

Theme Issue Article

Transcriptional regulation of the plasminogen activator inhibitor type I – with an emphasis on negative regulation

Yoshikuni Nagamine

Friedrich Miescher Institute for Biomedical Research, Novartis Research Foundation, Basel, Switzerland

Summary

By inhibiting plasminogen activators uPA and tPA, inducing uPA-uPAR internalization and interfering with the interaction between extracellular matrix protein vitronectin and $\alpha\beta3$ integrin, plasminogen activator inhibitor type I (PAI-1) is active in the regulation of various biological processes involving extracellular proteolysis and tissue remodeling. PAI-1 is expressed in many cell types under the control of a variety of signals, depending on cell type. The most prominent and important of these sig-

nals are TGF β , hypoxia and insulin. Although the signaling pathways were largely elucidated, recent investigations have revealed more complicated aspects. The pathways interact at the level of both transcription factors and regulatory elements on the promoter. Furthermore, the engagement of negative factors in these pathways has been shown to be important, adding complexity and versatility to PAI-1 gene regulation.

Keywords

Review, plasminogen activator, gene regulation, negative regulation

Thromb Haemost 2008; 100: 1007–1013

Introduction

Plasminogen activator inhibitor type 1 (PAI-1) is a secreted protein belonging to the serpin (serine protease inhibitor) family. Its primary targets are urokinase-type and tissue-type plasminogen activators (uPA and tPA) that convert proenzyme plasminogen to an active serine protease plasmin. PAI-2 is another PA-specific inhibitor, mainly expressed in the cytoplasm and with an yet uncertain physiological target. Plasmin is a serine protease but, unlike PAs, it has a wide range of substrate specificity, including various extracellular matrix proteins, blood clot fibrin and precursors of growth factors and other proteases (for reviews see [1, 2]). Thus, both plasmin formation and its action are highly influenced by PAI-1 levels. However, the biological role of PAI-1 is not restricted to the modulation of plasminogen activation by PAs. Due to its high affinity for the extracellular protein vitronectin, it may interfere with the interaction between vitronectin and its ligand $\alpha\beta3$ integrin (3). Furthermore, because PAI-1 binding induces internalization of the surface uPA-receptor/uPA complex, PAI-1 may affect uPA-induced intracellular signaling (4, 5). This latter reaction is an important regulatory component of multicellular processes involving cell remodeling, such as angiogenesis, wound healing and possibly metastasis. High ex-

pression of PAI-1 leads to various pathological conditions depending on the site of expression, such as hyperthrombosis in blood vessels, nephropathy in kidney, and keloidal scar formation in skin wounds (for a review see [6]).

To deal with these pathological conditions, it is very important to know how the PAI-1 promoter is regulated. PAI-1 is expressed in almost all cell types, prominently in endothelial cells, adipocytes and hepatocytes. Reflecting the diversity of cell types in which PAI-1 is induced and the biological roles of the inhibitor, its expression is controlled by a variety of signals, including both steroid and peptide hormones, cytokines and mechanical as well as physical (environmental) stresses. The most investigated of these signals are tumor growth factor beta (TGF β), insulin and hypoxia and these are the subject of this short review. Further signals are listed in Table 1 together with corresponding intracellular signals and cis-elements in the PAI-1 promoter.

As in many biological systems, gene regulation here is under the control of both positive and negative regulators, which endows the system with a rapid or sensitive response to external signals, depending on whether the opposing signals are arranged in parallel or in series, respectively. Here, I will stress the involvement of negative regulators that modulate positive signals.

Correspondence to:
Yoshikuni Nagamine
Friedrich Miescher Institute
Maulbeerstrasse 66
CH-4058 Basel, Switzerland
Tel.: +41 61 697 6669, Fax: +41 61 697 3976
E-mail: yoshikuni.nagamine@fmi.ch

Received: May 8, 2008
Accepted after minor revision: July 18, 2008

Prepublished online: November 13, 2008
doi:10.1160/TH08-05-0294

TGF β signaling

Positive regulation

TGF β and related cytokines act on most cell types, controlling proliferation, differentiation and apoptosis. TGF β interacts and activates receptor serine/threonine kinases, heterodimers of type I and type II receptors. The activated receptor phosphorylates Smad2 or Smad3, which binds to Smad4. The Smad complex enters the nucleus and activates various target genes, recognizing specific sequences in the promoters (7). PAI-1 is one of most prominent and studied targets of TGF β (8).

Functional analysis of the PAI-1 promoter for TGF β induction has identified a major regulatory region between -800 to -500 with respect to the transcription initiation site. Several cis-elements have been identified in this region that mediate TGF β induction. However, the results vary somewhat between studies, which may reflect cell type differences and the context of the template used for the analysis.

Riccio et al. (9) using a PAI-1 promoter of 800 nt linked to the SV40 promoter in HepG2 cells showed that two neighboring sequences with high homology to consensus binding sites for the CCAAT-binding transcription factor nuclear factor I (CTF/NF-I) (TGGCTGCATGCCC: -560 to -548) and the ubiquitous factor (USF) (E-box, CACGTG: -568 to -563) are responsible for TGF β induction. Dennler et al. (10) demonstrated in the same cells that three TGF β -responsive elements {AG(C/A)CAGACA}, termed "CAGA boxes", located at -740 to -732 (AGCCAGACA), -590 to -582 (AGACAGACA) and -286 to -278 (AGACAGACA), cooperatively mediated TGF β induction by interacting with Smad3/Smad4 heterodimers. Song et al. (11) showed in Hep3B cells that a 12-bp element (AGACAAGGTTGT) at -736 to -725, which partially overlaps a CAGA-box, mediated TGF β induction when multiple copies were located upstream of a heterologous promoter. In this promoter context, sequence downstream of AGAC apparently contributed to Smad3/Smad4 binding, as mutation of this element without affecting AGAC abrogated TGF β induction. Synergism between the Smad3/Smad4 complex and other factors in TGF β induction was first reported by Hua et al. (12). They showed in HT 1080-derived cells that the transcription factor E-box-binding protein TFE3 synergized with the Smad3/Smad4 complex to mediate TGF β induction in a manner dependent on Smad3 phosphorylation, with the former binding to an E-box (-568 to -563) and the latter to a sequence immediately upstream containing CAGA boxes (-590 to -572). Feng et al. (13) found that TGF β induced the binding of Smad3 to a transcriptional adaptor CBP/p300, suggesting the CBP/p300 bridges the Smad3/Smad4 complex to the transcriptional machinery.

There are two SP1 sites in the proximal region of the PAI-1 promoter: (GGGTGGGG) at -77 to -70 and (CCTGCCC) at -46 to -40. Datta et al. (14) showed that TGF β induces the interaction between Smad 3 and SP1 and that SP1 is essential for this induction. The sequence around this promoter proximal regulatory region is well conserved between human and mouse, but the sequence around the two upstream E-boxes is not. This suggests that SP1 sites are more important than upstream E-boxes in TGF β regulation.

LIIM-only protein 4 (LMO4) is highly expressed in the epithelial components at locations of active mesenchymal-

epithelial interaction. LMOs interact strongly with various co-activators, co-repressors, competitors, and other transcription factors thereby modulating the expression of the target genes. Lu et al. (15) considered the possibility that LMO4 is involved in mesenchymal-epithelial signaling and found, using a reporter gene containing multiple copies of TGF β -responsive elements in HEK293T cells, that LMO4 enhances TGF β induction. They also showed that LMO4 enhances TGF β -induced PAI-1 gene expression in mouse mammary gland NMuMG cells (15). The enhancement occurred through LMO4 interaction with the Smad3/Sam4 complex. Just how this interaction enhanced transcription remains to be seen as LMO4 had no effect on TGF β -induced Smad phosphorylation or Smad3/Smad4 complex formation (15). It has been proposed that LMO4 affects TGF β -induced transcription by modulating Smad protein-involving multiprotein complexes with transcriptional coactivators and corepressors. This proposal is based on the observation of a biphasic effect of LMO4 on TGF β -induced transcription in transient transfection assays, suggesting that LMO4 stoichiometry with respect to other components is critical in TGF β -induced transcription. Whether the endogenous PAI-1 promoter responds to LMO4 in a similar biphasic manner is not known.

Negative modulation

The TGF β -induced PAI-1 gene can be modulated by other molecules through their interaction with activated Smad proteins. Song et al. (16) showed in HepG3 cells that TGF β induction can be repressed by glucocorticoids through direct binding of liganded glucocorticoid receptor (GR) to the carboxyl terminal transactivation domain of Smad3. Importantly, this inhibition by GR did not require GR recognition elements in the promoter, suggesting that any genes regulated by TGF β through involvement with Smad3 can be repressed by glucocorticoids. Inhibition of TGF β signaling by glucocorticoids was not reciprocal, as GR/GRE-dependent reporter gene expression was not affected at all by TGF β (16). Later, the same group found that overexpression of steroid receptor coactivator-1 (SRC-1) and GR-interacting protein-1 (GRIP-1) could enhance liganded GR-mediated repression of TGF β -induced reporter gene expression (17). This enhancement occurs through their direct interaction with the MH2 transactivation domain of Smad3. Interestingly, without the presence of glucocorticoids, Smad-mediated transcription was positively regulated by simple overexpression of SRC1 and GRIP-1. It may be that the Smad3/Smad4 complex bound by SRC-1 or GRIP-1 acquired a different conformation, making it more potent in the transactivation of the promoter but at the same time more sensitive to repression by liganded GR. This study, however, did not examine the effect of overexpression of these proteins on the induction of the endogenous PAI-1 gene by TGF β .

Smad7 is a negative regulator of TGF β signaling, suppressing the phosphorylation of Smad2 and 4 by blocking their interaction with the type I TGF β receptor. Boon et al. (18) found that Kruppel-like factor 2 (KLF2), a transcription factor exclusively expressed in endothelial cells, markedly suppressed TGF β -induced PAI-1 gene expression in HUVEC cells. This was the result of the concerted effects of KLF2, induction of Smad7 transcription and suppression of AP1 activation. KLF2 is a very im-

portant zinc-finger transcription factor in vascular biology that suppresses pro-inflammatory responses and attenuates angiogenesis (reviewed in [19]). High levels of PAI-1 expression have been implicated in the pathological development of the vascular system. Suppression of TGF β -induced PAI-1 gene expression by KLF2 may, therefore, be critical for regulation of vascular homeostasis.

Hypoxia signaling

Positive regulation

A condition of low oxygen supply, hypoxia, occurs in various pathological situations such as sepsis, ischemia, atherosclerotic lesions leading to myocardial infarction and deep-vein thrombosis (20). Hypoxia is also conspicuous in solid tumors, where it hampers radiotherapy (21). Hypoxia induces the expression of various genes by increasing the levels of the transcription factor HIF-1 (hypoxia-inducible factor-1), which is a dimer of HIF-1 α and HIF-1 β . At a normal oxygen concentration, oxygen-dependent prolyl and asparaginyl hydroxylases reduce (prolyl hydroxylated) HIF-1 α levels through von-Hippel-Lindau tumor suppressor-mediated proteasomal degradation or attenuate transactivation activity of (asparaginyl hydroxylated) HIF-1 α by suppressing its interaction with the p300 transcription co-activator (22, 23). Under hypoxic conditions, hydroxylation-free stable HIF-1 α accumulates and the resulting active HIF-1 induces transcription of various genes recognizing specific hypoxia-responsive elements in the promoter. The consensus HIF-1 recognition sequence (HRE) is BACGTGSK (with B=G/C/T, S=G/C, and K=G/T) (24, 25). Since the first report that PAI-1 mRNA may be strongly induced by hypoxic conditions in human endothelial cells (26), several different mechanisms by which the PAI-1 gene is upregulated have been reported. Kietzmann et al. (20) identified putative hypoxia-responsive elements HRE1 (-175 to -168: 5'-CACGTACA-3') and HRE2 (-165 to -158: 5'-CACGTGTC-3') in rat hepatoma cells, of which the latter was most critical for induction by mild hypoxia (8% O₂) and HIF-1 binding. Fink et al. (27) showed in HepG2 that an HRE (-194 to -187: 5'-CACGTACA-3') was sufficient for hypoxia-induced PAI-1 gene induction. Sato et al. (28, 29) showed that hypoxia induced PAI-1 gene expression by increasing the level of endothelial PAS domain protein-1 (EPAS1), an HIF-1 α -related protein, in human lung adenocarcinoma A549 cells. In this induction, an HRE (-194) and an adjacent GT-box (-78), which is recognized by transcription factors SP1 and SP3, independently but also cooperatively mediated hypoxia induction (28). It is noteworthy that SP1 was also seen to cooperate with Smad3 in TGF β induction of the PAI-1 gene (14), suggesting a key role in bridging different signals to the transcription machinery.

In freshly prepared human keloid-derived fibroblasts, Zhang et al. (30) found that hypoxia induced PAI-1 gene expression by elevating HIF-1 protein levels. Interestingly, this induction was also strongly attenuated by the PI3 kinase inhibitors LY294002 and Wortmannin and the protein tyrosine kinase inhibitor genistein. In both cases, inhibition was due to suppression of the increase in hypoxia-induced HIF-1 α protein levels. In this system, the Erk MAP kinase pathway inhibitor PD98059 also suppressed PAI-1 mRNA induction, but without affecting HIF-1 levels.

These results suggest that PI3 kinase/PKB and tyrosine kinases are involved in the HIF-1 α stabilization mechanism, while Erk kinase may act either at HIF-1 binding to HRE or its interaction with the transcriptional machinery. Later, the same group showed that hypoxia-mediated activation of PI3 kinase and PKB was via activation of Etk/Bmx, a member of the Tec family of non-receptor protein tyrosine kinases, and that the level of Etk/Bmx was augmented in the keloid (31). It remains to be seen how PKB activation leads to an increase in the HIF-1 α protein level and how Erk kinases activate HIF-1.

Like TGF β signaling, the HIF-1/HRE system can be used by or interact with other signaling pathways. Kietzmann et al. (32) showed in rat hepatoma cells that insulin induced PAI-1 gene expression through the HRE element by augmenting HIF-1 α protein levels in a manner dependent on PI3 kinase and PKB activation at a normal oxygen concentration. This suggests that HIF-1 levels are the target for regulation of the PAI-1 gene by signals other than hypoxia. A similar regulatory mechanism was observed in PAI-1 gene induction by insulin-like growth factor (IGF-1) in HepG2 human hepatocytes, involving the use of HRE (-194 to -187) in the promoter and the enhancement of HIF-1 α protein levels (33). The induction could be suppressed by inhibition of PI3 kinase and Erk1/2 kinases, as well as by overexpression of dominant negative Raf-1. It was shown in primary rat hepatocytes as well as in human hepatocyte HepG2 cells that the same HRE site could be a target for CREB binding to mediate PAI-1 gene induction by glucagon/cAMP, suggesting that HRE is a novel type of cAMP-responsive element (34, 35). This induction was observed irrespective of hypoxic conditions, indicating that CREB and HIF act independently on this element.

Negative modulation

Negative regulators are involved in hypoxia-mediated PAI-1 gene induction in some systems. Samoylenko et al. (36) showed in primary rat hepatocytes that upstream stimulatory factor-2a (USF-2a) interacts with HRE1 and downregulates the PAI-1 promoter. This is suggestive of a competition between HIF-1 and USF-2a in PAI-1 gene regulation. Gross et al. (37) showed that hypoxia induced the activation of both endogenous and transfected PAI-1 promoters in mouse skin endothelial (SEND) cells by reducing the level of the negative transcription factor Net. The underlying mechanism involves its ubiquitination and proteasome-dependent degradation. Thus, hypoxia has opposing effects on HIF-1 α and Net, the former being stabilized and the latter destabilized. Net, together with Elk-1 and Sap-1, is a member of the ternary complex factor subfamily of Ets transcription factors but, unlike the other two members, acts negatively on target genes. Previously, it was found in MEF cells that Net can act negatively on the PAI-1 promoter, utilizing one or all of three potential Ets-binding sites [GGA(A/T)] located between -519 and -319 with respect to the transcription start site (38). The relative importance of HIF-1 and Net in hypoxia-mediated PAI-1 gene may depend on cell type or species. Whereas an HRE (-194 to -187) in the promoter is essential and sufficient for hypoxia-mediated induction (27), as described above, RNAi-mediated knockdown of Net in SEND cells abrogated PAI-1 induction (37). The role of Net in hypoxia-mediated PAI-1 gene regulation in human cells has not been reported.

Table 1: PAI-1 gene regulators.

Regulators	Cells ^a	Cis-elements ^b	Trans-factors	Features	Reference
Wound (scrape)	Rat keratinocytes			Erk-dependent	(69)
Oxidative stress	GH4 (rat pituitary)	-61: TGAGTTCA	AP-1		(70)
Ca ²⁺	HepG2	-195: CACGTACA	HIF-1	via HIF-1 α induction	(71)
DNA alkylating agents	NIH3T3 (mouse fibroblast)	-160 : ACACATGCCT-CAGCAAGTCC	p53	via ATR and ATM kinases	(72, 73)
TPA	HT1080, HeLa, HepG2	-82 to -65	AP-1, AP-2, SPI-like		(74, 75)
Fatty acid	HepG2	72 bp: -599 to -528	SPI-like	PKC dependent	(76)
IL1 / oncostatin M	Cortical astrocytes	-61 : TGAGTTCA	c-fos/c-jun	via induction of c-fos	(77)
Hyperglycemia	Rat primary GMC	SPI sites -76 : GGTGG and -44 : CTGCC	SPI	SPI glycosylation involved	(78-80)
Glucocorticoid	trophoblast, adipocytes, HepG2				(81-83)
Retinoic acid	VSMC			Dependent on genistein-sensitive tyrosine kinase	(84)
TNF α	BAEC, 3T3-L1	-15 kb: TGGAATTCT (BAEC)	NF κ B?	via TGF β induction in 3T3-L1	(85, 86)
Thrombospondin-1	HDMEC			Negative regulation	(87)
Thrombin	HK2 (kidneyPTEC)			JNK/API pathway: PKC/src dependent	(88)
Angiotensin II	Rat primary VSMC, rat primary mesangial cells	Rat -89 to -50 (=human -91 to -50)	SPI & API	MEKK1-dependent	(89, 90)
Fibrin fragment	Rat primary lung fibroblast	-59: TGAGTTCA	AP-1 (c-fos/JunD)	Conserved cis element	(91)
Circadian regulation	BAEC, HUVEC 293T	Two E-boxes CACGTG at -684 and -565 (human gene). Two RORE (TGACCT): -418 and -265	CLOCK/BMAL, CLOCK/CLIF Rev-erba	Negative regulation	(92-94)
Cytochalasin D/ Colchicine	Rat VSMC (R22)			MEK-dependent, sensitive to genistein and herbimycin A	(95)
Ethanol	HUVEC	800 to -549		Inhibition seen at 0.02 %	(96,97)

BAEC, Bovine aortic endothelial cells; GMC, glomerular mesangial cells; HDMEC, human dermal microvascular endothelial cells; HUVEC, human umbilical endothelial cells; PTEC, proximal tubular epithelial cell; VSMC, vascular smooth muscle cells. ^a unless otherwise mentioned, the cells are human. ^b unless otherwise mentioned, the numbering is on the human PAI-1 gene promoter. For TGF β a, hypoxia and insulin, see text.

Insulin signalling (especially in insulin-resistant conditions)

Insulin is a large polypeptide of 51 aminoacids composed of two chains connected by disulfide linkage. The hormone is produced by pancreatic β -cells and acts on various target tissues, including adipose, muscle and liver. Of these, adipocytes are prominent targets of insulin action (>200,000 receptors per cell) and one of the main sources of PAI-1 in obesity and type 2 diabetes ([39-41], reviewed in [42]). The main action of insulin in target tissues is to regulate carbohydrate and lipid metabolism and protein synthesis, but it also induces many genes not directly linked to these

processes through insulin-induced multiple signaling pathways (43-45). In the context of PAI-1 gene regulation, the most important aspect to be discussed is elevated PAI-1 expression, especially in adipocytes, under pathological insulin-resistant conditions, associated with obesity and type-2 diabetes (reviewed in [42]). Under insulin-resistant conditions, cells do not respond to normal concentrations of insulin by increasing the capacity of glucose and lipid metabolism. However, under these conditions cells maintain the ability to respond to insulin by change in gene expression patterns, indicating that insulin receptors are not completely silenced. Several lines of evidence suggest that insulin together with other signals plays a role in elevated PAI-1 expression in insulin-resistant conditions (see below).

Both positive and negative signals are released from the insulin receptor

Insulin acts on target tissues by binding to its specific insulin receptor tyrosine kinase (IR) on the membrane, which is composed of two α - and two β -subunits held together by disulfide linkages. Ligand binding to α -subunits, which are located outside of the cell, induces conformational changes in the cytoplasmic domains of the β -subunits so that they become active tyrosine kinase and undergo autophosphorylation at several tyrosine residues in the β -subunit. These phosphotyrosine residues recruit several signaling molecules leading to the initiation of multiple signaling pathways (46, 45). Of these, the most relevant for PAI-1 gene regulation are Shc and the IRS1/2. The Shc signal is relayed to the Ras/Erk signaling pathway, whereas recruited docking protein IRSs further recruit various signaling proteins, including PI3 kinase. The two pathways exert opposite effects on the PAI-1 promoter. The mitogenic Erk signaling pathway activates the PAI-1 gene (47) by activating the transcription factor AP1 (48) and HIF-1 (49), whereas the metabolic PI3 kinase signaling pathway has a negative effect (50, 51), partly by activating the transcription factor E2F (52) (see below).

How is the balance disturbed?

Insulin-resistant conditions are primarily the consequence of the total or partial impairment of insulin receptor function. With total impairment, ligand-induced autophosphorylation of IR is strongly reduced and consequently IR-mediated signaling pathways are not responsive to insulin (45). In the case of partial impairment, ligand-induced autophosphorylation of IR is not reduced but the relaying of ligand-induced signals to some downstream signaling pathways, mostly those mediated by IRS, is selectively blocked as a result of serine/threonine phosphorylation of IRS; this suppresses its activating tyrosine phosphorylation by IR (53). The PI3-K pathway is reported to be compromised in adipocytes (54) and skeletal muscles (55, 56) from type 2 diabetes patients or skeletal muscles from insulin-resistant db/db mice (57), but insulin-induced Erk activation is not affected. As the concentration of insulin circulating in the body is markedly augmented (hyperinsulinemia) under insulin-resistant conditions (at least at an early stage) (58), it may follow that the PAI-1 gene receives more positive signals in insulin-resistant conditions. Several kinases have been shown to be responsible for IRS serine/threonine phosphorylation, such as IKK β , PKC ζ , mTOR, and S6K1 (reviewed in [59, 60]), and some of these mediate the effect of fatty acid and tumor necrosis factor alpha (60). Although the detailed mechanism has not been fully elucidated, caveolar dysfunction also induces selective impairment of the PI3 kinase axis of insulin signaling. Caveolae are pot-like microdomains on the plasma membrane in which IR is localized (61), and their dysfunction as induced by cholesterol depletion (an essential component of caveolae) leads to the impairment of PI3 kinase signaling (52). Insulin resistance is often preceded by obesity, which is a consequence of an increase in both the number and the size of fat cells, resulting in a decrease in cholesterol concentration at the membrane (62). This suggests that cholesterol depletion-induced caveolar dysfunction is one of the causes of insulin resistance. But then how is PI3 kinase linked to the negative regulation of the PAI-1 gene? Several reports describe the activation of E2F, a cell-

cycle regulating transcription factor (63, 64). In an inactive state, E2F is complexed with retinoblastoma protein (pRB). Phosphorylation of pRB by cyclin-dependent kinases releases active E2F from the complex. Koziczak et al. (65) reported that free, active E2F is actually a negative regulator of the PAI-1 gene. In nuclear run-on experiment in cells expressing tamoxifen-activatable E2F, they showed that tamoxifen could still suppress PAI-1 gene expression in the presence of a protein synthesis inhibitor, indicating that the PAI-1 gene is the primary target of E2F (66). Deletion analysis of the PAI-1 promoter showed only a stepwise reduction in E2F-mediated suppression, with p53 recognition site being one of the candidate E2F target sites (66). Further analysis is required in order to understand the precise molecular mechanism by which E2F keeps the target genes silent.

The way to restore the negative regulation

The proposed mechanism of elevated PAI-1 expression in insulin-resistant conditions implies that E2F is preferentially complexed with pRB, even after insulin treatment, but that it is still possible to reduce PAI-1 expression by physically dissociating the E2F-pRB complex, thereby releasing free E2F at the time of insulin surge. Indeed, data of Venugopal et al. (52) support this possibility. The authors prepared a cell-penetrating interfering peptide in which a region of the E2F1 protein responsible for pRB interaction was linked to a stretch of the HIV virus TAT protein that had the capacity to penetrate the cell. The conjugate peptide effectively entered the cell and disrupted the E2F-pRB complex. When caveolar dysfunction-induced insulin resistant 3T3-L1 adipocytes were treated with this peptide simultaneously with insulin treatment, insulin induction of PAI-1 was suppressed (52). It may also follow that efficient insulin induction of PAI-1 expression requires effective downregulation or suppression of the PI3 kinase pathway leading to E2F activation. This situation was clearly observed during adipocyte differentiation using the 3T3-L1 system (50). In preadipocytes, insulin did not significantly induce PAI-1. However, after cells had fully differentiated, when the level of free E2F was low even after insulin treatment, the PAI-1 gene was markedly induced by insulin. A reduction in E2F activity was achieved in this system cooperatively by reduced E2F1 expression, enhanced pRB expression and reduced pRB phosphorylation (50).

Perspectives

Various cis-regulatory elements have been identified in the PAI-1 promoter, including SP1 sites, AP1 sites, HREs, Smad complex binding sites and E-boxes. However, since the last review of PAI-1 gene regulation in 2005 (67), the list of new signals and corresponding cis-elements has not been extended. In contrast, as discussed here, a number of studies have reported crosstalk between signaling pathways at the level of transcription factors and cis-elements, revealing the complexity of PAI-1 gene regulation. Furthermore, we have also observed various negative regulators acting directly on the promoter or indirectly by the modulation of the activity of positive trans-acting factors. A picture of the complexity of PAI-1 gene regulation through interactions between regulatory network components has only just begun to emerge and this will surely expand in the future. We

have not yet seen any involvement of micro RNAs in PAI-1 gene regulation and the epigenetic regulation of the PAI-1 gene has not been addressed. It is noteworthy that expression of SATB1, a chromatin remodeling protein, is highly correlated with the progression of breast tumors and that PAI-1 is one of the many genes whose expression is affected by SATB1 (68).

References

- Binder BR, Christ G, Gruber F, et al. Plasminogen activator inhibitor 1: physiological and pathophysiological roles. *News Physiol Sci* 2002; 17: 56–61.
- Durand MK, Bodker JS, Christensen A, et al. Plasminogen activator inhibitor-I and tumour growth, invasion, and metastasis. *Thromb Haemost* 2004; 91: 438–449.
- Seiffert D, Smith JW. The cell adhesion domain in plasma vitronectin is cryptic. *J Biol Chem* 1997; 272: 13705–13710.
- Cubellis MV, Wun TC, Blasi F. Receptor-mediated internalization and degradation of urokinase is caused by its specific inhibitor PAI-1. *EMBO J* 1990; 9: 1079–1085.
- Degryse B, Sier CF, Resnati M, et al. PAI-1 inhibits urokinase-induced chemotaxis by internalizing the urokinase receptor. *FEBS Lett* 2001; 505: 249–254.
- Lijnen HR. Pleiotropic functions of plasminogen activator inhibitor-1. *J Thromb Haemost* 2005; 3: 35–45.
- Kretzschmar M, Massague J. SMADs: mediators and regulators of TGF-beta signaling. *Curr Opin Genet Dev* 1998; 8: 103–111.
- Westerhausen DR, Jr, Hopkins WE, Billadello JJ. Multiple transforming growth factor-beta-inducible elements regulate expression of the plasminogen activator inhibitor type-1 gene in Hep G2 cells. *J Biol Chem* 1991; 266: 1092–1100.
- Riccio A, Pedone PV, Lund LR, et al. Transforming growth factor beta 1-responsive element: closely associated binding sites for USF and CCAAT-binding transcription factor-nuclear factor I in the type 1 plasminogen activator inhibitor gene. *Mol Cell Biol* 1992; 12: 1846–1855.
- Dennler S, Itoh S, Vivien D, et al. Direct binding of Smad3 and Smad4 to critical TGF-beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *Embo J* 1998; 17: 3091–3100.
- Song CZ, Siok TE, Gelehrter TD. Smad4/DPC4 and Smad3 mediate transforming growth factor-beta (TGF-beta) signaling through direct binding to a novel TGF-beta-responsive element in the human plasminogen activator inhibitor-1 promoter. *J Biol Chem* 1998; 273: 29287–29290.
- Hua X, Liu X, Ansari DO, et al. Synergistic cooperation of TFE3 and smad proteins in TGF-beta-induced transcription of the plasminogen activator inhibitor-1 gene. *Genes Dev* 1998; 12: 3084–3095.
- Feng XH, Zhang Y, Wu RY, et al. The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation. *Genes Dev* 1998; 12: 2153–2163.
- Datta PK, Blake MC, Moses HL. Regulation of plasminogen activator inhibitor-1 expression by transforming growth factor-beta -induced physical and functional interactions between smads and Sp1. *J Biol Chem* 2000; 275: 40014–40019.
- Lu Z, Lam KS, Wang N, et al. LMO4 can interact with Smad proteins and modulate transforming growth factor-beta signaling in epithelial cells. *Oncogene* 2006; 25: 2920–2930.
- Song CZ, Tian X, Gelehrter TD. Glucocorticoid receptor inhibits transforming growth factor-beta signaling by directly targeting the transcriptional activation function of Smad3. *Proc Natl Acad Sci USA* 1999; 96: 11776–11781.
- Li G, Heaton JH, Gelehrter TD. Role of steroid receptor coactivators in glucocorticoid and transforming growth factor beta regulation of plasminogen activator inhibitor gene expression. *Mol Endocrinol* 2006; 20: 1025–1034.
- Boon RA, Fledderus JO, Volger OL, et al. KLF2 suppresses TGF-beta signaling in endothelium through induction of Smad7 and inhibition of AP-1. *Arterioscler Thromb Vasc Biol* 2007; 27: 532–539.
- Atkins GB, Jain MK. Role of Kruppel-like transcription factors in endothelial biology. *Circ Res* 2007; 100: 1686–1695.
- Kietzmann T, Roth U, Jungermann K. Induction of the plasminogen activator inhibitor-1 gene expression by mild hypoxia via a hypoxia response element binding the hypoxia-inducible factor-1 in rat hepatocytes. *Blood* 1999; 94: 4177–4185.
- Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 2001; 93: 266–276.
- Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 2001; 294: 1337–1340.
- Lando D, Peet DJ, Gorman JJ, et al. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* 2002; 16: 1466–1471.
- Kvietikova I, Wenger RH, Marti HH, et al. The transcription factors ATF-1 and CREB-1 bind constitutively to the hypoxia-inducible factor-1 (HIF-1) DNA recognition site. *Nucleic Acids Res* 1995; 23: 4542–4550.
- Gerber HP, Condorelli F, Park J, et al. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 1997; 272: 23659–23667.
- Gertler JP, Perry L, L'Italien G, et al. Ambient oxygen tension modulates endothelial fibrinolysis. *J Vasc Surg* 1993; 18: 939–46.
- Fink T, Kazlauskas A, Poellinger L, et al. Identification of a tightly regulated hypoxia-response element in the promoter of human plasminogen activator inhibitor-1. *Blood* 2002; 99: 2077–2083.
- Sato M, Tanaka T, Maemura K, et al. The PAI-1 gene as a direct target of endothelial PAS domain protein-1 in adenocarcinoma A549 cells. *Am J Respir Cell Mol Biol* 2004; 31: 209–215.
- Sato M, Tanaka T, Maeno T, et al. Inducible expression of endothelial PAS domain protein-1 by hypoxia in human lung adenocarcinoma A549 cells. Role of Src family kinases-dependent pathway. *Am J Respir Cell Mol Biol* 2002; 26: 127–134.
- Zhang Q, Wu Y, Chau CH, et al. Crosstalk of hypoxia-mediated signaling pathways in upregulating plasminogen activator inhibitor-1 expression in keloid fibroblasts. *J Cell Physiol* 2004; 199: 89–97.
- Chau CH, Clavijo CA, Deng HT, et al. Etk/Bmx mediates expression of stress-induced adaptive genes VEGF, PAI-1, and iNOS via multiple signaling cascades in different cell systems. *Am J Physiol Cell Physiol* 2005; 289: C444–454.
- Kietzmann T, Samoylenko A, Roth U, et al. Hypoxia-inducible factor-1 and hypoxia response elements mediate the induction of plasminogen activator inhibitor-1 gene expression by insulin in primary rat hepatocytes. *Blood* 2003; 101: 907–914.
- Dimova EY, Moller U, Herzig S, et al. Transcriptional regulation of plasminogen activator inhibitor-1 expression by insulin-like growth factor-1 via MAP kinases and hypoxia-inducible factor-1 in HepG2 cells. *Thromb Haemost* 2005; 93: 1176–1184.
- Kvietikova I, Wenger RH, Marti HH, et al. The hypoxia-inducible factor-1 DNA recognition site is cAMP-responsive. *Kidney Int* 1997; 51: 564–566.
- Dimova EY, Jakubowska MM, Kietzmann T. CREB binding to the hypoxia-inducible factor-1 responsive elements in the plasminogen activator inhibitor-1 promoter mediates the glucagon effect. *Thromb Haemost* 2007; 98: 296–303.
- Samoylenko A, Roth U, Jungermann K, et al. The upstream stimulatory factor-2a inhibits plasminogen activator inhibitor-1 gene expression by binding to a promoter element adjacent to the hypoxia-inducible factor-1 binding site. *Blood* 2001; 97: 2657–2666.
- Gross C, Buchwalter G, Dubois-Pot H, et al. The ternary complex factor net is downregulated by hypoxia and regulates hypoxia-responsive genes. *Mol Cell Biol* 2007; 27: 4133–4141.
- Buchwalter G, Gross C, Wasyluk B. The ternary complex factor Net regulates cell migration through inhibition of PAI-1 expression. *Mol Cell Biol* 2005; 25: 10853–10862.
- Samad F, Loskutoff DJ. The fat mouse: a powerful genetic model to study elevated plasminogen activator inhibitor 1 in obesity/NIDDM. *Thromb Haemost* 1997; 78: 652–655.
- Mavri A, Alessi MC, Bastelica D, et al. Subcutaneous abdominal, but not femoral fat expression of plasminogen activator inhibitor-1 (PAI-1) is related to plasma PAI-1 levels and insulin resistance and decreases after weight loss. *Diabetologia* 2001; 44: 2025–2031.
- Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Obes Relat Metab Disord* 2004; 28: 1357–1364.
- Alessi MC, Poggi M, Juhan-Vague I. Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. *Curr Opin Lipidol* 2007; 18: 240–245.
- Whitman M, Kaplan DR, Schaffhausen BS, et al. Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* 1985; 315: 239–242.
- Johnston AM, Pirola L, Van Obberghen E. Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. *FEBS Lett* 2003; 546: 32–36.
- Youngren JF. Regulation of insulin receptor function. *Cell Mol Life Sci* 2007; 64: 873–891.

Acknowledgement

I would like to thank Dr. Thomas Gelehrter for a very informative discussion and Dr. Patrick King for critical reading of the manuscript.

46. Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. *Trends Cell Biol* 2002; 12: 65–71.
47. Samad F, Pandey M, Bell PA, et al. Insulin continues to induce plasminogen activator inhibitor 1 gene expression in insulin-resistant mice and adipocytes. *Mol Med* 2000; 6: 680–692.
48. Griffiths MR, Black EJ, Culbert AA, et al. Insulin-stimulated expression of c-fos, fra1 and c-jun accompanies the activation of the activator protein-1 (AP-1) transcriptional complex. *Biochem J* 1998; 335: 19–26.
49. Dimova EY, Kietzmann T. The MAPK pathway and HIF-1 are involved in the induction of the human PAI-1 gene expression by insulin in the human hepatoma cell line HepG2. *Ann N Y Acad Sci* 2006; 1090: 355–367.
50. Venugopal J, Hanashiro K, Nagamine Y. Regulation of PAI-1 gene expression during adipogenesis. *J Cell Biochem* 2007; 101: 369–380.
51. Mukai Y, Wang CY, Rikitake Y, et al. Phosphatidylinositol 3-kinase/protein kinase Akt negatively regulates plasminogen activator inhibitor type 1 expression in vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2007; 292: H1937–1942.
52. Venugopal J, Hanashiro K, Yang ZZ, et al. Identification and modulation of a caveolae-dependent signal pathway that regulates plasminogen activator inhibitor-1 in insulin-resistant adipocytes. *Proc Natl Acad Sci USA* 2004; 101: 17120–17125.
53. Zick Y. Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochem Soc Trans* 2004; 32: 812–816.
54. Smith U, Axelsen M, Carvalho E, et al. Insulin signaling and action in fat cells: associations with insulin resistance and type 2 diabetes. *Ann NY Acad Sci* 1999; 892: 119–126.
55. Cusi K, Maezono K, Osman A, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 2000; 105: 311–320.
56. Krook A, Bjornholm M, Galuska D, et al. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* 2000; 49: 284–292.
57. Shao J, Yamashita H, Qiao L, et al. Decreased Akt kinase activity and insulin resistance in C57BL/KsJ-Lepr^{db/db} mice. *J Endocrinol* 2000; 167: 107–115.
58. Olefsky JM, Nolan JJ. Insulin resistance and non-insulin-dependent diabetes mellitus: cellular and molecular mechanisms. *Am J Clin Nutr* 1995; 61: 980S–986S.
59. Zick Y. Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. *Sci STKE* 2005; 2005: pe4.
60. Herschkovitz A, Liu YF, Ilan E, et al. Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. *J Biol Chem* 2007; 282: 18018–18027.
61. Anderson RG. The caveolae membrane system. *Annu Rev Biochem* 1998; 67: 199–225.
62. Le Lay S, Krief S, Farnier C, et al. Cholesterol, a cell size-dependent signal that regulates glucose metabolism and gene expression in adipocytes. *J Biol Chem* 2001; 276: 16904–16910.
63. Gille H, Downward J. Multiple ras effector pathways contribute to G(1) cell cycle progression. *J Biol Chem* 1999; 274: 22033–22040.
64. Brennan P, Babbage JW, Thomas G, et al. p70(s6k) integrates phosphatidylinositol 3-kinase and rapamycin-regulated signals for E2F regulation in T lymphocytes. *Mol Cell Biol* 1999; 19: 4729–4738.
65. Koziczak M, Krek W, Nagamine Y. Pocket protein-independent repression of urokinase-type plasminogen activator and plasminogen activator inhibitor 1 gene expression by E2F1. *Mol Cell Biol* 2000; 20: 2014–2022.
66. Koziczak M, Muller H, Helin K, et al. E2F1-mediated transcriptional inhibition of the plasminogen activator inhibitor type 1 gene. *Eur J Biochem* 2001; 268: 4969–4978.
67. Nagamine Y, Medcalf RL, Munoz-Canoves P. Transcriptional and posttranscriptional regulation of the plasminogen activator system. *Thromb Haemost* 2005; 93: 661–675.
68. Han HJ, Russo J, Kohwi Y, et al. SATB1 reprograms gene expression to promote breast tumour growth and metastasis. *Nature* 2008; 452: 187–193.
69. Providence KM, Higgins PJ. PAI-1 expression is required for epithelial cell migration in two distinct phases of in vitro wound repair. *J Cell Physiol* 2004; 200: 297–308.
70. Vulin AI, Stanley FM. Oxidative stress activates the plasminogen activator inhibitor type 1 (PAI-1) promoter through an AP-1 response element and cooperates with insulin for additive effects on PAI-1 transcription. *J Biol Chem* 2004; 279: 25172–25178.
71. Liu Q, Moller U, Flugel D, et al. Induction of plasminogen activator inhibitor I gene expression by intracellular calcium via hypoxia-inducible factor-1. *Blood* 2004; 104: 3993–4001.
72. Parra M, Jardi M, Koziczak M, et al. p53 phosphorylation at serine 15 is required for transcriptional induction of the plasminogen activator inhibitor-1 (PAI-1) gene by the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine. *J Biol Chem* 2001; 276: 36303–36310.
73. Vidal B, Parra M, Jardi M, et al. The alkylating carcinogen N-methyl-N'-nitro-N-nitrosoguanidine activates the plasminogen activator inhibitor-1 gene through sequential phosphorylation of p53 by ATM and ATR kinases. *Thromb Haemost* 2005; 93: 584–591.
74. Descheemaeker KA, Wyns S, Nelles L, et al. Interaction of AP-1, AP-2, and Sp1-like proteins with two distinct sites in the upstream regulatory region of the plasminogen activator inhibitor-1 gene mediates the phorbol 12-myristate 13-acetate response. *J Biol Chem* 1992; 267: 15086–15091.
75. Arts J, Grimbergen J, Bosma PJ, et al. Role of c-Jun and proximal phorbol 12-myristate-13-acetate-(PMA)-responsive elements in the regulation of basal and PMA-stimulated plasminogen-activator inhibitor-1 gene expression in HepG2. *Eur J Biochem* 1996; 241: 393–402.
76. Chen Y, Billadello JJ, Schneider DJ. Identification and localization of a fatty acid response region in the human plasminogen activator inhibitor-1 gene. *Arterioscler Thromb Vasc Biol* 2000; 20: 2696–2701.
77. Kasza A, Kiss DL, Gopalan S, et al. Mechanism of plasminogen activator inhibitor-1 regulation by oncostatin M and interleukin-1 in human astrocytes. *J Neurochem* 2002; 83: 696–703.
78. Du XL, Edelstein D, Rossetti L, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A* 2000; 97: 12222–12226.
79. Goldberg HJ, Scholey J, Fantus IG. Glucosamine activates the plasminogen activator inhibitor 1 gene promoter through Sp1 DNA binding sites in glomerular mesangial cells. *Diabetes* 2000; 49: 863–871.
80. Goldberg HJ, Whiteside CI, Fantus IG. The hexosamine pathway regulates the plasminogen activator inhibitor-1 gene promoter and Sp1 transcriptional activation through protein kinase C-beta 1 and -delta. *J Biol Chem* 2002; 277: 33833–33841.
81. Ma Y, Ryu JS, Dulay A, et al. Regulation of plasminogen activator inhibitor (PAI)-1 expression in a human trophoblast cell line by glucocorticoid (GC) and transforming growth factor (TGF)-beta. *Placenta* 2002; 23: 727–734.
82. Morange PE, Aubert J, Peiretti F, et al. Glucocorticoids and insulin promote plasminogen activator inhibitor 1 production by human adipose tissue. *Diabetes* 1999; 48: 890–895.
83. Healy AM, Gelehrter TD. Induction of plasminogen activator inhibitor-1 in HepG2 human hepatoma cells by mediators of the acute phase response. *J Biol Chem* 1994; 269: 19095–19100.
84. Watanabe A, Kanai H, Arai M, et al. Retinoids induce the PAI-1 gene expression through tyrosine kinase-dependent pathways in vascular smooth muscle cells. *J Cardiovasc Pharmacol* 2002; 39: 503–512.
85. Hou B, Eren M, Geilanter CA, et al. Tumor necrosis factor alpha activates the human plasminogen activator inhibitor-1 gene through a distal nuclear factor kappaB site. *J Biol Chem* 2004; 279: 18127–18136.
86. Samad F, Yamamoto K, Pandey M, et al. Elevated expression of transforming growth factor-beta in adipose tissue from obese mice. *Mol Med* 1997; 3: 37–48.
87. Kellouche S, Mourah S, Bonnefoy A, et al. Platelets, thrombospondin-1 and human dermal fibroblasts cooperate for stimulation of endothelial cell tubulogenesis through VEGF and PAI-1 regulation. *Exp Cell Res* 2007; 313: 486–499.
88. Pontrelli P, Ranieri E, Ursi M, et al. jun-N-terminal kinase regulates thrombin-induced PAI-1 gene expression in proximal tubular epithelial cells. *Kidney Int* 2004; 65: 2249–2261.
89. Chen HC, Feener EP. MEK1,2 response element mediates angiotensin II-stimulated plasminogen activator inhibitor-1 promoter activation. *Blood* 2004; 103: 2636–2644.
90. Motojima M, Ando T, Yoshioka T. Sp1-like activity mediates angiotensin-II-induced plasminogen-activator inhibitor type-1 (PAI-1) gene expression in mesangial cells. *Biochem J* 2000; 349: 435–441.
91. Olman MA, Hagood JS, Simmons WL, et al. Fibrin fragment induction of plasminogen activator inhibitor transcription is mediated by activator protein-1 through a highly conserved element. *Blood* 1999; 94: 2029–2038.
92. Schoenhard JA, Smith LH, Painter CA, et al. Regulation of the PAI-1 promoter by circadian clock components: differential activation by BMAL1 and BMAL2. *J Mol Cell Cardiol* 2003; 35: 473–481.
93. Maemura K, de la Monte SM, Chin MT, et al. CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. *J Biol Chem* 2000; 275: 36847–36851.
94. Wang J, Yin L, Lazar MA. The orphan nuclear receptor Rev-erb alpha regulates circadian expression of plasminogen activator inhibitor type 1. *J Biol Chem* 2006; 281: 33842–33848.
95. Samarakoon R, Higgins PJ. MEK/ERK pathway mediates cell-shape-dependent plasminogen activator inhibitor type 1 gene expression upon drug-induced disruption of the microfilament and microtubule networks. *J Cell Sci* 2002; 115: 3093–3103.
96. Grenett HE, Wolkowicz PE, Benza RL, et al. Identification of a 251-bp fragment of the PAI-1 gene promoter that mediates the ethanol-induced suppression of PAI-1 expression. *Alcohol Clin Exp Res* 2001; 25: 629–636.
97. Cullen JP, Sayeed S, Kim Y, et al. Ethanol inhibits pulse pressure-induced vascular smooth muscle cell migration by differentially modulating plasminogen activator inhibitor type 1, matrix metalloproteinase-2 and -9. *Thromb Haemost* 2005; 94: 639–645.