic activation of uPA. Fourth, the activity of tPA is vastly stimulated by its binding to fibrin and therefore plasmin generation by tPA is strictly regulated by binding to fibrin. Fifth and most importantly, the activity of both tPA and uPA is regulated at the level of plasminogen activator inhibitors (PAI) (for a review, see [1]).

The first evidence for the existence of these inhibitors was gained from experiments in the 1970s (2, 3) and later on, these molecules and their respective genes were purified and cloned

from various biological sources (4–8). Plasminogen activator inhibitors include PAI-1 and PAI-2, and later on, protein C inhibitor (PAI-3), protease nexin-1, and neuroserpin were shown to possess plasminogen activator inhibitory activity. All these molecules belong to the protein family of SERPINs (for a review, see [9]). Among these five molecules, PAI-1 appears to be the primary and most specific and fast acting inhibitor of plasminogen activation *in vivo*.

## PAI-I as a multifunctional protein in vascular cell biology

Besides its ability to inhibit uPA and tPA, PAI-1 also undergoes other molecular interactions. PAI-1, although circulating and primarily measured in blood, is associated with the extracellular matrix, mainly by binding to vitronectin. It may therefore block cell adhesion due to interference between vitronectin and  $\alpha v \beta 3$  integrin or the uPA receptor (uPAR). Further, PAI-1 was found to induce uPA-uPAR internalization because the ternary PAI-1-uPA-uPAR complex can bind to and is cleared from the cell surface by scavenger receptors from the low-density lipoprotein receptor related protein family (LRP) including very-low-density lipoprotein receptor (VLDLR). All these molecular interactions suggest a highly complex scenario in which PAI-1 may not only affect pericellular proteolysis and cell adhesion but may also be involved in initiating or modulating intracellular signalling cascades (for a review, see [1, 10]).

The physiological importance of PAI-1 is emphasized by several clinical studies. In the context of PAI-1 as an inhibitor of fibrinolysis, decreased plasma PAI-1 levels cause bleeding complications (11). A high plasma PAI-1 level is a risk factor for myocardial infarction and coronary heart disease, deep venous thrombosis as well as acute and chronic inflammatory lung dis-

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orders (12). Additionally, enhanced plasma PAI-1 levels are found in patients with metabolic syndrome and type 2 diabetes mellitus (13, 14), which both are associated with a disturbed carbohydrate and lipid metabolism (15). In fact, overexpression of PAI-1 in transgenic mice causes venous occlusion (16), showing a causal relation between a high PAI-1 level and thrombosis. In the context of PAI-1 in ECM turnover, an initial study in the early 1990s showed that high levels of PAI-1 in tumor cells were associated with a poor prognosis in patients with breast tumors (17). Since then, tumor PAI-1 has been shown to be one of the most informative biochemical prognostic markers in several cancer types (18). The possibility of a causal role of PAI-1 in cancer spread is supported by several observations in animal models (for a review, see [19]).

Because of this, the signalling reactions leading to PAI-1 synthesis, i.e. the upstream events, have an important impact on the biological role of PAI-1 in different cell types and the entire organism. Further, the exciting phenomenon that PAI-1 may act as a signalling molecule by itself indicates that pathways triggered by PAI-1, i.e. downstream events, may be important for the life and death of a number of cells and tissues (Fig. 1). In regard to this, some reports have shown that PAI-1 protects different cell lines against spontaneous and drug-induced apoptosis, whereas other studies found that PAI-1 may act as a pro-apoptotic factor. Confusing as these apparently conflicting observations may be, they do indicate the complexity of PAI-1 actions and add to the concept that PAI-1 may contribute to cell-specific biological

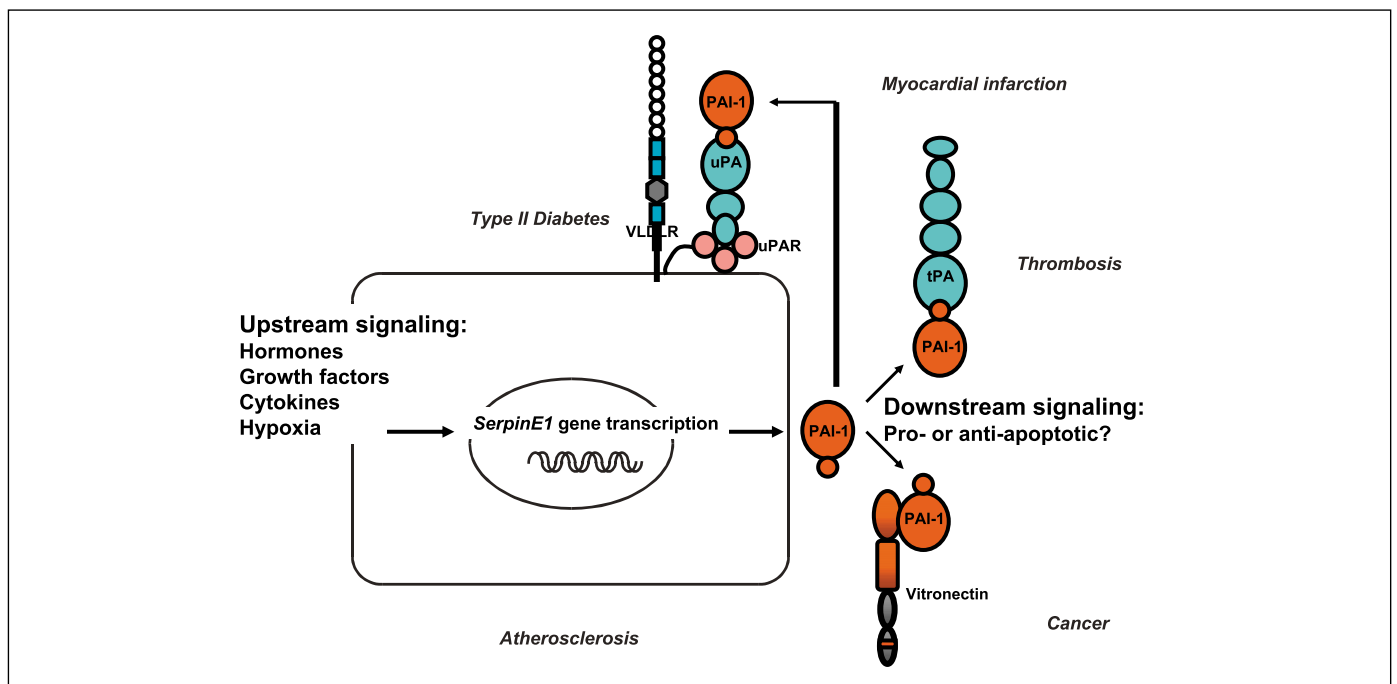
functions especially with respect of cellular proliferation or apoptosis. Obviously, the upstream and downstream events are interrelated, as changed pericellular PAI-1 levels caused by upstream effects will contribute to the downstream effects.

In this Theme Issue, comprised of a series of review articles and original manuscripts, important new findings on PAI-1 are highlighted which are related to different aspects of cell signalling events upstream and downstream of PAI-1 and the influence of these pathways and PAI-1 itself on apoptosis and cell proliferation.

## PAI-1 gene regulation

It was already shown in the 1980s that a number of hormones, cytokines, growth factors and environmental factors regulate the temporal expression of PAI-1 in responsive cells and tissues, although the detailed molecular mechanisms remained largely unknown at the time (for a review, see [20]). In the meantime, improved techniques and cell culture studies allowed a number of signalling pathways and transcription factors to be unravelled which have an impact on PAI-1 expression. Here, the review by Kruithof (22) gives an overview about positive and negative elements that influence PAI-1 gene expression, particularly focussing on inflammatory mediators and regulators.

It was found that transforming growth factor (TGF)- $\beta$ 1 is fundamental in the pathogenesis of fibrotic diseases including atherosclerosis and cardiac hypertrophy/fibrosis by enhancing the expression of PAI-1 (21). The fact that TGF- $\beta$ 1-induced



**Figure 1: PAI-1 acts as a multifunctional protein.** The molecular interactions of PAI-1 suggest a highly complex scenario in different processes. PAI-1 may not only affect fibrinolysis and pericellular proteolysis as well as cell adhesion via binding to vitronectin but is also involved in modulating intracellular signalling cascades. This may occur due to binding and clearance of the PAI-1-uPA-uPAR complex by receptors from the low-density lipoprotein receptor related protein family. In addition, high plasma PAI-1 levels are associated with atherosclerosis, myocardial

infarction, type 2 diabetes mellitus, and PAI-1 was shown to be one of the most informative prognostic markers in several cancer types. Therefore, the signalling reactions leading to PAI-1 synthesis, i.e. the upstream events have an important impact on the role of PAI-1. Further, pathways triggered by PAI-1, i.e. downstream events, may also be important for life and death of cells and tissues. Obviously, the upstream and downstream events are interrelated, as changed pericellular PAI-1 levels caused by upstream effects will contribute to the downstream effects.

neointimal growth can be attenuated upon PAI-1 deficiency led to the concept that interference with this pathway may represent a therapeutic opportunity. However, the pictures appear to be more complex and several signalling cascades often use common components and cooperate at several points to achieve fine tuning. The review by Samarakoon and Higgins (23) describes recent data suggesting the essential cooperation among the epidermal growth factor receptor-MAP kinase cascade, the Rho/ROCK pathway and SMADs in the TGF- $\beta$ 1-induced PAI-1 expression in vascular smooth muscle cells. Likewise, the paper by Diebold et al. (24) shows that exposure of pulmonary artery smooth muscle cells to thrombin enhances PAI-1 expression via a signaling cascade involving the thrombin receptor PAR-1, the small GTPase Rac1 and the transcription factors hypoxia-inducible factor-1 (HIF-1) and nuclear factor kappaB (NF $\kappa$ B), the latter bound to the HIF-1 $\alpha$  promoter. These findings ultimately link thrombin signaling with hypoxic and inflammatory events.

Although PAI-1 is highly expressed in vascular endothelial and smooth muscle cells, it can also be secreted from a variety of other cells, among them are hepatocytes, adipocytes and platelets (25–29). With respect to the enhanced PAI-1 levels found in patients with type 2 diabetes mellitus (13, 14) in which glucose and lipid homeostasis (30) is misbalanced, a number of studies have been performed with adipocytes and hepatocytes. Under normal conditions, glucose and lipid homeostasis is mainly achieved by the adequate regulation of hepatic metabolism. Thus, disturbances in the metabolic function of the liver will not only have profound effects on PAI-1 expression but also on the synthesis of acute phase proteins, the xenobiotic metabolism as well as the maintenance of the biomatrix components. It is commonly accepted that the perturbations in PAI-1 expression in the liver largely depend on the aberrant activation of signaling pathways, and the review by Dimova and Kietzmann (31) describes the regulation of PAI-1 expression in liver cells and discusses potential cross-talks between metabolic, hormonal and environmental signals. In view of the situations where PAI-1 levels are found to be elevated the question arises whether there are conditions under which PAI-1 expression can be inhibited, and the review by Nagamine (32) discusses such regulatory mechanisms.

## Role of PAI-1 in cell apoptosis

Concerning the downstream effects of PAI-1, Soeda et al. (33) summarize current findings regarding the role of PAI-1 as anti-apoptotic and neurotrophic factor in the central nervous system, where the anti-proteolytic activity of PAI-1 helps to prevent tPA-induced neuronal death. Deficiency of PAI-1 thereby reduces expression of the anti-apoptotic Bcl-2 and Bcl-XL but increases pro-apoptotic Bcl-2 family proteins Bcl-XS and Bax as well as activates caspase-3, cytochrome c release from mitochondria and DNA fragmentation in differentiated rat pheochromocytoma (PC-12) cells. Further, in PC12 cells PAI-1 appears to act independently of its function as a protease inhibitor by initiating signalling through the Jun and MAPK/ERK pathways.

Apoptosis of vascular cells is an important process that occurs during blood vessel remodelling under both physiological and pathological conditions. Cells in all layers of the vessel wall

sense and respond to different stress factors such as mechanical forces as well as inflammatory and thrombotic events. The second article by Diebold et al. (34) reviews the multifunctional role in various processes associated with vascular remodelling in the systemic and pulmonary vasculature. Interestingly, the findings from experiments with vascular smooth muscle cells from PAI-1<sup>-/-</sup> mice show that these cells are more prone to apoptosis than those from wild type mice, and the extent of apoptosis correlates positively with the generation of plasmin (35). By contrast, endothelial cells isolated from aortas of PAI-1<sup>-/-</sup> mice displayed a higher proliferation rate associated with strong activation of the protein kinase B (PKB/Akt) pathway which is crucially maintaining cell survival. This double-edged action of PAI-1 in apoptosis is then emphasized in the review by Balsara and Ploplis (36). Although a number of recent reports highlighted the involvement of the MAPK or PKB/Akt pathway for the regulation of either the apoptotic or anti-apoptotic PAI-1 action, the review by Schneider et al. (37) summarizes findings showing that the impact of PAI-1 to influence apoptosis requires not only the MAPK or PKB/Akt pathways but also PAI-1 action as a protease inhibitor, its interaction with vitronectin, and the possibility that PAI-1 may directly bind and inhibit caspase-3.

The review by Lademann and Rømer (38) summarises observations performed by these authors on increased sensitivity of fibrosarcoma cells established from PAI-1<sup>-/-</sup> mice towards chemotherapeutics induced apoptosis as compared to wild-type mice. The authors discuss their findings in relation to reports by others on similar or conflicting observations, including effects of PAI-1 on the PI3K/Akt signalling pathway. Their results suggest one possibility why high PAI-1 levels in tumors could be a marker for poor prognosis: Cancer cells in tumors associated with high PAI-1 levels are less prone to apoptosis.

## Perspectives

Whereas the studies of the upstream signalling events have established quite clearly a set of regulatory pathways determining PAI-1 gene expression, the reports concerning the effects of PAI-1 on apoptosis and cell survival remain apparently mutually conflicting, both with respect to the influence of PAI-1 on end points and on intracellular signalling pathways. The apparently conflicting observations concerning apoptosis and proliferation may possibly arise from differences in the relative expression levels of interacting molecules (plasminogen activators, vitronectin, endocytosis receptors), differences in the experimental design, the cell type, the stage of the tumor or may be PAI-1 dose-dependent. Further progress seems to require, among other things, the use of agents specifically inhibiting each of PAI-1's molecular interactions and/or PAI-1 variants mutationally inactivating a specific molecular interactions. Also, search for abnormalities in frequencies of apoptosis in PAI-1<sup>-/-</sup> mice may eventually solve these questions. Although a complete consistent picture with respect to the apoptotic or anti-apoptotic actions of PAI-1 cannot be reached so far and a number of mechanistic details need to be clarified, we think that the PAI-1 field is still exciting and hope that the contributions of this Theme Issue will not only be useful for the “insiders” but also attract “new people” to this area of biomedical research.

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