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Transcriptional regulation of the plasminogen activator inhibitor type 1 – with an emphasis on negative regulation

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Summary
By inhibiting plasminogen activators uPA and tPA, inducing uPA-uPAR internalization and interfering with the interaction between extracellular matrix protein vitronectin and αvβ3 integrin, plasminogen activator inhibitor type 1 (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 signals are TGFβ, 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

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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 αvβ3 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 expression 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 (TGFβ), 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.

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TGFβ signaling

Positive regulation

TGFβ and related cytokines act on most cell types, controlling proliferation, differentiation and apoptosis. TGFβ 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 TGFβ (8).

Functional analysis of the PAI-1 promoter for TGFβ 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 TGFβ 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-abox, CACGTG: –568 to –563) are responsible for TGFβ induction. Dennler et al. (10) demonstrated in the same cells that three TGFβ-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 TGFβ 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 TGFβ induction when multiple copies were located upstream of a heterologous promoter. In this promoter context, sequence downstream of AGAC apparently contributed to Samd3/Samd4 binding, as mutation of this element without affecting AGAC abrogated TGFβ induction. Synergism between the Smad3/Smad4 complex and other factors in TGFβ 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 TGFβ 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 TGFβ induced the binding of Smad3 to a transcriptional adaptor CBP/p300, suggesting the CBP/p300 bridges the Smad3/Samd4 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 TGFβ 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 TGFβ 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 TGFβ-responsive elements in HEK293T cells, that LMO4 enhances TGFβ induction. They also showed that LMO4 enhances TGFβ-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 TGFβ-induced Smad phosphorylation or Smad3/Smad4 complex formation (15). It has been proposed that LMO4 affects TGFβ-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 TGFβ-induced transcription in transient transfection assays, suggesting that LMO4 stoichiometry with respect to other components is critical in TGFβ-induced transcription. Whether the endogenous PAI-1 promoter responds to LMO4 in a similar biphasic manner is not known.

Negative modulation

The TGFβ-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 TGFβ 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 TGFβ through involvement with Smad3 can be repressed by glucocorticoids. Inhibition of TGFβ signaling by glucocorticoids was not reciprocal, as GR/GRE-dependent reporter gene expression was not affected at all by TGFβ (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 TGFβ-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 TGFβ.

Smad7 is a negative regulator of TGFβ signaling, suppressing the phosphorylation of Smad2 and 4 by blocking their interaction with the type I TGFβ receptor. Boon et al. (18) found that Kruppel-like factor 2 (KLF2), a transcription factor exclusively expressed in endothelial cells, markedly suppressed TGFβ-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-

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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 TGFβ-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-1α and HIF-β. At a normal oxygen concentration, oxygen-dependent prolyl and asparaginyl hydroxylases reduce (prolyl hydroxylated) HIF-1α levels through von-Hippel-Lindau tumor suppressor-mediated proteasomal degradation or attenuate transactivation activity of (asparaginyl hydoxylated) HIF-1α by suppressing its interaction with the p300 transcription co-activator (22, 23). Under hypoxic conditions, hydroxylation-free stable HIF-1α 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% O_2) 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-1α-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 TGFβ 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-1α 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-1α 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-1α protein level and how Erk kinases activate HIF-1.

Like TGFβ 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-1α 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-1α 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-1α 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.

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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)
Ca2+	HepG2	–195:CACGTACA	HIF-1	via HIF-1α 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, SP1-like		(74, 75)
Fatty acid	HepG2	72 bp: –599 to –528	SP1-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	SP1 sites –76 : GGTGG and –44 : CTGCC	SP1	SP1 glycosylation involved	(78–80)
Glucocorticoid	trophoblast, adipocytes, HepG2				(81–83)
Retinoic acid	VSMC			Dependent on genistein-sensitive tyrosine kinase	(84)
TNFα	BAEC, 3T3-L1	–15 kb:TGGAATTTCT (BAEC)	NFκB?	via TGFβ induction in 3T3-L1	(85, 86)
Thrombospondin-1	HDMEC			Negative regulation	(87)
Thrombin	HK2 (kidneyPTEC)			JNK/AP1 pathway: PKC/src dependent	(88)
Angiotensin II	Rat primary VSMC, rat primary mesangial cells	Rat –89 to –50 (=human –91 to –50)	SP1 & AP1	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, gromerular 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 TGFß, 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).

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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 IKKβ, PKCζ, 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 net work components has only just begun to emerge and this will surely expand in the future. We

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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).

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