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Theme Issue Article

The 'PAI-1 paradox' in vascular remodelling

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Summary

Vascular remodelling is a complex phenomenon associated with restructuring of the vessel wall as a consequence of disruption of vascular homeostasis. Alterations of the vascular wall have been linked to a variety of cardiovascular disorders including atherosclerosis, vascular injury and pulmonary hypertension. Plasminogen activator inhibitor-1 (PAI-1) is a member of the serpin (serine proteinase inhibitor) family and acts as an important inhibitor of fibrinolysis by interfering with the plasminogen system. In addition to its anti-fibrinolytic effects, PAI-1 appears to modulate cellular responses linked to vascular remodelling. Since PAI-1 levels have been shown to be altered in various disorders associated with vascular remodelling of the systemic and pulmonary vascular bed, this serpin may play a pivotal role in the pathogenesis of these diseases.

Keywords

Plasminogen activator inhibitors, atherosclerosis, restenosis, remodelling, pulmonary hypertension

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Introduction

The vessel wall is a dynamic organ composed of endothelial (EC) and vascular smooth muscle cells (VSMC) as well as fibroblasts, which are arranged in distinct layers of cells interacting in a complex autocrine-paracrine manner. The vessel wall senses and actively responds to diverse vascular stress factors, including mechanical forces, vasoactive and other humoral factors, as well as inflammatory and thrombotic events. Moreover, microenvironmental changes such as modulation of oxygen supply can also lead to disruption of vascular homeostasis (1–3).

Vascular tissue remodelling is an active process essential for the response to pathological stimuli. It includes changes in structure and function of the vessel wall via modulation of cell adhesion, migration, proliferation, production and degradation of extracellular matrix (ECM), and cell death. These processes are implicated in a variety of disorders in different vascular beds including atherosclerosis, neointima formation, restenosis as well as pulmonary vascular remodelling associated with pulmonary hypertension (PH) (2–6). Vascular injury frequently occurs early in the development of vascular remodelling and often results in the activation of the coagulation cascade thus leading to a prothrombotic state and fibrin deposition. In addition, members of the coagulation system have been shown to interact with the vessel wall and have thus been implicated to play a role in promoting vascular remodelling processes on top of their pro-coagulatory activity (7–12).

Plasminogen activator inhibitor-1 (PAI-1) appears to be a key factor at the intersection between thrombosis, fibrin deposition, ECM degradation and the balance between proliferation and cell death. PAI-1 was initially described to control the plasminogen activation system, a proteolytic cascade implicated in various physiological and pathological processes including vascular thrombosis, inflammation, wound healing, tumour invasion, and neovascularization (8, 13, 14). In addition to platelets, which contain a large pool of PAI-1 mostly in an inactive form, macrophages, vascular cells as well as many other cell types produce and secrete PAI-1. Increased PAI-1 activity in plasma attenuates endogenous fibrinolysis and shifts the dynamic balance towards fibrin generation rather than fibrinolysis (15, 16). Additionally, PAI-1 has been shown to interact directly with vascular cells and has been suggested to affect the balance between cell proliferation and cell death (17–19).

The exact mechanisms how PAI-1 can affect vascular cell physiology and thus vascular structure and function are not completely clear. In fact, conflicting results exist with regard to the role of PAI-1 in both promoting and preventing vascular remodelling processes, a phenomenon that is sometimes referred to as the '*PAI-1 paradox*' (20). This review will summarize the potential effects of PAI-1 on the vascular wall, its impact on the vascu-

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lar proliferative balance and its importance in disorders associated with vascular remodelling such as neointima formation, atherosclerosis and pulmonary hypertension.

Mechanisms of interaction of PAI-1 with the vascular wall

PAI-1 is a member of the serine protease inhibitor (serpin) gene family and the principle physiological inhibitor of urokinase-type plasminogen activator (u-PA) and tissue-type plasminogen activator (t-PA), but also of other proteases such as thrombin, activated protein C, matriptase-3, and DESC-1 (differentially expressed squamous cell carcinoma-1) (8). u-PA as well as t-PA activates plasminogen to generate plasmin by proteolytic cleavage. The serine protease plasmin degrades many blood plasma proteins, most notably fibrin. In contrast to t-PA, which is mainly involved in intravascular fibrinolysis, u-PA exerts not only proteolytic effects but also intracellular signaling functions by binding to its high affinity receptor on the cell surface (21).

PAI-1 is secreted by vascular cells as an unstable active form which spontaneously converts into the inactive (latent) form with a half-life of 1–2 hours (h) at 37°C (22). Active PAI-1 interacts very rapidly at its reactive center loop (RCL) located at amino acids 320–351 (Fig. 1) with u-PA at Arg-346 (13, 23, 24) to form an inactive u-PA-PAI-1 complex which is internalized by the LDL receptor related protein-1 (LRP-1) and degraded in lysosomes (25, 26).

In addition, PAI-1 prevents the interaction between u-PA and its receptor urokinase-type plasminogen activator receptor (u-PAR) by forming a trimeric complex which is internalized into the cell together with the α-2 macroglobulin receptor and its ligand (26–28). The free u-PAR is then recycled to the cell surface where binding and activation of a second u-PA molecule can occur (29), meaning that PAI-1 levels can influence the activity of u-PA by modulating the amount of free u-PA. Moreover, clearance of the u-PAR-u-PA-PAI-1 complex by LRP-1 is accompanied by a net decrease of u-PAR on the cell surface (25, 30, 31). In addition to LRP-1, u-PAR undergoes ligand (u-PA-PAI-1)-induced internalization and recycling by CD222, a mannose-6-phosphate/insulin-like growth factor-II receptor (M6P/IGF-IIR) which binds to u-PAR at its N-terminal region (30, 32, 33).

PAI-1 as well as u-PAR can interact with the matrix protein vitronectin at the somatomedin B domain (SMB) (34, 35) (Fig. 1). Indeed, most of the active PAI-1 in plasma was found to be in complex with vitronectin (36). PAI-1 binding to vitronectin stabilizes itself in the active conformation, alters its specificity, and localizes it to areas of vascular injury (37, 38). In addition, the sole RGD (Arg-Gly-Asp) sequence for integrin binding is close to the SMB domain (39), indicating that PAI-1 cannot only compete with u-PAR binding at the SMB domain but also disturbs integrin binding to vitronectin and that it can do so independently of its inhibitory activity towards u-PA (40–43).

On the other hand, PAI-1-u-PA binding has been shown to loosen PAI-1 binding to vitronectin, thus possibly reversing the anti-adhesive properties of PAI-1 (27, 44). Thus, PAI-1 interacts in a complex manner with the vascular wall thereby controlling protein degradation in the basement membrane, affecting adhesion-dependent cell signaling and modulating cellular migration (45), making it a good candidate for targeted regulation of remodelling processes in the vasculature.

PAI-1 in pulmonary vascular remodelling

Vascular remodelling, together with vasoconstriction, is a hallmark of pulmonary hypertension (PH). These remodelling processes involve enhanced pulmonary artery smooth muscle cell (PASMC) and EC proliferation as well as restructuring of the ECM and thrombosis, due to disturbances in the balance between vasodilators and vasoconstrictors, growth inhibitors and mitogenic factors, as well as anti-thrombotic and pro-thrombotic determinants (46). Given the properties of PAI-1 to modulate cell adhesion and ECM composition, PAI-1 may represent a plausible candidate in the pathogenesis of pulmonary vascular remodelling. However, data regarding the involvement of PAI-1 in pulmonary vascular remodelling are scarce. Most studies investigated the levels of components of the coagulation and fibrinolytic systems in plasma samples from patients with PH. A correlation between the loss of fibrinolytic activity and the degree of elevation of mean pulmonary artery pressure in both primary and secondary PH has been reported suggesting a link between abnormalities in the coagulation system and PH (47). In patients with primary PH arterial PAI-1 levels were considerably higher than mixed venous PAI-1 levels (48), suggesting the lung to be a major source of PAI-1 under these conditions. Interestingly, the pro-thrombotic state in patients with primary PH was particularly pronounced in female patients with elevated plasma levels of the major components of the plasminogen activation system (PAI-1, u-PA, t-PA) (49, 50). Elevated levels of t-PA and PAI-1 proteins were detected in plasma from patients with chronic thromboembolic PH (50), although the fibrinolytic potential was not altered in the endothelium in thrombus-free areas of pulmonary arteries from these patients as compared to controls (51).

In contrast, another study reported that basal plasma levels of t-PA antigen, t-PA activity, and PAI-1 activity, respectively, did

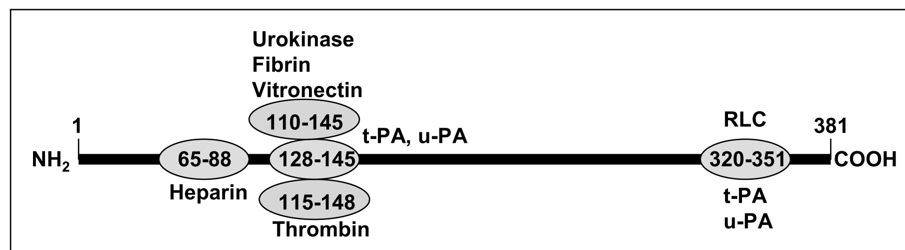

Figure 1: Scheme of PAI-1 protein structure. The binding sites for heparin, vitronectin, fibrin, urokinase, u-PA, t-PA and thrombin are shown. Plasminogen activators initially interact with PAI-1 at the reactive center loop (RCL) to form a stable complex. Numbers refer to the amino acid positions (modified according to [107]).

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not alter significantly between patients with different forms of PH and healthy subjects (52). However, after venal occlusion, mean t-PA activity levels increased to a higher extent in control subjects compared to patients with primary PH or Eisenmenger's syndrome due to congenital heart disease, and this was accompanied by increased fibrinogen plasma levels and a diminished fibrinolytic response compared with healthy subjects.

On the other hand, elevated PAI-1 mRNA and protein expression was reported in vascular cells in thrombus-rich pulmonary arteries of patients with pulmonary thromboembolism compared to thrombus-free areas as was demonstrated by immunohistochemical analysis (53) suggesting a contribution of PAI-1 to stabilization of the thrombemboli and possibly pulmonary vascular remodelling. Confirming this, several in-vitro studies reported increased levels of PAI-1 in PASMC in response to stimuli associated with pulmonary vascular remodelling such as thrombin, hypoxia, urotensin-II or oxidative stress (10, 54–58). Addition of an inhibitory antibody against PAI-1 reduced thrombin-stimulated proliferation of PASMC (Diebold et al., unpublished data) indicating that up-regulation of PAI-1 promotes proliferation of PASMC. However, a recent study reported decreased pulmonary tissue PAI-1 levels in patients with idiopathic (i.e. primary) PH compared to controls (59). In-vitro studies showed that treatment with supraphysiological concentrations of recombinant PAI-1 decreased PASMC proliferation and adhesion to vitronectin, but increased PASMC migration whereas reverse effects were observed in PAI-1 knock-down cells (59) pointing towards complex effects of PAI-1 on PASMC physiology.

The levels of PAI-1 have also been studied in animal models of pulmonary vascular remodelling. In a pneumonectomy-monocrotaline model of hypertensive pulmonary vascular disease in rats, mRNA expression analysis revealed a significant increase in the expression of PAI-1 between normal and diseased lungs. In addition, immunohistologic examination demonstrated that monocrotaline injection increased PAI-1 expression in pulmonary vessels (60). Recently, it was reported that in a piglet model of chronically obstructed pulmonary artery PAI-1 expression was elevated and returned to normal within two days after reperfusion. Interestingly, elevated PAI-1 levels have been associated with increased endothelial cell apoptosis in this model (61).

PH can also be induced upon exposure to chronic hypoxia. In-vitro studies showed that hypoxia is able to upregulate PAI-1 levels in various cell types including PASMC (58, 62). Indeed, PAI-1 has been identified as a direct target gene of the hypoxia-inducible transcription factor HIF (63). In line, exposure to short-term hypoxia increased PAI-1 activity and PAI-1 levels in pulmonary tissue with macrophages being the main source of PAI-1 (64). Furthermore, hypoxia increased pulmonary fibrin deposition, and this response was abrogated in PAI-1-deficient mice (64) implicating that PAI-1 may promote pulmonary vascular remodelling. However, similar studies have not been conducted under conditions of chronic hypoxia and prevalent PH. Interestingly, u-PA-deficient mice were protected against the development of pulmonary vascular remodelling and PH in response to hypoxia, whereas deficiency of u-PAR only moderately decreased pulmonary vascular remodelling (63, 65). However, the coagulation profile of mice under short-term hypoxia indicated that in contrast to elevated levels of PAI-1, which might mediate the pulmonary vascular response to hypoxia, pulmonary u-PA expression was down-regulated (64).

Thus, although the pathophysiology of pulmonary vascular remodelling appears to suggest an involvement of PAI-1, the amount of studies investigating the role of PAI-1 in these disorders is still limited. In addition, the results reported to date appear to be conflicting and even contradictory in some instances, indicating the need for further studies to better understand the involvement of PAI-1 in the complex pathophysiology of the pulmonary vasculature.

PAI-1 in systemic vascular remodelling

Role of PAI-1 in response to vascular injury

Alterations of the vessel wall following mechanical injury often result in neointima formation which may lead to restenosis. An association between vascular remodelling and PAI-1 has been described in initial studies where PAI-1 mRNA and protein expression were enhanced in the vascular wall adjacent to an arterial thrombus induced by mechanical injury of the rat carotid artery (66).

In contrast to these rather descriptive initial studies, perivascular electric and transluminal mechanical injury resulted in improved vascular wound healing and promoted neointimal proliferation in a mouse model of PAI-1 deficiency (67) (Table 1). These changes have been attributed to increased migration of PAI-1$^{-/-}$ smooth muscle cells from the uninjured borders into the necrotic center of the arterial wound. Similarly, PAI-1$^{-/-}$ and vitronectin$^{-/-}$ mice showed enhanced neointima proliferation two to four weeks following carotid artery ligation in comparison to wild-type mice as a result of more extensive VSMC proliferation (68). On the other hand, decreased neointimal growth accompanied by enhanced thrombus formation was observed upon localized PAI-1 over-expression by adenoviral PAI-1 gene transfer, or by seeding PAI-1 over-expressing VSMC on denuded rat carotid arteries (69, 70). Finally, PAI-1 originating from bone marrow-derived cells was shown to inhibit neointima formation after ferric (III) chloride-induced vascular injury (71). Thus, these studies suggest that PAI-1 prevents the formation of neointima upon vascular injury, and that decreased levels of PAI-1 allow neointimal growth and vascular remodelling.

In contrast, in a study using copper-induced arterial injury, a model associated with enhanced oxidative stress, PAI-1-deficient mice were reported to exhibit enhanced fibrinolytic potential, attenuated thrombotic lesion development, and a lack of neointima formation (72). Similarly, increased expression of PAI-1 in the arterial wall due to viral gene transfer promoted neointima growth after balloon injury, and this response was associated with fibrin(ogen) accumulation and increased cell proliferation (70). In a rat obese diabetic model, angioplasty promoted neointima lesions with prominent fibrin deposition and numerous proliferating VSMC. Infusion of a catalytic DNA enzyme targeting PAI-1 reduced PAI-1 levels within 48 h at sites of injury, and correlated with decreased neointimal growth (73). Similarly, pharmacological inhibition of PAI-1 prevented neointima formation in a mouse model of arterial injury (74). Furthermore, PAI-1–deficient mice were shown to be essentially

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Table 1: Studies on the effects of PAI-1 on vascular pathology in different injury models. The numbers refer to the references cited in the text. Abbreviations: PAI-1$^{-/-}$ (Plasminogen activator inhibitor-1 knockout); Vn$^{-/-}$ (vitronectin knockout); ApoE$^{-/-}$ (Apolipoprotein E knockout); SM (smooth muscle); LDLR$^{-/-}$ (low density lipoprotein receptor knockout); RV (retroviral); PAI-Tg (transgene plasminogen activator inhibitor); VSMC (vascular smooth muscle cells); N/A (not assessed).

Mice genotype	Injury model	Fibrinolysis	Time [weeks]	Effects on vascular pathology	Referenced study
PAI-1-/-, Vn-/-	Ferric (III) chloride/carotid artery ligation	In Vn-/- attenuated	4	Neointima formation suppressed in Vn-/- and PAI-1-/-	(104)
PAI-1-/-	Copper-induced oxidative damage	N/A	1 and 3	Neointima formation suppressed	(72)
PAI-1 from bone-marrow-derived cells	Ferric (III) chloride	In PAI-1-/- increased	3	Neointima formation enhanced	(71)
PAI-1-/-, Vn-/-	Ferric (III) chloride	In Vn-/-, PAI-1-/- attenuated	3	Mean time of thrombotic occlusion elevated in PAI-/- compared to Vn-/- and WT	(105)
PAI-1-/-	Perivascular electric/ transluminal mechanical	In PAI-1– /-increased	1 and 3	Neointima formation suppressed	(67)
Rat RV transduced PAI-1	Synthetic carotid artery	N/A	4	Neointima formation suppressed	(69)
ApoE-/-: PAI-1-/-	Ferric (III) chloride	Increased	8	Neointima formation enhanced	(85)
SM-22αPAI transgene ApoE-/-	Atherosclerotic model	N/A	7.5–30	VSMC cellularity and migration decreased	(83)
ApoE-/-: PAI-1-/-	Atherosclerotic model	Increased	5,10 and 25	Larger plaques at the advanced stage	(86)
PAI-1-/-, PAI-1Tg^{+}, or PAI-1-/- cross-bred into ApoE-/- or LDLR-/-	Atherosclerotic model	Attenuated	6,15 and 30	No differences in size and histological appearance of lesions	(84)
ApoE-/-: PAI-1-/-	Atherosclerotic model	No difference	52	Neointima not affected as assessed by aortic arch thickness	(106)
PAI-1Tg^{+}	Perivascular electric	Attenuated	2	Neointima formation not affected as assessed by intimal thickness and VSMC motility	(77)

protected from transforming growth factor (TGF)-β1-induced intimal expansion (75). These findings indicate that PAI-1 promotes the formation of neointima and vascular remodelling in response to vascular injury.

Another study, however, reported only a non-significant trend towards neointima reduction after carotid artery ligation in PAI-1$^{-/-}$ mice which may be related to the short observation period of one and two weeks after injury (76). In line, transgene-induced elevations of PAI-1 levels had no significant effect on neointimal growth two weeks after electrical injury (77), although occlusive and persistent thrombus formation was observed upon PAI-1 overexpression.

These findings indicate that fibrin deposition, besides being important for maintaining the vascular wall integrity in the process of haemostasis, may also play a prominent role in neointima proliferation. Although PAI-1 expression and fibrin deposition both occur after vascular injury, it has still not been experimentally confirmed that the vascular effects of PAI-1 are actually mediated by stabilization of the provisional fibrin matrix (11). It has been suggested that PAI-1 may inhibit neointima formation in the absence of fibrin (67, 77), and may enhance it in the presence of fibrin (72, 78). However, the observed effects of PAI-1 on fibrin deposition are insufficient to explain all the effects of PAI-1 on neointima formation.

Role of PAI-1 in atherogenesis

Atherogenesis is a complex process which involves repetitive vascular injury, lipid accumulation, and platelet and fibrin deposition. Increased expression of PAI-1 has been demonstrated in atherosclerotic lesions (79, 80), and has been proposed to play an important role in atherogenesis (Table 1) (81). In line, a threefold upregulation of PAI-1 plasma levels in mice deficient in apolipoprotein E (ApoE), prone to develop atherosclerosis, compared to wild-type mice has been reported (82).

Subsequently, overexpression of PAI-1 within VSMC reduced the cellularity of neointimal lesions in ApoE$^{-/-}$ mice, supporting the hypothesis that enhanced PAI-1 expression in atheroma could promote plaque rupture by decreasing the cellular content of the fibrous cap (83). In line, neointimal cellularity of vascular lesions was decreased in ApoE$^{-/-}$ mice over-expressing PAI-1 in smooth muscle cells compared with ApoE$^{-/-}$ control mice. This effect has been attributed to disturbances in the abil-

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ity to migrate, since VSMC explanted from transgene-positive mice exhibited attenuated migration (83). However, bone marrow cell-derived PAI-1 did not alter plaque size in $ApoE^{-/-}$ mice apparently because VSMC, rather than macrophages, are the dominant source of PAI-1 in the atherosclerotic plaque (84).

In contrast, decreased neointima formation was observed in PAI-1-deficient mice crossed with $ApoE^{-/-}$ mice as compared to $PAI\text{-}1^{+/+}$: $ApoE^{-/-}$ mice subjected to ferric chloride-induced injury of the carotid artery, suggesting a protective effect of PAI-1 upon lesion growth after oxidative injury in hyperlipidemic mice (85). However, other studies in mouse models where PAI-1-deficient mice were crossed with ApoE-deficient or LDL receptor (LDLR)-deficient mice which are also prone to the development of atherosclerosis (86), did not show an effect of PAI-1 on the formation of atherosclerotic lesions, suggesting that PAI-1 may only become prevalent on the development of atherosclerosis upon additional vascular injury, and that hyperlipidemia itself is not sufficient to account for a contribution of PAI-1 to atherogenesis.

Cellular mechanisms of PAI-1 in vascular remodelling processes

Despite frequent associations between the levels of PAI-1 expression and either improved or worsened vascular remodelling in response to a variety of stress situations and disorders, a causal relationship between PAI-1 and such processes has yet to be definitively established. However, several in-vitro studies attempted to dissect the mechanisms of PAI-1's role in controlling migration and/or proliferation of vascular cells.

PAI-1 as an inhibitor of vascular proliferative responses

In accordance with several of the in-vivo models cited above, PAI-1 may exert protective or even inhibitory effects with regard to the development of vascular remodelling indicating that PAI-1 may decrease migration and/or proliferation or promote cell detachment or even cell death (Fig. 2).

One explanation regarding the differing actions of PAI-1 on vascular proliferative activity has been related to the relative amounts of the inhibitor that are in active *versus* inactive conformations (87). PAI-1 can be cleaved and thus inactivated by several proteolytic molecules such as plasmin or matrix metalloproteinase-3 (MMP-3). PAI-1 cleavage products lacking the C-terminal reactive center loop (RCL) and part of the heparin binding domain at the amino terminus (Fig. 1) have been shown to decrease endothelial angiogenic responses. This has been associated with increased phosphorylation of JNK and subsequent activation of caspase-3 and even cell death. Furthermore, cleaved PAI-1 blocked fibroblast growth factor-2 (FGF-2) signaling through FGF receptor 1 and syndecan-4, thus inhibiting cell migration, tubulogenesis, and proliferation (88). These findings suggest that enhanced levels of cleaved and thus probably inactive PAI-1 for example due to increased plasmin activity favor a pro-apoptotic, anti-proliferative state in endothelial cells. On the other hand, PAI-1 has been suggested to reduce endothelial proliferative responses due to inhibition of matrix degradation through plasmin (39) or MMP (89) or through thrombin, which is a potent mitogen for VSMC (90). Thus, the balance between PAI-1's inhibitory action on plasmin and other proteases and the cleavage of PAI-1 by these proteases may play a critical role in interfering with vascular proliferative responses.

However, in addition to PAI-1 mutants defective in proteinase inhibitor activities also PAI-1 mutants defective in vitronectin binding rescued inhibitory effects on endothelial proliferation by PAI-1, suggesting that not only inhibition of proteinase activities but also vitronectin binding is contributing to proliferation inhibition by PAI-1 (87). In support of the latter mechanism, it was shown that endothelial cells grow only poorly on a vitronectin surface compared to other matrix molecules. This seemed to be due to binding of PAI-1 to the vitronectin surface, thus disabling the cells from loosening their matrix contacts as a prerequisite to proceed through mitosis (91). Furthermore, PAI-1 reduced cell adhesion to vitronectin by disrupting endothelial or smooth muscle cell actin polymerization and/or

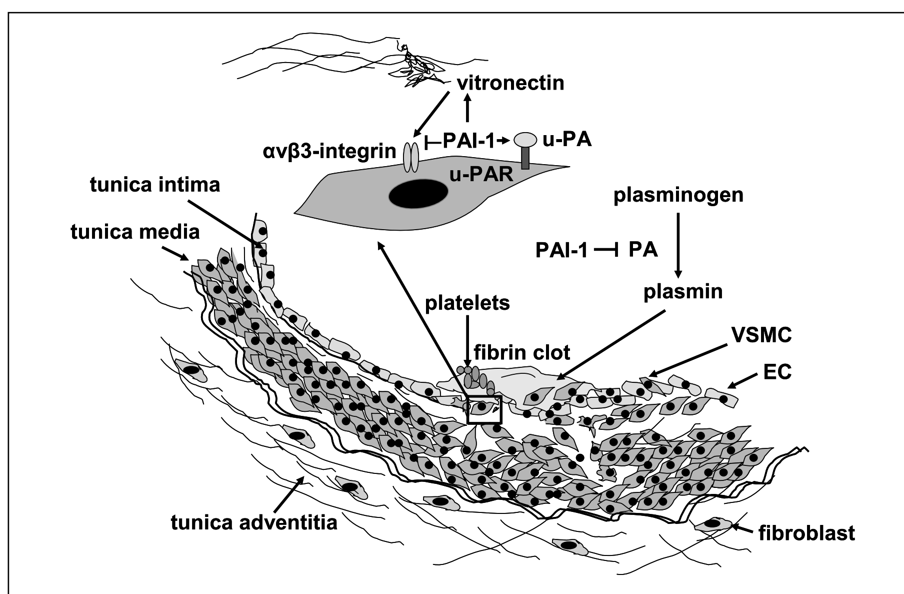

Figure 2: Scheme of the role of PAI-1 in neointima formation. Inhibition of fibrinolysis by PAI-1 may promote fibrin deposition, which may serve as a provisional matrix for migration of VSMC. As a consequence, the intimal layer is populated with proliferating and migrating VSMC which results in neointimal growth. On the cellular level, PAI-1 binds vitronectin and blocks its interactions with u-PAR (urokinase-type plasminogen activator receptor) and αvβ3 integrin thus inhibiting migration and causing cell detachment. EC: endothelial cells, PA. plasminogen activator, u-PA: urokinase-type plasminogen activator.

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focal adhesion assembly (92). Interestingly, the adhesion of endothelial but not smooth muscle cells to vitronectin in the presence of PAI-1 required both polymerized microtubules and actin, demonstrating cell-specific differences in the action of PAI-1 and the particular importance of the cytoskeleton for integrin-mediated adhesion of endothelial cells.

In addition to PAI-1's ability to disrupt u-PAR-vitronectin interactions, PAI-1 also interferes with integrin-vitronectin interactions due to binding to the u-PA present in u-PA-u-PAR-integrin complexes on the cell surface (27, 43). Binding of PAI-1 to u-PA can lead to deactivation and internalization of integrins bound to u-PAR, and this can result in cell detachment (93). Furthermore, PAI-1 binding to u-PA induces internalization of the PAI-1-u-PA-u-PAR complex thus reducing the amount of available u-PA and thereby cell migration. In this way, PAI-1 is able to regulate both pericellular proteolysis and the concentrations of u-PA and u-PAR on the cell surface (93).

Interestingly, detachment of vitronectin binding of vascular cells by PAI-1 was not only correlated with a loss of cells but also with activation of caspase-3 as a sign of programmed cell death (38, 44). Thus, PAI-1 inhibition of adhesion seems to limit cellular migration on one side, and may lead to cell detachment and anoïkis (94) on the other side. In support, endothelial cells deficient in PAI-1 showed enhanced proliferation and phosphorylation of Akt, inactivation of the tumor suppressor PTEN and caspase-9 and a decrease in procaspase-3 and cleaved caspase-3, and this effect was dependent on LRP. This suggests that PAI-1 may promote programmed cell death by preventing activation of the Akt survival pathway (95). Indeed, using an inducible expression system for active PAI-1 it was shown that PAI-1 can trigger activation of the caspase system (96).

PAI-1 as a promoter of vascular proliferative responses

As indicated above, PAI-1 has also been shown to promote vascular remodelling processes and to contribute to neointimal growth in various conditions (43, 64, 97). Consistently, at the cellular level, several studies reported a stimulatory effect of PAI-1 on vascular cell proliferation and/or angiogenesis (87, 98, 99). In line, lack of PAI-1 completely abolished angiogenesis, and microvessel outgrowth from PAI-1$^{-/-}$ aortic rings could be restored by adding exogenous PAI-1 (100). Interestingly, addition of recombinant PAI-1 led to a bell-shaped angiogenic response suggesting that PAI-1 is proangiogenic at physiological concentrations which have been reported to be between 0.1–100 ng/ml in mouse plasma. However, at higher levels PAI-1 seems to be antiangiogenic (100). Interestingly, a partial restoration of angiogenesis was observed after addition of PAI-1 mutants defective in vitronectin binding, whereas angiogenesis was not restored upon addition of PAI-1 mutants exhibiting normal vitronectin binding but were unable to control plasmin activity. These findings suggest that proteolytic activity is required for promoting proliferative responses associated with angiogenesis by PAI-1. However, at high non-physiological concentrations these latter PAI-1 mutants were able to also induce angiogenesis, suggesting that at such concentrations the interaction with vitronectin may take over the effect and regulate angiogenesis, or that u-PA proteolysis is reduced (98, 100).

In contrast to these observations, it was shown that in a model of TGFβ1-induced neointimal growth PAI-1 enhances cell migration by interacting with vitronectin and u-PA-u-PAR, rather than by an antiproteolytic activity (101). As stated above, PAI-1 binds to u-PA and promotes formation of the complex between $\alpha_V\beta_3$ integrin, u-PA and u-PAR, and the reaction between PAI-1 and u-PAR enhances endocytotic internalization of the complex by LRP-1 and consequent resurfacing of free u-PAR which is again able to form a new complex (44, 93, 102). Interestingly, both active and inactive (e.g. cleaved) PAI-1 have been shown to activate the Jak/Stat signaling system and stimulate cell migration dependent on LRP-1 indicating that LRP-1 acts as the signaling molecule mediating the migration-promoting activities of PAI-1 (103). Furthermore, in PAI-1 overexpressing VSMC increased proliferation was associated with activation of nuclear factor kappaB (NFκB) and ERK as well as Raf-1 (18). Although the exact upstream regulatory mechanisms have not been elucidated in this study it suggests that PAI-1, possibly via LRP-1, stimulates signalling cascades commonly used to regulate cellular proliferative responses.

On the other hand, PAI-1 deficiency has been associated with activated caspase-3 in atherosclerotic plaques from ApoE$^{-/-}$ mice (19). Similarly, over-expression of PAI-1 has been linked with increased proliferation of VSMC due to a direct inhibitory effect of PAI-1 on the activity of caspase-3, but not caspase-8 (17, 18). Indeed, PAI-1 has been shown to form a high-affinity complex with caspase-3, thereby directly inhibiting caspase-3 activity. However, a mutant PAI-1 with limited capacity for binding to plasminogen activators did not inhibit caspase-3 activity (18). Moreover, PAI-1 was associated with increased activity of the FLICE-like inhibitory protein (FLIP) which can disrupt apoptosis mediated by death receptors suggesting that PAI-1 may protect against apoptosis, and thus promote proliferation and other vascular remodelling processes.

The finding that the anti-apoptotic effect of PAI-1 is connected to binding of plasminogen activators, and that lower concentrations of PAI-1 enhance proliferation primarily by controlling proteolytic activity suggest that PAI-1 at low concentrations acts anti-apoptotic and possibly increases proliferation via its proteolytic activity. The observed apoptotic effects of PAI-1 may be achieved with higher concentrations of PAI-1 where the interaction with vitronectin becomes more prevalent, and cell detachment and anoïkis can occur. This dose-dependent effect of PAI-1 together with the concept of a 'proteolytic balance' in which a critical protease-inhibitor equilibrium is necessary for cell migration and differentiation may at least partially explain some of the contradictory observations regarding the role of PAI-1 in controlling proliferative responses.

Conclusion

The serine protease inhibitor PAI-1 plays a multifunctional role in various processes associated with vascular remodelling in the systemic and pulmonary vasculature. However, discordant effects of PAI-1 in either promoting or repressing vascular remodelling have been described. The complex vascular functions of PAI-1 appear to depend on the vascular bed, type of lesion, and the experimental and clinical conditions which affect the various PAI-1 functions in one or more aspects. In fact these different conditions may exist in different parts of the same injured artery

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and/or at different time points after vascular injury. Additionally, the ability of PAI-1 to interact with a variety of molecules may also be responsible for its opposing effects on vascular remodelling. Thus, novel genetic in-vivo models introducing targeted disruption or enhancement of PAI-1 within specific cell types, as well as models expressing PAI-1 mutants lacking specific domains required for its function and/or interaction with other molecules will be needed for elucidation of the complex mechanisms of action of PAI-1 in vascular pathology.

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