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Role of Plasminogen Activator Inhibitor-1 in Senescence and Aging

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Abstract

The average age of the US population continues to increase. Age is the most important determinant of disease and disability in humans, but the fundamental mechanisms of aging remain largely unknown. Many age-related diseases are associated with an impaired fibrinolytic system. Elevated plasminogen activator inhibitor-1 (PAI-1) levels are reported in age-associated clinical conditions including cardiovascular diseases, type 2 diabetes, obesity and inflammation. PAI-1 levels are also elevated in animal models of aging. While the association of PAI-1 with physiological aging is well documented, it is only recently that its critical role in the regulation of aging and senescence has become evident. PAI-1 is synthesized and secreted in senescent cells and contributes directly to the development of senescence by acting downstream of p53 and upstream of insulin-like growth factor binding protein-3. Pharmacologic inhibition or genetic deficiency of PAI-1 was shown to be protective against senescence and the aging-like phenotypes in *kl/kl* and *N^ω-nitro-L-arginine methyl ester*-treated wild-type mice. Further investigation into PAI-1's role in senescence and aging will likely contribute to the prevention and treatment of aging-related pathologies.

Keywords

PAI-1; senescence; aging

Advanced age contributes to the development of frailty and disease in humans, but the fundamental mechanisms that drive the physiological aging are incompletely understood.^{1,2} As the elderly population of the United States and other Western countries continues to grow, the need to investigate and understand these mechanisms has never been greater. While aging is inevitable, therapeutic interventions that target aging-related pathways could delay or even prevent the onset of chronic conditions that lead to aging and thus increase the overall healthspan of the population.

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A substantial body of evidence indicates that many age-related diseases are associated with an impaired fibrinolytic system. The main enzyme of fibrinolysis, plasmin, is generated from the inactive precursor plasminogen by the serine proteases tissue type and urokinase-type plasminogen activators (t-PA and uPA, respectively). Plasmin degrades fibrin clots in the vasculature and can remodel the extracellular matrix directly and through the activation of matrix metalloproteinases.^{3,4} Plasminogen activator inhibitor-1 (PAI-1) is the primary physiological inhibitor of both t-PA and uPA and therefore is the main regulator of plasminogen activation system. Over the years, studies have shown that PAI-1's influence is not limited to its canonical function in fibrinolysis, but also includes other diverse biological processes such as cell adhesion and migration, cell-matrix interactions, signaling pathways, replicative senescence, proteolysis, and physiological aging.

Fibrinolytic activity is known to decrease with age,^{5–7} in part due to increase in PAI-1 activity.⁸ While age-dependent increases in plasma PAI-1 levels are well documented, it is recently that this protein is being viewed as a critical determinant of aging. In this review, we will discuss how PAI-1 is associated with physiological aging and examine the molecular mechanisms of how PAI-1 mediates its effects on aging-related pathologies (► Table 1).

Elevated PAI-1 Levels Are Associated with Age-Related Conditions

Increased PAI-1 activity levels lead to reduced plasmin generation and as a result, promote both a prothrombotic and profibrotic environment, both of which are clinical conditions related to aging. Elevated levels of PAI-1 are associated with veno-occlusive disease, ischemia, arterial thrombosis, and venous thrombosis.^{9–11} PAI-1 is also predictive of both initial¹² and recurrent¹³ myocardial infarction. An excess of PAI-1 results in collagen accumulation and the development of fibrotic disorders. PAI-1 levels are also increased in patients with aortic valve stenosis, the most common vascular disease affecting the elderly population. PAI-1 expression in the valves is proportional to the rate of valve calcification.¹⁴

Plasma PAI-1 levels significantly increase in several clinical conditions that arise because of aging, including obesity,¹⁵ type 2 diabetes mellitus (T2DM),¹⁶ and inflammation.¹⁷ In humans, PAI-1 is independently correlated with many markers of adiposity, including body mass index (BMI), visceral fat mass, serum triglyceride levels, and liver steatosis.^{18–20} PAI-1 predicts the development of T2DM independent of other known risk factors, including age, sex, ethnicity, BMI, smoking, family history of diabetes, and insulin sensitivity.^{21,22} In addition to the age-dependent increases of PAI-1 in plasma, PAI-1 accumulates in atherosclerotic plaques,^{23–26} a finding that is exaggerated in T2DM.²⁷ Interestingly, Pandolfi et al have found that PAI-1 expression is markedly augmented in the vessel walls of patients with T2DM even before the development of atherosclerotic plaques, strongly suggesting that local PAI-1 gene expression is an important factor for increased cardiovascular risk in diabetes.²⁸ The PAI-1 promoter contains response elements for several proinflammatory cytokines. Factors such as interleukin-1, transforming growth factor- β (TGF- β), lipopolysaccharide,¹⁷ tumor necrosis factor- α ,²⁹ and endotoxin³⁰ can upregulate PAI-1 expression during inflammation. PAI-1 can also promote neutrophil recruitment by regulating cell migration.³¹

Similar to humans, animal studies have demonstrated an age-associated elevation in the plasma levels and activity of PAI-1.³² PAI-1 accumulates in murine heart³³ and porcine aortic valves³⁴ as a function of age. Moreover, restraint stress- induced PAI-1 expression is dramatically enhanced in aged animals.^{32,35,36}

PAI-1 Is Elevated in Animal Models of Aging

Increased levels of PAI-1 expression have been observed in mouse models of “accelerated aging,” including *Klotho* (*kl/kl*) and *BubR1*^{H/H} mice. *kl/kl* mice stop growing at the age of 3 weeks with an average lifespan of 56 days, and develop pathologies that resemble human aging including emphysema, arteriosclerosis, and skin atrophy.³⁷ *kl/kl* mice have an age-dependent increase in plasma PAI-1 levels as well as increased expression of PAI-1 mRNA and protein accumulation in the kidney, aorta, and heart.³⁸ Similarly, *BubR1*^{H/H} mice develop various aging-like phenotypes that include a shortened lifespan, sarcopenia, cataracts, arterial stiffening, reduced stress tolerance, and impaired wound healing.³⁹ Interestingly, the most dramatic increase in PAI-1 expression is observed in the tissues most affected by aging in *BubR1*^{H/H} mice: adipose tissue, smooth muscle, and eyes.⁴⁰

PAI-1 Is a Critical Determinant of the Age- Related Abnormalities

It is not immediately clear whether the increase in PAI-1 levels with aging represents a biomarker of aging, or if PAI-1 actually regulates organismal aging. Recent work from our group provides the first demonstration that modulation of PAI-1 levels can actually prolong survival and prevent the development of age-related diseases in a mouse model of accelerated aging. To investigate the role of PAI-1 in the aginglike pathology, we modulated PAI-1 levels in the established murine model of accelerated aging, *kl/kl* mice. Both partial and complete PAI-1 deficiency had a pronounced effect on the longevity of *kl/kl* mice. The median survival of *kl/kl* mice increased 2.8-fold by partial deletion of PAI-1, and 4.2-fold in PAI-1 -deficient *kl/kl* mice (►Fig. 1). This improvement in survival was also associated with evidence of increased overall vigor and health, as *kl/klpai-1*^{-/-} mice exhibited near normal weight gain over time and spontaneous physical activity. In addition, a PAI-1 deficiency restored the fertility of *kl/kl* mice and preserved organ structure. Thus, partial or complete loss of PAI-1 expression prevented age-dependent enlargement of air spaces and associated alveolar destruction, and protected against age-induced ectopic calcification in kidney and heart tissues without altering serum levels of phosphate, calcium, alkaline phosphatase, and aldosterone.

Likewise, modulation in PAI-1 levels can influence the development of hypertension and arteriosclerosis. Hypertension is one of the most prevalent age-related conditions (80% of people older than 65 years have measurable high blood pressure.).⁴¹ Both genetic deficiency and pharmacological inhibition of PAI-1 attenuated the increases in systolic blood pressure and aortic arteriosclerosis that develop in mice treated with long-term nitric oxide synthases inhibition^{42–44} (►Fig. 2A-D).

The critical role of PAI-1 in the regulation of organismal aging is also suggested from previous work from our laboratory indicating that transgenic overexpression of a

functionally stable form of PAI-1 is sufficient to induce multiple age-dependent phenotypic abnormalities that resemble age-related pathology, including alopecia, systemic amyloidosis,⁴⁵ and perivascular fibrosis and coronary arterial thrombosis⁴⁶ (►Fig. 2E-K).

Effects of PAI-1 on Molecular Mechanisms of Aging

PAI-1 Promotes Cellular Senescence

Although aging is associated with increased susceptibility to all major chronic diseases, the fundamental mechanisms that give rise to age-related diseases remain largely unknown. Since the discovery of senescence in cultured cells, cellular senescence has been suggested to be linked to aging-related diseases. Accumulation of senescent cells has been detected in various aged tissues and organs⁴⁷ as well as in atherosclerotic plaques of coronary arteries obtained from patients with ischemic heart disease.⁴⁸ Furthermore there is a direct correlation between replicative potential of cells in culture and lifespan potential of the species from which they are derived. Thus, cells derived from progeroid patients exhibited accelerated cellular senescence in vitro.⁴⁹ In addition to the earlier observations, a recent study provided evidence that senescent cells are directly contributing to age-related dysfunction. Baker et al observed that removal of senescent cells can prevent or delay tissue dysfunction in *BubR1^{H/H}* progeroid mice.³⁷

PAI-1 expression has been reported to increase during replicative senescence in various cell types^{50–55} and is a validated senescence indicator. Together with proinflammatory cytokines, chemokines, and growth factors secreted by senescent cells, PAI-1 is a part of the senescence phenotype (►Table 2), termed the senescence-messaging secretome (SMS). It is not immediately clear whether SMS proteins represent biomarkers of replicative senescence, or if these proteins actually regulate organismal aging by signaling to other cells.

Until recently, PAI-1 has been regarded as a marker that merely correlates with, rather than contributes to, senescence.^{56,57} However, recent works suggested that PAI-1 is not merely a senescence marker, but both necessary and sufficient for the induction of replicative senescence in vitro.^{58,59} Not only is PAI-1 capable of inducing replicative senescence in mouse embryonic fibroblasts⁵⁹ and MCF-7 breast cancer cells,⁶⁰ but PAI-1-deficient fibroblasts and endothelial cells can also escape growth arrest and senescence. Conversely, overexpression of PAI-1 in proliferating keratinocytes induces growth arrest.⁶¹ Importantly, results published by Kortlever et al indicate that PAI-1 is a critical downstream target of the tumor-suppressor p53 in the induction of replicative senescence in vitro.⁵⁹ In addition, a positive association between PAI-1 and p53 also has been reported in vivo.^{62,63} Analysis of PAI-1 in healthy subjects suggests that p53 polymorphism plays a crucial role in determining PAI-1 levels in aging.⁶⁴

Recently, Elzi et al identified a downstream target of PAI-1, which provides a potential mechanism to explain the role of PAI-1 in promoting cellular senescence in vitro.⁶⁰ The expression of insulin-like growth factor binding protein-3 (IGFBP3) is increased in response to senescent stimuli (UV, hypoxia, etoposide, and H₂O₂) and in doxorubicin-induced senescence. PAI-1 secreted by nascent senescent cells inhibits the t-PA-dependent proteolysis of IGFBP3 (►Table 2). As PAI-1 is the main physiological inhibitor of t-PA,

IGFBP3 is thought to be a critical downstream target of PAI-1-induced senescence. Simply stated, PAI-1 promotes senescence by limiting the metabolism of IGFBP3 (►Fig. 3).

Data from our group provided the first demonstration that modulation of the SMS can actually prevent the development of senescence in a mouse model of accelerated aging. PAI-1-deficient had a profound impact on senescence in *kl/kl* mice. We observed that both partial and complete PAI-1 deficiency lowered levels of the SMS factors IGFBP3 and interleukin-6 in plasma of *kl/kl* mice to levels seen in wild-type (WT) mice (►Table 2). PAI-1 deficiency also provided partial protection of telomere integrity in numerous tissues and prevented the nuclear accumulation of the senescence marker p16^{Ink4a} in *kl/kl* mice⁶⁵ (►Table 2). Inhibition of PAI-1 activity also protected against the development of senescence in another aging-related model. Pharmacological inhibition of PAI-1 prevented telomere shortening and reduced p16^{Ink4a} expression in aortas of mice with N^ω-nitro-L-arginine methyl ester (L-NAME)-induced senescence⁴² (►Table 2).

PAI-1 Mediates Senescence by Affecting the Dynamics of Intercellular Communication

Nelson et al recently reported that senescent cells induced a DNA damage response in neighboring cells through gap junction-mediated cell-cell communication and secretion of reactive oxygen species. Continuous exposure to senescent cells induced senescence in replication-competent fibroblasts nearby.⁶⁶ This mechanism may actually be PAI-1 dependent, as Heberlein et al have two recent publications indicating that PAI-1 plays a critical role in gap junction formation and that myoepithelial junction formation is reduced in PAI-1-deficient mice fed a high fat diet.^{67,68} The findings that gap junction formation and signaling may be enhanced or impaired via PAI-1 regulation of the PA system open new perspectives that PAI-1 mediates senescence by affecting the dynamics of intercellular communication.

Conclusions

While aging is the major risk factor that is related to the development of chronic and acute diseases in humans, the fundamental molecular mechanisms driving physiological and pathophysiological aging need to be investigated further. However, numerous studies have implicated PAI-1 in the aging process. Circulating and tissue levels of PAI-1 increase with age and impair the biological processes such as fibrinolysis, cell migration, senescence, and aging. Age-dependent elevations in PAI-1 levels have been associated with the clinical conditions observed with advanced aging including cardiovascular diseases (thrombosis, atherosclerosis, and arteriosclerosis), organ fibrosis, inflammation, obesity, and diabetes. Senescent cells accumulate in aged tissues and cellular senescence is thought to contribute to aging. PAI-1 was shown to be a critical determinant of replicative senescence and contributes to senescence in vivo by impairing the proteolytic clearance of the SMS factor IGFBP3. Furthermore, we have recently shown that pharmacological inhibition or genetic deficiency of PAI-1 was protective against senescence and aging-like phenotypes in *kl/kl* mice as well as arteriosclerosis and senescence in L-NAME-treated WT mice. These preliminary data suggest that inhibition of PAI-1 activity offers a therapeutic approach to

prevent or reduce tissue senescence and organismal aging, which in turn would extend the healthspan of populations.

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Fig. 1.

Effect of PAI-1 deficiency on lifespan of Klotho mice. Log-rank analysis shows that the survival curves for WT, *kl/kl*, *kl/klpai-1^{+/-}* and *kl/klpai-1^{-/-}* mice differ significantly ($p < 0.0001$). PAI-1, plasminogen activator inhibitor-1; WT, wild-type.

Fig. 2.

A novel plasminogen activator inhibitor (PAI-1) TM5441 attenuates N^{ω} -nitro-L-arginine methyl ester (L-NAME)-induced periaortic fibrosis. Photomicrograph of Masson trichome-stained aortic cross-sections illustrate the extent of fibrosis in (A) untreated wild-type (WT) mice, (B) WT mice treated with TM5441, (C) WT mice that received L-NAME, and (D) WT mice coadministered with L-NAME and TM5441. Transgenic mice overexpressing human PAI-1 developed perivascular fibrosis and coronary arterial thrombosis. Panels (E) through (F) show thrombi detected in three different mice. Panels (H) through (K) display serial heart sections to detect the extent of coronary thrombi. The schematic below the

photomicrographs illustrates sections at 0 (starting point), 30, 90, and 180 μm to follow the thrombus in this large-sized coronary artery that bifurcates at the 90 μm -section into two branches that are also thrombosed.

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Fig. 3. Proposed schematic model illustrating role of extracellular PAI-1 in regulating senescence. CDKs, cyclin dependent kinases; IGFBP3, insulin-like growth factor binding protein-3; PAI-1, a plasminogen activator inhibitor; TGF- β , transforming growth factor- β ; t-PA, tissue plasminogen activator

Table 1

Physiological manifestations of aging influenced by PAI-1

• Emphysema
• Arterial thrombosis
• Hair loss
• Amyloid deposition
• Arteriosclerosis/hypertension
• Calcification
• Infertility

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Table 2

Senescence factors affected by PAI-1

-
- IGFBP3
 - p16^{Ink4a}
 - Telomere length
 - IL-6
 - TGF- β
 - Gap junctions
-

Abbreviations: IGFBP3, insulin-like growth factor binding protein-3; IL-6, interleukin 6; TGF- β , transforming growth factor- β ;

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