

Commentary

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Small Molecule Targeting of PAI-1 Function: A New Therapeutic Approach for Treatment of Vascular Stenosis

Tessa M. Simone, Jaclyn Archambeault and Paul J. Higgins*

Center for Cell Biology & Cancer Research, Albany Medical College, Albany, New York 12208, USA

Plasminogen activator inhibitor-1 (PAI-1; SERPINE1) is a clade E1 member of the serine protease inhibitor (SERPIN) superfamily and the major physiologic inhibitor of the urokinase (uPA) and tissue-type (tPA) plasminogen activators. Elevated PAI-1 expression is a significant causative factor in vascular disease and a major contributor to the pathophysiology of diabetes, metabolic syndrome, stroke, atherosclerosis and restenosis, particularly in the setting of increased vessel TGF- β 1 [1-3].

PAI-1 is unique relative to other SERPINs existing in the structurally and functionally distinct active, latent and cleaved conformations [4,5]. PAI-1 is initially synthesized in an active state, capable of interacting with its proteinase targets, but is unstable (half-life of 2 hours at 37° C, pH 7.4) and converts spontaneously into a latent form [6]. Latency requires insertion of the N-terminus of the PAI-1 reactive center loop into β -sheet A forming a new β -strand (s4A) which creates an unusual loop structure and conformational change in the reactive center, ultimately preventing interaction with proteinases [7-9]. Alternatively, PAI-1 can be proteolytically-cleaved at the sissile P1-P1' bond causing the N-terminal end of the reactive center loop to insert into β -sheet A, while the C-terminus of the reactive site loop forms strand s1C in β -sheet C. These structural rearrangements produce a 70Å separation of the P1 and P1' residues, thereby, preventing PAI-1 from complexing with the target proteinase due to spatial distortion, ultimately allowing for increased plasmin activation [10-12]. While neither cleaved nor latent PAI-1 forms complexes with their target proteases, all three conformations bind the low-density lipoprotein receptor-related protein-1 (LRP1) and initiate Jak/Stat signaling [13].

Elevated PAI-1 mRNA and protein expression are evident in the carotid vascular wall adjacent to thrombi induced by implantation of indwelling polyethylene tubing [14]. Furthermore, adenoviral delivery of PAI-1 potentiated neointima formation after catheter-induced injury while copper-stimulated neointima formation was reduced in PAI-1-null mice [15,16]. In a mouse model of carotid artery ligation, PAI-1 protein levels are elevated in neointimal lesions 14-days after restriction. Regions expressing PAI-1 also express smooth muscle cell α -actin (Figure 1A,C), suggesting that PAI-1 is associated with smooth muscle cells (VSMCs). PAI-1 involvement in the pathological response to healing is reflected in its expression in the developing neointima in the ligated artery, but not the contralateral control vessel (Figure 1B,D), as well as in balloon-injured carotid arteries (Figure 1E). These findings implicate PAI-1 as a significant factor in the development of restenosis and provided the impetus for development of low-molecular weight PAI-1 antagonists. Tiplaxtinin (PAI-039), the most well studied small molecule PAI-1 inhibitor, attenuates asthmatic episodes, obesity, diabetes, cancer cell motility and angiogenesis [17-24]. The mechanism by which Tiplaxtinin antagonizes the anti-fibrinolytic activity of PAI-1 appears to involve inhibition of complex formation between PAI-1 and its target protease with promotion of PAI-1 cleavage [25,26]. This has translational implications as PAI-1 deficiency in various cell types promotes plasmin-dependent apoptosis [27-31]. A decrease in PAI-1 antiproteolytic activity, through functional blockade or proteolytic

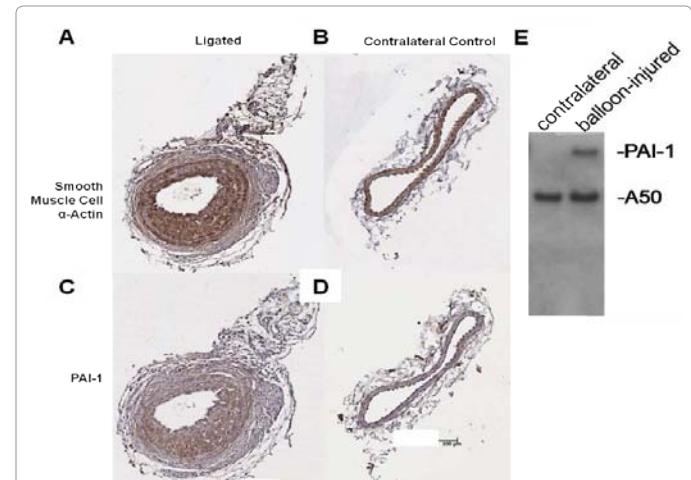


Figure 1: PAI-1 expression is upregulated within vascular smooth muscle cells of neointimal lesions. A-D.) Paraffin sections (5 μ m) of ligated (A&C) and contralateral control (B&D) mouse, common, carotid arteries were subjected to PAI-1 (C&D) or smooth muscle cell α -actin (A&B) staining. Images taken with a 10x objective. E.) Rats were subjected to balloon-catheter endothelial denudation injury of the left common carotid artery. One week later, RNA was extracted from the injured carotids as well as the contralateral control arteries. Northern blot analysis of mRNA levels using 32P-labeled cDNA probes to PAI-1 and A50 (loading control).

cleavage, may subsequently increase VSMC apoptosis due to plasmin generation. One mechanism suggests that PAI-1 might contribute to neointimal growth by facilitating VSMC survival. Recent findings indicate that Tiplaxtinin induces VSMC apoptosis in a dose-dependent manner and this response was attenuated by the addition of TGF- β 1. However, with the exception that PAI-1 binds and prevents the cleavage and activation of caspase-3, the role of PAI-1 in preventing VSMC apoptosis remains unexplored [31]. One attractive possibility is that PAI-1 might promote cell survival through the PI3K/Akt signaling axis and both PAI-1 and TGF- β 1 stimulate Akt phosphorylation. Since PAI-1 is a highly upregulated gene in the TGF- β 1 response set, TGF- β 1 may activate Akt through PAI-1 or, at least, induce PAI-1 expression through an Akt-dependent pathway. Given the ubiquitous role PAI-1 plays in the etiology and progression of several chronic and acute fibrotic disorders, the therapeutic efficacy of small molecule PAI-

***Corresponding author:** Dr. Paul J. Higgins, Center for Cell Biology & Cancer Research, Albany Medical College, 47 New Scotland Avenue, Albany, New York 12208, USA, Tel: 518-262-5168; FAX: 518-262-5669; E-mail: higginp@mail.amc.edu

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1 inhibitors, such as Tiplaxtinin, may have translational adaptability beyond the scope of vascular disease.

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