

REVIEW

Plasminogen activator inhibitor-1 and asthma: role in the pathogenesis and molecular regulation

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Summary

Plasminogen activator inhibitor (PAI)-1 is a major inhibitor of the fibrinolytic system. PAI-1 levels are markedly increased in asthmatic airways, and mast cells (MCs), a pivotal cell type in the pathogenesis of asthma, are one of the main sources of PAI-1 production. Recent studies suggest that PAI-1 may promote the development of asthma by regulating airway remodelling, airway hyperresponsiveness (AHR), and allergic inflammation. The single guanosine nucleotide deletion/insertion polymorphism (4G/5G) at –675 bp of the PAI-1 gene is the major genetic determinant of PAI-1 expression. Plasma PAI-1 level is higher in people with the 4G/4G genotype than in those with the 5G/5G genotype. A strong association between the 4G/5G polymorphism and the risk and the severity of asthma has been suggested. Levels of plasma IgE and PAI-1 and severity of AHR are greater in asthmatic patients with the 4G/4G genotype than in those with the 5G/5G genotype. The PAI-1 promoter with the 4G allele renders higher transcription activity than the PAI-1 promoter with the 5G allele in stimulated MCs. The molecular mechanism for the 4G allele-mediated higher PAI-1 expression is associated with greater binding of upstream stimulatory factor-1 to the E-box adjacent to the 4G site (E-4G) than to the E-5G. In summary, PAI-1 may play an important role in the pathogenesis of asthma. Further studies evaluating the mechanisms of PAI-1 action and regulation may lead to the development of a novel prognostic factor and therapeutic target for the treatment and prevention of asthma and other PAI-1-associated diseases.

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Introduction

Plasminogen activator inhibitor (PAI)-1 is a key regulator of the fibrinolytic system which comprises plasminogen that can be converted to plasmin. Plasmin degrades fibrin and other protein substrates, and it also activates the matrix metalloproteinase (MMP) system, which is involved in degrading extracellular matrix (ECM). Two physiologic plasminogen activators (PAs) have been identified, tissue type PA (tPA) and urokinase-type PA (uPA). Inhibition of the fibrinolytic system may occur either at the level of the PA, by specific PAIs, including PAI-1, -2, and -3, or at the level of plasmin, mainly by α_2 -antiplasmin. PAIs belong to the family of serine protease inhibitors. Among them, PAI-1 has the greatest inhibitory effect. PAI-1 plays a crucial role in various physiological processes, including fibrinolysis, ECM turnover, tissue repair, blood coagulation, thrombolysis, ovulation, embryogenesis, angiogenesis, and cell adhesion and migration through its interactions with PAs, uPA receptors, and vitronectin [1]. Recent studies from our laboratory and

others indicate that PAI-1 may play an important role in pathogenesis of asthma.

Expression of plasminogen activator inhibitor-1 in asthma

Under normal conditions, PAI-1 is present in plasma and tissues at low concentration. Elevated levels of PAI-1 are often observed in a variety of pathologic conditions and clinical settings, such as infection, stroke, myocardial infarction, diabetes, obesity, sepsis, cancers, and so on. Abnormal PAI-1 expression is also observed in asthma.

Elevated plasminogen activator inhibitor-1 levels in asthma

Increased PAI-1 production has been observed in both murine or rat asthma models and asthmatic patients. In a murine asthma model, PAI-1 production was increased fourfold in lung tissue and fivefold in bronchoalveolar

lavage fluid (BALF) after challenge with ovalbumin (OVA) for 4 weeks compared with mice challenged with saline [2]. Wagers et al. [3] also reported that PAI-1 activity in lung tissues from mice with allergic airway inflammation was increased compared with that in healthy mice, which was accompanied by a marked decrease in PA activity. PAI-1 mRNA expression was up-regulated in bronchial wall at 2 weeks after chronic allergen exposure in the asthmatic mice [4]. Similar results were observed in a rat asthma model [5]. Elevated PAI-1 levels were also described in asthmatic patients by a number of reports. Recurrent wheeze in early childhood has been presented as an independent risk factor for the later development of asthma. Levels of PAI-1 are increased in patients with recurrent wheeze in comparison with control subjects [6]. In the lung tissues of asthmatic patients, the numbers of PAI-1-positive MCs are increased than in control subjects, suggesting that PAI-1 expression is elevated in asthmatic patients [7]. Xiao et al. [8] reported that in several lung diseases, including cystic fibrosis, COPD and asthma, sputum PAI-1 levels were increased compared with healthy controls. The plasma levels of PAI-1 in asthmatic patients were statistically higher than those in controls. In house dust mite-sensitive allergic asthmatics (HDM-AAs), the average plasma levels of PAI-1 was 49.7 ng/mL, and in healthy controls it was only 26.0 ng/mL ($P < 0.0001$) [9]. Significant elevation of concentration of PAI-1 was also observed in induced sputum supernatants of HDM-AAs [10]. The increased expression of PAI-1 in asthmatics suggests that PAI-1 may play an important role in pathogenesis of asthma.

Mast cells as an important source of plasminogen activator inhibitor-1 in the asthmatic airways

MCs are the major effector cells in asthma through release of various mediators after stimulation [11]. Human mast cell line (HMC-1) cells were stimulated by a combination of phorbol myristate acetate (PMA) and calcium ionophore A23187, the differential expression of MC-derived genes was studied using cDNA microarray. Among the inducible genes, PAI-1 gene was induced at the highest level. PAI-1 mRNA and protein expression are activated markedly in both PMA/A23187-stimulated HMC-1 cells and IgE-stimulated primary cultured human MCs [7]. PAI-1 gene promoter activity is elevated by 23-fold in calcium ionophore-stimulated HMC-1 cells compared with in resting cells [12]. Nakajima et al. [13] also demonstrated that *PAI-1* is one of the most highly up-regulated genes in IgE-stimulated human MCs. In asthmatic patients, the density of PAI-1-positive MCs is higher than in healthy controls [7]. In a rat asthma model, increased inflammatory cells in lungs were observed, and among them, the highest accumulation was MCs. Immunohistochemical staining

showed that the greatest intensification of immunopositive reaction of PAI-1 was demonstrated by numerous inflammatory cells, especially in MCs [5]. In addition to calcium ionophore and IgE-mediated stimulation, TGF- β and C5a also induce PAI-1 expression in MCs [14, 15]. Both are important mediators released by MCs. These suggest that at least some mediators secreted by stimulated MCs may induce PAI-1 expression in MCs through an autocrine mechanism. In a murine asthma model, PAI-1 production is approximately 50% less in the airways of MC-deficient W/W^v mice after OVA challenge compared with that in wild-type (WT) mice (S. H. Cho & C. K. Oh, unpublished data). A number of mediators released by activated MCs are strong inducers of PAI-1 expression by numerous cell types in airways. Therefore, the reduction of PAI-1 production in MC-deficient mice results from both direct lack of production due to deficient MCs and decreased production by other cell types in airways due to decreased inflammation. Taken together, these findings indicate that MCs are important to production of PAI-1 in asthmatic airways through both direct and indirect effects.

Other sources of plasminogen activator inhibitor-1 in asthmatic airways

Besides MCs, there are other cell types that may contribute to the production of PAI-1 in the asthmatic airway. In a murine chronic allergen-induced asthma model, the indices of airway remodelling and hyperreactivity were increased, and gene expression of PAI-1 and TGF- β 1 was significantly up-regulated in airway epithelium at 2 weeks after chronic OVA exposure [4]. *In vitro* studies showed that TGF- β induces PAI-1 expression in human bronchial epithelial cell lines, BEAS2B cells and NCI-H292 [16, 17]. TGF- β treatment resulted in reduced plasmin generation in bronchial epithelial cells accompanied by increase in PAI-1. Eosinophilic inflammation is a key feature of asthma. The accumulated eosinophils in asthmatic bronchial walls secrete TGF- β , which induces PAI-1 expression in bronchial epithelial cells through a paracrine mechanism [16]. Lung fibroblasts are another source of PAI-1 production in airways, which is induced by eosinophils-derived mediators [18]. Endothelial cell is another important source of PAI-1 production. In a rat model of asthma, the very strong expression of PAI-1 was observed in the endothelium of lung [5]. Other cell types that are known to contribute to PAI-1 production in asthmatic airways may include neutrophils, platelets, and macrophages [8, 19, 20]. Moreover, the PAI-1 levels in plasma of asthmatic patients are increased compared with healthy controls, which may also contribute to PAI-1 production in asthmatic airways [9].

Plasminogen activator inhibitor-1 in the pathogenesis of asthma

PAI-1 is involved in various physiological functions and associated with many diseases. Increasing evidence suggest that PAI-1 also plays an important role in the pathogenesis of asthma through regulating airway remodelling, airway hyperresponsiveness (AHR), and allergic inflammation.

Plasminogen activator inhibitor-1 and airway remodelling

Airway remodelling is characterized by increased airway smooth muscle mass, mucus gland hypertrophy, deposition of ECM components, thickening of the reticular basement membrane, and angiogenesis [21]. The fibrinolytic system plays a key role in the development of airway remodelling [22]. ECM degradation is mediated mainly by the fibrinolytic system and the MMP system. Plasmin directly degrades various types of ECM proteins including fibronectin, laminin, proteoglycan, fibrin, and collagens [23]. It can also activate MMPs, which in turn degrade ECM proteins [24]. As discussed above, MCs are an important source of PAI-1 in the asthmatic airway. More importantly, unlike fibroblasts, macrophages, endothelial cells, and other inflammatory cells which tend to produce both t-PA and its inhibitor PAI-1 simultaneously, MCs release t-PA when in resting state and PAI-1 upon activation. Consequently, activated MC-derived PAI-1 converts a fibrinolytic environment to a fibrosis-dominant condition *in vitro* by surpassing t-PA activity, which is dominant during the resting state [7, 25]. In the asthmatic airway, the number of activated MCs and PAI-1-positive MCs is markedly increased [7, 26]. PAI-1 promotes ECM deposition in the airways of a murine model of asthma [2]. The levels of collagen deposition were approximately 50% less in lung tissues from PAI-1-deficient mice compared with WT mice after OVA challenge. PAI-1 also promotes fibrin deposition in the lung. The amount of irreversible deposition of fibrin in the lung was approximately 75% less in PAI-1-deficient mice than WT mice. The major MMP released in the asthmatic airway is MMP-9 [27]. Studies in a murine asthma model showed that peribronchial fibrosis was less apparent in MMP-9-deficient mice than in WT mice [28]. MMP-9 activity in lung tissues and BALF from PAI-1-deficient mice was threefold higher than in WT mice after OVA challenge [2]. These results suggest that PAI-1 promotes fibrin and ECM deposition by both inhibiting fibrinolysis and activation of MMP-9.

PAI-1 may also contribute to airway remodelling by regulating angiogenesis. Angiogenesis is an important aspect of airway remodelling in asthma [29]. Vascular endothelial growth factor (VEGF) is thought to be the most important pro-angiogenic growth factor. The number of VEGF-positive cells is increased in the airways of

asthmatics compared with control subjects, and increased angiogenesis of the asthmatic mucosa is related to the elevated VEGF expression [30]. Elevated levels of VEGF and its receptors were also found in the asthmatic airway [31]. The elevated production of other pro-angiogenic factors bFGF and angiogenin have been reported in asthmatics also [30, 32]. Proteolytic balance and cell migration are two important aspects during angiogenesis. Recent evidence indicates that PAI-1 is a multi-functional regulator of angiogenesis through modulation of ECM degradation and cell adhesion and migration [33, 34]. Studies using the PAI-1-deficient mice in tumour or non-tumour-based model showed decreased angiogenesis, suggesting that angiogenesis is critically dependent on PAI-1 [35, 36]. PAI-1 regulates adhesion, migration, and proliferation of vascular smooth muscle cells [37, 38]. A small molecule inhibitor of PAI-1, PAI-039 (tiplaxtinin), inhibits the tumour angiogenesis *in vivo* partially through inhibiting smooth muscle cells adhesion and migration [39]. PAI-1 is also involved in angiogenesis through regulating expression and activity of VEGF. In PAI-1-deficient mice, the expression of VEGF is significantly reduced compared with control mice [40]. In glioma patients the protein levels of PAI-1 correlated strongly with levels of VEGF in the tumour tissues, and exogenous treatment of human glioma cells with recombinant PAI-1 proteins results in an upregulation of VEGF expression *in vitro* [41]. PAI-1 is also found to affect activity of VEGF by regulating release of VEGF from heparin/heparan sulphate of cell surface and activation of pro-VEGF [42]. The potential role of PAI-1 in airway angiogenesis of asthma, however, remains to be determined.

Plasminogen activator inhibitor-1 and airway hyperresponsiveness

AHR is a consistent and defining feature of asthma. Although the precise mechanisms of AHR remain unclear, there is evidence that airway remodelling plays an important role in the pathophysiology of AHR [43]. Recently it was found that PAI-1 contributes to the pathogenesis of AHR in allergic diseases. The PAI-1-dependent nasal hyperresponsiveness was reported in a murine nasal allergy model [44]. The dose of histamine inducing nasal hyperresponsiveness in PAI-1-deficient mice was approximately 0.1% of that in WT mice after OVA challenge. Chronic inhalation of endotoxin lipopolysaccharide (LPS) causes all of the classic features of asthma, such as airflow obstruction, airway inflammation, AHR, and airway remodelling. Four weeks after LPS inhalation, AHR and the expansion of the subepithelial area in the medium and large airways were observed in WT but not in PAI-1-deficient mice [45]. The airway surface of normal lung exhibits active fibrinolysis; however, the fibrinolysis is

impaired in the asthmatic airway, which potentiates the accumulation of fibrin [3]. PAI-1-dependent fibrin deposition in lung tissues has been demonstrated in asthmatic mice [2]. Recent studies indicate that fibrin deposition on the surface of asthmatic airway contributes to the pathogenesis of AHR. There are large amounts of fibrin on the surfaces of the distal airways and alveoli in a patient who died of status asthmatics and in mice with induced allergic airway inflammation, which indicates the apparent decrease in fibrinolytic activity on the asthmatic airway surfaces. The similar results were observed in the airway surfaces of mice with acute allergic airway inflammation, which were accompanied by increased PAI-1 activity. The constrained fibrinolysis in asthmatic airway is likely to be the result of increased PAI-1 activity. Delivery of nebulized fibrinolytic agent, tPA, to the airway surface of mice with allergic airway inflammation was found to reduce AHR [3]. These results suggest that the fibrinolytic system, including PAI-1, plays an important role in the pathogenesis of AHR in airway, which is mediated by regulating the ECM deposition, especially fibrin deposition.

Plasminogen activator inhibitor-1 and allergic inflammation

Fibrinolytic components are associated with not only fibrinolysis, but also with inflammation. PAI-1 can inhibit integrin-mediated leucocyte migration, but support IL-8-mediated neutrophil migration [46, 47]. In the LPS-challenged mice, PAI-1 was found to influence the early-phase of the inflammatory response through control of T lymphocyte traffic [48]. In the model of bleomycin-induced lung injury, overexpression of PAI-1 enhanced the accumulation of leucocytes in the lung [49]. In Gram-negative pneumonia, PAI-1 deficiency reduced accumulation of neutrophils in the lungs [50]. However, in the murine asthma model, no obvious difference in the numbers of peribronchial eosinophils and goblet cells were observed between WT and PAI-1-deficient mice [2]. Similar results were observed in the mice that received chronic LPS inhalation [45]. These results suggest that the role of PAI-1 in the regulation of chemotaxis of inflammatory cells varies in different types of the immune response. Gyetko *et al.* [51, 52] reported that uPA is required to generate both the T-helper type 1 (Th1) and Th2 immune responses to different infections. Therefore, it is possible that PAI-1 regulates the Th1/Th2 immune responses by affecting the activity of uPA. Recently, Sejima *et al.* [44] reported that PAI-1 plays the key role in the regulation of the Th1/Th2 response in a murine allergic rhinitis model. PAI-1 production in the nasal mucosa and nasal lavage fluid is increased in this model. In WT mice, the frequency of sneezing, nasal rubbing, nasal hyperresponsiveness, production of specific IgG1

and IgE, IL-4 and IL-5 increased significantly after intranasal OVA challenge. In PAI-1-deficient mice, these reactions were not observed, but specific IgG2a and IFN- γ increased significantly. The immune response appears to be a dominant Th2 response in WT mice; however, in PAI-1-deficient mice, the Th2 response was suppressed, shifting the immune response to a Th1-dominant response. These results suggest an important role of PAI-1 in regulating the allergic immune response. In a murine asthma model, plasminogen was found to be the key in the recruitment and migration of inflammatory cells into the lungs [53]. This suggests that the fibrinolytic system may play an important role in allergic inflammation of asthma. However, the accurate mechanisms of the fibrinolytic system, including PAI-1, in the regulation of the allergic inflammatory response in asthma need to be determined further.

The 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene in asthma

The PAI-1 gene is a highly polymorphic gene and more than 180 single nucleotide polymorphisms (SNPs) have been identified (<http://www.ncbi.nlm.nih.gov/projects/SNP>). Among SNPs of the PAI-1 gene, the 4G/5G polymorphism, which is characterized by a single guanosine nucleotide insertion/deletion variation at -675 bp of the PAI-1 promoter, has been extensively studied, whereas far less is known about the functions of other polymorphisms. Recently, several PAI-1 SNPs other than the 4G/5G polymorphism, including rs2227631 (-844 G/A), rs6465787, rs2227674, rs2227683, rs2227666, and rs2227694, are found to be associated with plasma PAI-1 levels, and the rs6092 (Ala15Thr) and rs7242 SNPs may affect PAI-1 functions [54, 55].

The 4G/5G polymorphism in other diseases

The 4G/5G polymorphism is a major genetic determinant of plasma PAI-1 levels. The plasma PAI-1 level is 25% higher in patients with 4G/4G than in those with 5G/5G, while 4G/5G has intermediate values [56–62]. In whole blood stimulation assay with LPS and peptidoglycan, subjects with 4G/4G and 4G/5G had 3.3-fold and 1.9-fold, respectively, increased PAI-1 expression than those with the 5G allele [63]. Endothelial cells, an important source of plasma PAI-1, with the 4G allele have greater induction of PAI-1 expression than those with the 5G allele after stimulation with IL-1 [56, 57], angiotensin II [64], or very low density lipoprotein [65]. This suggests that the 4G allele is associated with higher basal transcription and induction of PAI-1 gene expression.

The 4G/5G polymorphism is associated with a number of pathological conditions and diseases. The 4G allele appears to increase the risk of venous thrombosis [66],

and is associated with poor outcome in meningococcal disease [67]. The 4G/4G genotype confers an increase in the risk of mortality in adult patients with septic shock due to a greater organ failure, and it is also associated with higher IL-1, TNF- α , and PAI-1 plasma concentration and increased severity in these patients [68]. PAI-1 may play an important role in growth, angiogenesis, and metastasis of some types of tumours such as prostate cancer and breast cancer [69, 70]. The 4G/4G genotype is also significantly associated with increased risk of myocardial infarction [71], arterial hypertension [72], early onset of coronary heart disease [73], and pneumonia [63]. Most recently, in a subgroup of normolipidemic post-infarction patients, only the 4G/5G polymorphism was found to be associated with recurrent risk from a set of atherosclerosis-associated genetic polymorphisms and blood markers [74]. These are summarized in Table 1.

The 4G/5G polymorphism in asthma

For the first time, Cho et al. [75] reported that the 4G/5G polymorphism was linked to a propensity to develop allergic asthma. The nuclear families recruited from Nottingham, UK, on the basis of affected sibling pairs for asthma were studied, and the preferential transmission of the 4G allele to asthmatic children was demonstrated. This finding was confirmed by numerous later reports. Buckova et al. [76] reported that the 4G allele is associated with allergic asthma and other allergic disorders in Czech subjects and an increased frequency of the 4G allele in allergic patients was demonstrated. In the healthy control group, the frequency of 4G allele is 0.508, and 5G is 0.492. In people with AR and atopic eczema/dermatitis syndrome, the frequency of 4G and 5G is 0.606 and 0.394 respectively ($P=0.0035$), and in asthmatic patients it is 0.596 and 0.404 respectively ($P=0.0148$). Hizawa et al. [77] reported that the 4G allele is associated with the development of asthma and a synergistic interaction between the 4G/5G polymorphism and β -chain of the

Fc ϵ RI (FCER1B) -109C/T and -654C/T polymorphisms. The 4G/5G polymorphism is also associated with HDM-AA [9, 61, 78]. The frequency of the 4G allele is greater in HDM-AAs (0.69) than in healthy controls (0.55, $P=0.0034$). The 4G/5G polymorphism is also associated with an increased risk for HDM-AA. The adjusted ratio is 2.62 for the 4G/5G genotype and 3.48 for 4G/4G genotype compared with the 5G/5G genotype. The 4G/5G polymorphism is also associated with the severity of HDM-AA. Both non-specific and allergen-specific AHR are greater in 4G/4G homozygotes than in 5G/5G homozygotes. The total serum IgE level in 4G/4G homozygotes is greater than in 5G/5G homozygotes ($P<0.001$). This is consistent with the previous finding that a locus linked to IgE levels was mapped at chromosome 7q, which contains the PAI-1 gene [79]. The 4G/5G polymorphism also regulates the allergen-induced elevation of PAI-1 synthesis in HDM-AAs. At 30 min after allergen challenge, the mean increase in plasma PAI-1 concentration was significantly greater in HDM-AAs than in healthy controls ($P<0.001$). At 6 h, an increase in plasma PAI-1 concentration after allergen challenge was found in only 7.4% healthy controls, but in 88.9% HDM-AAs ($P<0.0001$). An increase in plasma PAI-1 level at 6 h was found in all HDM-AAs with 4G allele but only in 33.3% of the 5G homozygotes ($P<0.0001$). The synergistic interactions of the 4G/5G polymorphism with the CD14 C-159T polymorphism to affect susceptibility and severity are observed in HDM-AAs. Patients with the 4G/4G genotype and the CD14 CC genotype have the lowest forced expiratory volume in 1 s and the highest bronchial reactivity. These findings strongly indicate that elevated PAI-1 levels that are rendered by the 4G allele are linked to a propensity to develop asthma and the severity in asthmatics.

Molecular mechanisms of the 4G/5G polymorphism-dependent plasminogen activator inhibitor-1 expression

The 4G allele renders higher PAI-1 expression in response to IL-1 [56, 57], angiotensin II [64], very low density lipoproteins [65], and circadian clock protein heterodimers BMAL/CLOCK [69] than the 5G allele. In stimulated human MC line HMC-1 cells, the transcriptional activity of 4G-PAI-1 promoter was 32% higher than 5G-PAI-1 promoter ($P<0.001$), whereas their promoter activities were comparable in resting cells [80]. The molecular mechanisms involved in the increased synthesis of PAI-1 by the 4G allele in HepG2 cells were thought to be the binding of a potential repressor protein to the 5G allele, but not to the 4G allele [56, 57]. However, this suggested mechanism remains controversial. First, such a potential repressor has not yet been isolated and characterized, although it has been more than 10 years since it was reported. Furthermore, one report showed that there is no difference in the binding of nuclear proteins of HepG2

Table 1. Summary of some reported diseases associated with the 4G/5G polymorphism of PAI-1 gene

Diseases	References
Venous thrombosis	[66]
Meningococcal disease	[67]
Sepsis	[68]
Prostate cancer	[69]
Breast cancer	[70]
Myocardial infarction	[71]
Arterial hypertension	[72]
Coronary heart disease	[73]
Pneumonia	[63]
Asthma	[9, 61, 75–78]

PAI-1, plasminogen activator inhibitor-1.

cells to the 4G or 5G allele [81], and such a repressor was not found in adipocytes and human MCs [80, 82].

Bioinformatics analysis showed that there is an E-box motif (CACGTG) at –682 to –677 bp overlapping with the 4G/5G site by a guanosine nucleotide (E-4G/5G). The activity of 4G-PAI-1 promoter is higher than that of 5G-PAI-1 promoter in stimulated MCs; however, after mutagenesis by substituting the central dinucleotides CG with AT in E-4G/5G, the promoter activities of both 4G- and 5G-PAI-1 promoters were decreased, and more interestingly, their promoter activities are comparable. These results suggest that E-4G/5G is a positive regulatory element for PAI-1 expression in stimulated MCs, and more importantly, it is critical to 4G/5G polymorphism-dependent PAI-1 expression. By the use of supershift assay with antibodies against several potential E-box-binding factors, it was found that it is upstream stimulatory factor (USF)-1 that binds to the E-4G/5G as homodimer [80]. Similar results were observed in renal epithelial cells that showed an E-box motif of rat PAI-1 promoter (–165 to –160 bp) was bound only by USF-1 but not USF-2 [83]. However, in adipocytes and epidermal keratinocytes, the E-4G/5G was occupied by both USF-1 and USF-2 [82, 84]. These results suggest that binding pattern of USFs to the E-box is cell type-dependent. Further analysis indicates that USF-1 binds to the E-4G with higher affinity than to the E-5G. When the 3' flanking sequences of the E-5G were changed, the DNA-binding affinity of USF-1 was increased [80]. This suggests that the different 3' sequences of the E-4G and E-5G determine the different binding affinity of USF-1 to them, which is consistent with the previous findings that the flanking sequence of the E-box core motif contributes to the binding affinity of USFs [85]. Overexpression of WT USF-1 leads to increased PAI-1 promoter activity in HMC-1 cells, and the contrary effects were obtained with dominant negative (DN) USF. Moreover, overexpression of WT USF-1 or DN USF remedies the difference between the activities of the 4G- and 5G-PAI-1 promoters [80]. These results further indicate that USF-1 is the key transcription factor that regulates the 4G/5G polymorphism-dependent PAI-1 transcription in human MCs (Fig. 1). The varied binding of USF-1 to the promoter and subsequent different gene transcription levels caused by gene polymorphisms were also reported in genes of the central MHC, IKBL, and thymidylate synthase [86, 87]. However, the mechanism of USF-1 in regulating PAI-1 expression may be complicated. Besides E-4G/5G, which serves as the positive element, two more USF-1-binding sites were identified, which are located at –675 to –671 bp [12] and within –70 to –489 bp (Z. Ma & C. K. Oh, unpublished data) and serve as negative and positive elements respectively for PAI-1 expression in MCs. USFs are ubiquitously expressed basic helix-loop-helix family transcription factors and are critical to regulate various genes [88]. They are early-response

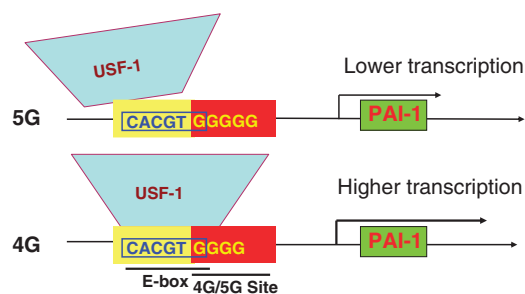


Fig. 1. Possible role of USF-1 in 4G/5G polymorphism-dependent PAI-1 expression. USF-1 binds to the E-box adjacent to the 4G site with higher affinity than the E-box adjacent to the 5G site, which results in greater transcription of 4G-PAI-1 gene than 5G-PAI-1 gene. USF-1, upstream stimulatory factor-1; PAI-1, plasminogen activator inhibitor-1.

transcription factors in human MCs [89]. The level of nuclear USF-1 protein is increased in a time-dependent manner in the stimulated MCs [80]. These new findings suggest a novel role of USF-1 in the pathogenesis of asthma by regulating the genotype-specific PAI-1 expression in MCs.

Conclusions

Increasing evidence indicates that PAI-1 may play an important role in pathogenesis of asthma, and the 4G/5G polymorphism of PAI-1 gene is associated with the risk and severity of asthma. Recent studies suggest that USF-1 may contribute to the development of asthma by regulating the 4G/5G polymorphism-dependent PAI-1 expression. Further studies to reveal the mechanisms of PAI-1 expression and action may lead to the development of a novel prognostic factor and therapeutic target for the treatment and prevention of asthma and other PAI-1-associated diseases.

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