

## Theme Issue Article

## Metabolic, hormonal and environmental regulation of plasminogen activator inhibitor-1 (PAI-1) expression: Lessons from the liver

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## Summary

Plasminogen activator inhibitor-1 (PAI-1) controls the regulation of the fibrinolytic system in blood by inhibiting both urokinase-type and tissue-type plasminogen activators. Enhanced levels of PAI-1 are found in patients with type 2 diabetes mellitus which is associated with a dysbalance in glucose and lipid homeostasis. Especially a defective insulin response in the liver contributes to the development of hyperglycemia, dyslipidemia and peripheral insulin resistance and may contribute to hepatic overexpression of PAI-1 in diabetes type 2. Furthermore, a substantial upregulation of PAI-1 expression has also been shown in a

variety of liver injury models. Thus, the liver appears to be not only a major site of PAI-1 synthesis in response to hormonal changes, but also in response to a variety of other pathological events. PAI-1 expression in liver largely depends on activation of signalling pathways and transcriptional regulators which may be the basis for a new level of cross-talk between different signalling pathways and thus may represent attractive therapeutic candidates. This article will primarily focus on the regulation of PAI-1 expression in liver cells and discuss potential cross-talks between metabolic, hormonal and environmental signals.

## Keywords

Fibrinolysis inhibitors, gene expression, plasminogen activator inhibitors, transcription factors, hypoxia

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## Introduction

A number of physiological and pathological processes among them fibrinolysis and matrix degradation are regulated by the tissue-type (tPA) and urokinase-type (uPA) plasminogen activators. Both PAs are serine proteases which catalyze the conversion of the inactive proenzyme plasminogen into active plasmin (1). In contrast to tPA and uPA, plasmin has a wide range of substrate specificity and degrades fibrin, basement membrane components such as laminin, collagen type IV and fibronectin as well as activates matrix metalloproteases and growth factors like HGF (hepatocyte growth factor) and transforming growth factor (TGF)- $\beta$  (2). The action of tPA and uPA is primarily antagonized by plasminogen activator inhibitors (PAIs). First evidence for the existence of these inhibitors was gained from experiments with conditioned medium from the rat hepatoma cell line HTC which showed the presence of a glucocorticoid-inducible fibrinolytic inhibitor (3, 4). This inhibitor could then also be purified from various biological sources and based on immunological properties at least two types of inhibitors were distinguished which became known as endothelial-type and placental-type PAI, respect-

ively (for review see [5]). With the cloning of cDNAs for each type (6–10) and immunological expression studies it became clear that both types are present in placenta (11) and the names PAI-1 and PAI-2 were adopted (12). In addition to PAI-1 and PAI-2 which belong to the serine protease inhibitor (serpin) superfamily, protein C inhibitor (PAI-3) and the protease nexin-1 also possess plasminogen activator inhibitor activity. From the PAIs known so far, PAI-1 appears to be the most important. It is a single-chain glycoprotein with an apparent molecular mass of 48 kDa and can be secreted from a variety of cells but the major producers are hepatocytes, vascular endothelial and smooth muscle cells, adipocytes and platelets (13–17). Although circulating and primarily measured in blood, PAI-1 is also a component of the extracellular matrix where it is found to interfere with the interaction between vitronectin and its cell surface ligand  $\alpha v \beta 3$  integrin (18). Further, PAI-1 was found to induce uPA-uPAR internalization and appears to be cleared from the circulation with the help of scavenger receptors from the low-density lipoprotein receptor related family (LRP) (19) and thus PAI-1 may affect intracellular signaling processes by itself (20, 21).

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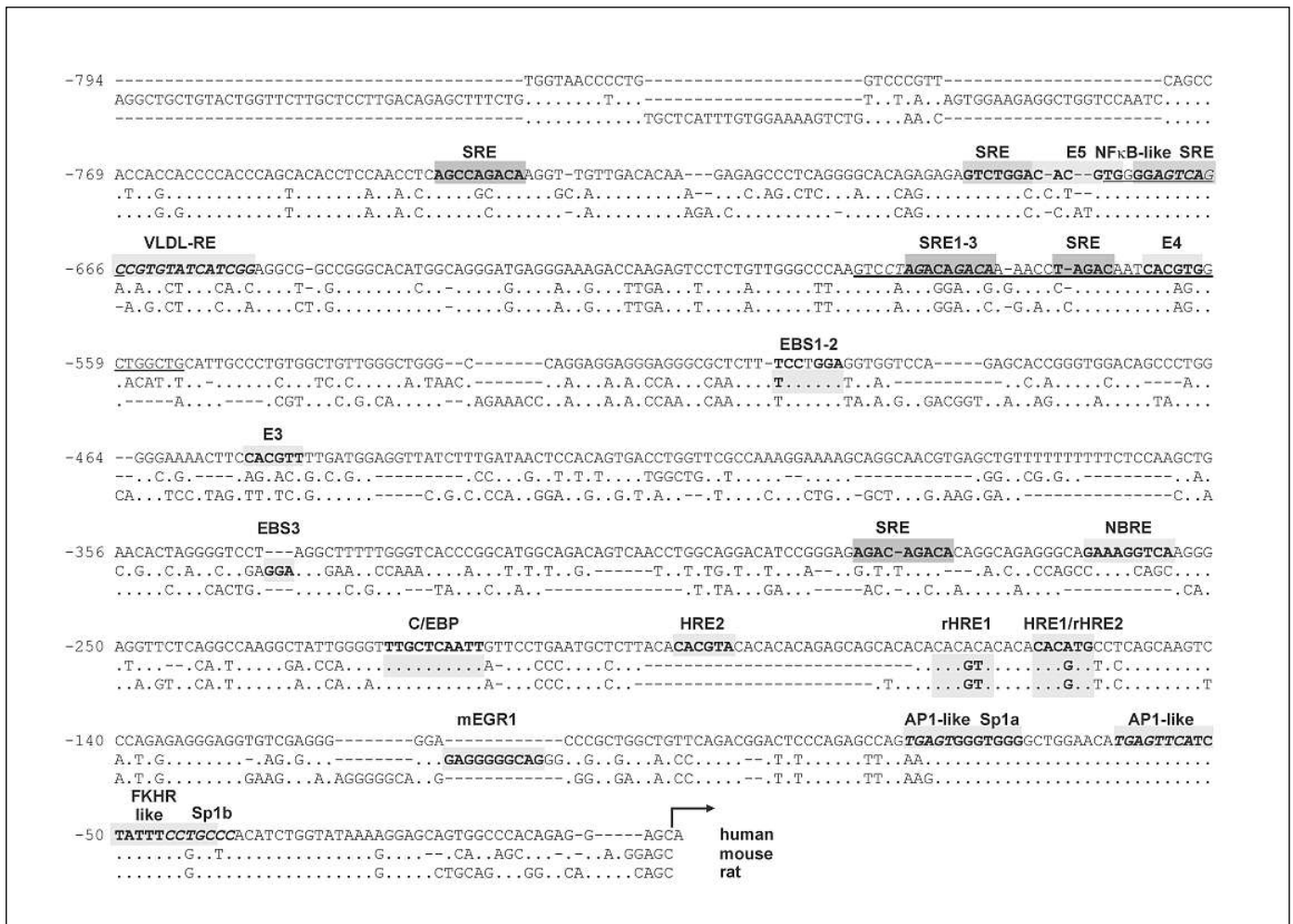
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The importance of PAI-1 is emphasized by several clinical studies: decreased PAI-1 levels cause bleeding diathesis, whereas increased PAI-1 levels are associated with conditions associated with hypoxia like atherosclerosis, coronary heart disease, deep-vein thrombosis, acute and chronic inflammatory lung disorders as well as cancer (for review see [22]).

Additionally, enhanced PAI-1 levels are found in patients with type 2 diabetes mellitus (23, 24) which is associated with a dysbalance in glucose and lipid homeostasis (25). Under normal conditions glucose and lipid homeostasis is mainly achieved by the adequate regulation of hepatic metabolism. This occurs primarily by the action of the pancreatic hormones insulin and glucagon. By contrast, it was shown that especially a defective insulin response in liver contributes to the development of hyperglycemia, dyslipidemia and peripheral insulin resistance (26–28). In addition, hepatic overexpression of PAI-1 in diabetes

type 2 was considered to contribute to the decreased basal membrane and extracellular matrix degradation and the resulting angiopathies (for review see [29]). Further, a substantial upregulation of PAI-1 expression has also been shown in a variety of liver injury models including hemorrhagic shock (30), bile duct ligation (31, 32), acetaminophen hepatotoxicity (33, 34) and alcohol-induced liver injury (35). Thus, the liver appears to be not only a major site of PAI-1 synthesis in response to hormonal changes, but also in response to a variety of other pathological events.

The liver is an organ composed of different cell types functioning in cooperation as a center of metabolism, a center of defence, a control station of the hormonal system and a blood reservoir as well as in all processes required for the formation and maintenance of its own cellular and extracellular structures (37, 38).



**Figure 1: Transcription factor binding sites in the human, mouse and rat PAI-1 promoters.** The mouse and rat promoters were aligned to the human –796 bp promoter using the Clustal Alignment algorithm. Dots indicate the positions of sequence identity, and dashes reflect gaps introduced into the sequence to obtain optimal sequence homology. The transcription factor binding sites identified in the respective promoters are boxed and in bold letters. AP1-like, Activator protein I-like binding element; C/EBP, CAAT enhancer binding protein response element; EBS, Ets/Net binding sites; mEGR I, mouse EGR I

binding element; E, E-box; FKHR-like, FoxO/forkhead like binding element; HRE, hypoxia response element; rHRE, rat HRE; NBRE, NGFI-B responsive element/Nurr77 response element; NF-κB-like, nuclear factor kappa B-like element (underlined); SP1a/Sp1b, stimulatory protein-I response element a and b, respectively; SRE, Smad 3–4 binding element/TGF-β response element; VLDL-RE, very-low-density lipoprotein response element. The P2.I (-592/-552) element encompassing SRE1–3, another SRE and E4 is underlined.

A major role of the liver in metabolism is determined by its glucostat function which keeps blood glucose levels constant. Thereby the parenchymal cells, the hepatocytes proper, rather than the non-parenchymal cells operate as glucose storing cells. They remove excess glucose after a meal and release glucose in-between meals for the use of the glucose-dependent erythrocytes and the central nervous system (CNS). In addition, they use nutrient- or muscle-derived amino acids to produce glucose and remove the ammonia by ureagenesis. Further, the liver is involved in cholesterol synthesis and processing of nutrient fat reaching the liver as chylomicrons and fatty acids. Moreover, ketone bodies can be produced as intermediates to economize the use of glucose in fasting periods (37, 39).

This glucostat function of the liver is controlled by a complex nervous-humoral network (40, 41). Glucose uptake is mainly stimulated by insulin and the parasympathetic liver nerves while utilization of the glucose stores and thus glucose output is stimulated by glucagon, glucocorticoids and the catecholamines adrenaline and noradrenaline. Moreover, glucose output and uptake are also controlled by the circulating concentrations of glucose, lactate and last but not least oxygen (37, 38).

Thus, disturbances in the metabolic function of the liver will have profound effects on the other functions of the liver such as the synthesis of PAI-1 and other plasma proteins, the xenobiotic metabolism as well as the maintenance of the biomatrix components.

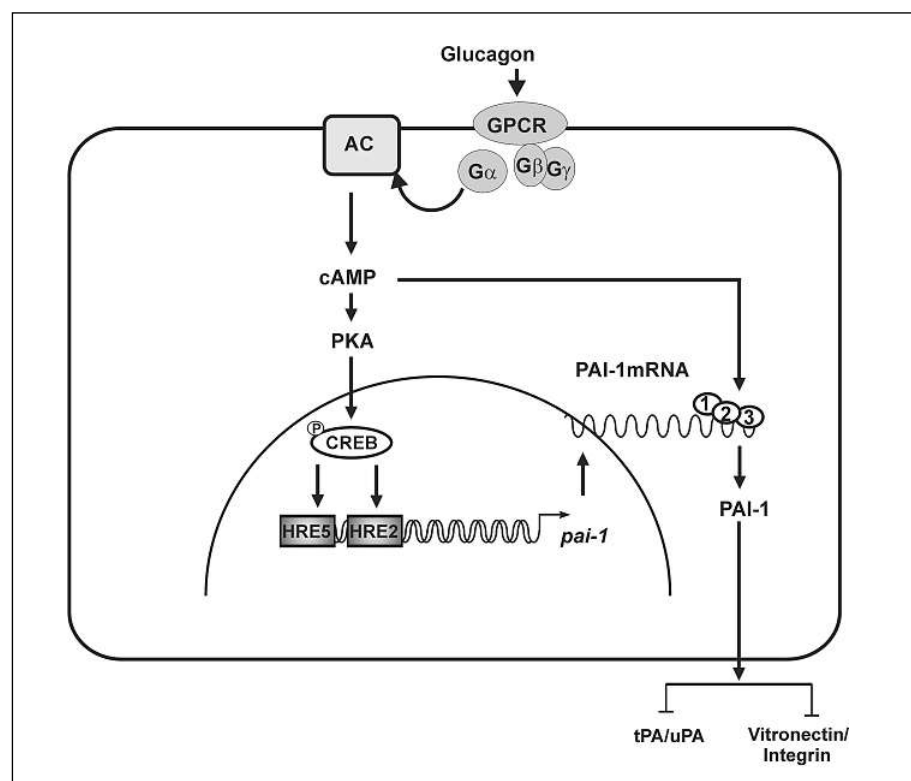
It is commonly accepted that the perturbations in PAI-1 expression in liver largely depends on the aberrant activation of signaling pathways and transcriptional regulators for which several binding sites have been identified within the promoters of the human, mouse and rat PAI-1 promoter (Fig. 1). Consequently,

these transcriptional regulators may be the basis for a new level of cross-talk between different signaling pathways and thus may represent attractive therapeutic candidates (36). Therefore, this article will primarily focus on the regulation of PAI-1 expression in liver cells and discuss potential cross-talks between metabolic, hormonal and environmental signals.

## Glucagon and cAMP

The 29 amino acid peptide glucagon acts via its receptor which belongs to the superfamily of G-protein-coupled receptors (42, 43). Although enhancement of  $Ca^{2+}$  and activation of protein kinase C have been described upon binding of glucagon to its receptor, the major signaling pathway in liver results in activation of the adenylate cyclase and subsequent enhancement of cAMP levels which in turn activate protein kinase A (PKA). This signaling pathway leads to the activation of hepatic glucose production by glycogenolysis and gluconeogenesis as well as to changes in the gene expression pattern mainly due to the PKA-dependent phosphorylation of the transcription factor cAMP responsive element binding protein (CREB) (44–46).

Indeed, first studies with primary rat hepatocytes showed that treatment with cAMP increased PAI-1 mRNA in these cells (47). In addition, increases of PAI-1 were also observed in the livers of rats upon injection of cAMP alone or in combination with dexamethasone (47). In addition, stimulation of the cAMP/PKA/CREB signaling cascade by starvation *in vivo* or by treatment of primary hepatocytes with glucagon *in vitro* induced PAI-1 gene expression. This response was associated with enhanced phosphorylation of CREB. Interestingly, binding of CREB did not occur at a cAMP responsive element (CRE). Instead, CREB was



**Figure 2: Regulation of PAI-1 expression in response to glucagon.** Glucagon binding to its receptor in liver results in activation of the adenylate cyclase and subsequent enhancement of cAMP levels and activation of protein kinase A (PKA). Upon PKA-dependent phosphorylation the transcription factor cAMP responsive element binding protein (CREB) can bind HRE2 and E5 to induce PAI-1 expression. In addition, the 3'-UTR from the PAI-1 mRNA can be destabilized in the presence of three cAMP regulatable 3'-UTR PAI-1 mRNA-binding proteins.

able to bind to three elements, hypoxia-responsive element 2 (HRE2) (-194/-187) and two classical E-boxes (E), E4 (-566/-559) and E5 (-681/-674), within the human PAI-1 promoter (Fig. 2) but only HRE2 and E5 appeared to be functionally active (48); In the rat PAI-1 promoter the HRE2 (-164/-157) is completely conserved and also contributed to the cAMP-dependent induction (48) but, the involvement of E5 could not be demonstrated due to the lack of E5 in the rat promoter.

The effect of cAMP or agonists that increase intracellular cAMP levels on PAI-1 expression appears to be cell type-specific, since the rat HTC hepatoma cell line responded with a decrease in PAI-1 levels upon stimulation with cAMP (49).

In addition, the different effects of cAMP on PAI-1 mRNA levels in the various cell types may in part be explained by an additional mode of post-transcriptional regulation. Interestingly, the 3'-UTR from the PAI-1 mRNA appears to be destabilized in the presence of cAMP in HTC rat hepatoma cells. At least two regions within the 3'-UTR could be identified from which the 3'-most 134 nucleotides were sufficient to mediate this effect also in a heterologous system (50). It was then found by ultraviolet cross-linking analyses that three cytosolic proteins of about 38–76 kDa could bind to that region (51). Although one of these mRNA-binding proteins was cloned (52), the exact identity of the other PAI-1 mRNA-binding proteins remains unknown. Thus, PAI-1 regulation by cAMP appears to be controlled at the transcriptional and post-transcriptional level.

Interestingly, the cAMP concentration is not only increased upon starvation but also during liver regeneration to stimulate DNA synthesis and the cell cycle (53, 54). In addition to its role in matrix remodelling and fibrinolysis, PAI-1 has been found to be an early response gene in the liver (55). In addition, PAI-1 can be induced in regenerating liver after partial hepatectomy which suggests that it is necessary for the modulation of the hepatocytes growth and differentiation. Indeed, tPA and uPA are known to be involved in the activation of HGF (56) and TGF- $\beta$  (57). Thus, the induction of PAI-1 by cAMP possibly represents a negative feedback for the regeneration process which may inhibit hepatocyte proliferation. Moreover, the induction by cAMP in the liver may have consequences for patients suffering from diabetes. In those patients, glucagon appears to be the dominant metabolic hormone compared to healthy individuals (58). This would indicate that the glucagon-mediated PAI-1 induction in the liver may contribute to the angiopathies occurring during diabetes. Indeed, PAI-1 was found to be overexpressed in patients suffering from non-proliferative diabetic retinopathy (59). Thus, the decreased matrix degradation due to PAI-1-induced inhibition of plasmin formation may contribute to these vessel abnormalities during diabetes.

## Catecholamines and angiotensin II

The receptors for catecholamines in liver are the  $\alpha$ 1-adrenergic receptors ( $\alpha$ 1AR) which like the glucagon receptor belong to the seven transmembrane domain G protein-coupled receptor (GPCR) family. Three  $\alpha$ 1AR subtypes are known (1A, 1B, and 1D) which couple to Gq and regulate phospholipase C (PLC) (60–62). Thus, hormone binding to the  $\alpha$ 1AR leads via Gq to ac-

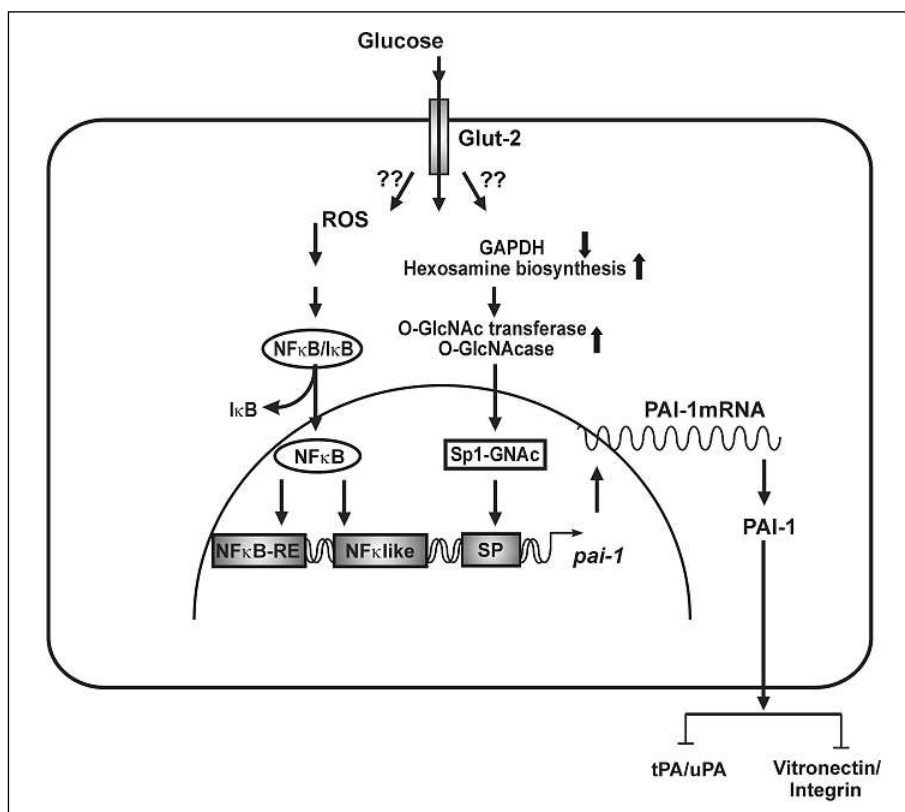
tivation of phospholipase C and generation of inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) which release intracellular  $\text{Ca}^{2+}$  and mediate activation of protein kinase C, respectively. Additionally,  $\alpha$ 1-AR has also been linked to other intracellular cascades in several extrahepatic cell types, including activation of phospholipase A2 (63), phospholipase D (64), and MAP kinases (65) and production of reactive oxygen species (66). These responses appear to be tissue- and cell type-specific and it remains obscure whether alternative signaling mechanisms are involved in hepatic  $\alpha$ 1-AR actions.

Interestingly, it was found in mice *in vivo* that noradrenaline increased PAI-1 expression in liver under special circumstances. While noradrenalin induced PAI-1 expression in the heart and aorta but not the kidney or liver of wild-type mice, it induced PAI-1 in liver and kidney when the angiotensin II receptor subtype AT(1a) was deficient. Although this study did not specifically address the mechanism through which noradrenaline increases PAI-1 expression, it implicated that the angiotensin II receptor subtype AT(1a) mediated signaling suppresses PAI-1 expression in liver and also in kidney. Interestingly, at the same time the noradrenaline effects on blood pressure were diminished upon AT(1a) deficiency (67).

This appears to be an interesting crosstalk, since the octapeptide angiotensin II which is best known for its action in the cardiovascular system also elicits a variety of responses including stimulation of cell proliferation on various organs among them the liver (68–71). Two major types of receptors, i.e. angiotensin AT1 (with the a and b subtypes) and AT2 receptors have been characterized and like the  $\alpha$ 1AR the AT1 receptors in hepatocytes are primarily coupled to the IP3/ $\text{Ca}^{2+}$ /PKC signal transduction pathway (72).

Since it was found *in vivo* that Ang II stimulated PAI-1 expression (73) in part through the AT(1b) receptor in kidney and liver (67), it can be speculated that the transcriptional mechanisms may be similar between kidney and liver. Although the transcription factors and PAI-1 promoter elements mediating Ang II-dependent induction in hepatocytes were not yet determined, it was found in kidney mesangial cells that the transcription factor Sp1 binding to two Sp1 binding sites (Sp1a, -76/-71 and Sp1b, -46/-41) (Fig. 3) in the human PAI-1 promoter was involved in the upregulation by Ang II (74). Similarly, it was reported that both Sp1a and AP-like sequences (-59/-52), respectively, mediated angiotensin II-stimulated PAI-1 promoter activation in a cooperative manner in vascular smooth muscle cells (75).

The catecholamine and angiotensin II modulated PAI-1 regulation may be important for regulation during liver fibrosis. It appeared also that the renin-angiotensin system mediates key actions involved in hepatic tissue repair and fibrosis, including myofibroblast proliferation, infiltration of inflammatory cells, and collagen synthesis. Thus enhanced PAI-1 expression may contribute to inhibition of collagenases and important growth factors like TGF- $\beta$  and HGF (76–78). However, it has also been proposed that inhibition of the renin-angiotensin system could prevent fibrosis progression in chronic liver diseases (79), but so far these antifibrotic effects in patients were not yet shown.



**Figure 3: Regulation of PAI-I expression in response to glucose.** Glucose is taken up by hepatocytes via the glucose transporter Glut-2 and an excessive intake of carbohydrates may result in the formation of reactive oxygen species (ROS) via a so far unknown mechanism. ROS in turn appear to activate NF- $\kappa$ B which then can induce PAI-I transcription via binding the NF- $\kappa$ B response element (NF- $\kappa$ B-RE) in the far upstream (-15kB) located enhancer or eventually via the NF- $\kappa$ B-like sequence at -678/-665 of the human PAI-I promoter. Glucose may also elicit an increased serine/threonine O-linked N-acetylglucosamination of Sp1. Then O-linked N-acetylglucosamination of Sp1 activates expression of PAI-I via binding of Sp1 to the Sp1 or Sp1c site in the promoter.

## Glucose

An excessive intake of carbohydrates as often seen with Western diets can contribute to fat accumulation via glucose- and insulin-regulated *de novo* lipogenesis in the liver.

In humans it was shown that combined hyperinsulinemia, hypertriglyceridemia and hyperglycemia increased the blood levels of PAI-1 and may thus contribute to hypofibrinolysis in type 2 diabetic patients, which underlines the importance of achieving glycemic and lipidemic control in those patients (80). The contributions of liver cells were then further investigated in the human HuH7 hepatocyte cell line which was treated with high glucose from 3 to 24 mM. Upon treatment with glucose in the presence of insulin, these cells displayed enhanced nuclear factor kappa-B (NF- $\kappa$ B) activity. Further, proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF $\alpha$ ) showed an additive effect with high glucose. Similar effects were obtained with the human PAI-1 promoter which appeared to be regulated by NF- $\kappa$ B (81). Finally, pretreatment of the cells with pyrrolidine dithiocarbamate (PDTC), which is thought to act as antioxidant, completely abolished the effect of high glucose and markedly attenuated that of TNF $\alpha$  (81). These data implicate that the responsiveness of the PAI-1 gene to glucose as well as to TNF $\alpha$  and IL-1 can be mediated via a common mechanism involving reactive oxygen species triggering NF- $\kappa$ B activation.

The role of reactive oxygen species as universal NF- $\kappa$ B activators was challenged by a number of studies and it became evident that this response occurs in a cell type-dependent manner (reviewed in [82, 83]). In addition, the experiments in which

PDTC antagonized the glucose effect do not specifically indicate involvement of reactive oxygen species because a recent study demonstrated that compounds considered being antioxidants such as PDTC and N-acetylcysteine (NAC) can inhibit NF- $\kappa$ B activity independent of their antioxidative properties. PDTC inhibited I $\kappa$ B-ubiquitin ligase activity while NAC blocked TNF receptor-induced signaling by lowering the receptor affinity (84). Thus, NF- $\kappa$ B activation does not seem to be a universal response to oxidative stress induced by high glucose but might partially contribute to this response in a cell type-specific manner.

So far, binding of NF- $\kappa$ B subunits to the PAI-1 promoter was found with a conserved NF- $\kappa$ B site in an about 15 kb upstream located enhancer mediating the TNF $\alpha$  response (see below) (85). In addition, another sequence at -678/-665 of the human PAI-1 promoter was shown to mediate the IL-1-dependent stimulation of an 805 bp PAI-1 promoter-driven reporter construct in HepG2 cells (86). Although this sequence was named NF- $\kappa$ B-like binding site due to sequence similarity, binding of NF- $\kappa$ B subunits was not directly determined (86). Thus, it remains entirely open whether binding of NF- $\kappa$ B to any of these sites in the PAI-1 promoter contributes to the glucose effect.

In addition to hepatocytes, the glucose-dependent induction occurs also in smooth muscle cells (87). Like in hepatocytes glucose activated the hexosamine pathway. However and in contrast to hepatocytes, high glucose elicited an increased serine/threonine O-linked N-acetylglucosamination of Sp1 (88). Furthermore, hyperglycemia increased expression of a 740 bp and 85 bp PAI-1 promoter Luc reporter containing two Sp1 sites (see above). When the two Sp1 sites were mutated, hyperglycemia did not increase expression of an 85-bp truncated PAI-1 promoter

Luc reporter (87). Thus, hyperglycemia induced hexosamine synthesis and then O-linked N-acetylglycosamination of Sp1, which activates expression of PAI-1 in vascular smooth muscle cells (88). In addition to Sp1, two other reports suggested that the glucose-increased PAI-1 gene transcription was dependent on activation of AP-1 in human (89) and rat vascular smooth muscle cells (90). Thus, to what extent NF- $\kappa$ B, Sp1, and AP-1 contribute to PAI-1 gene expression in response to glucose requires further investigations.

### Insulin and insulin-like growth factor-I (IGF-I)

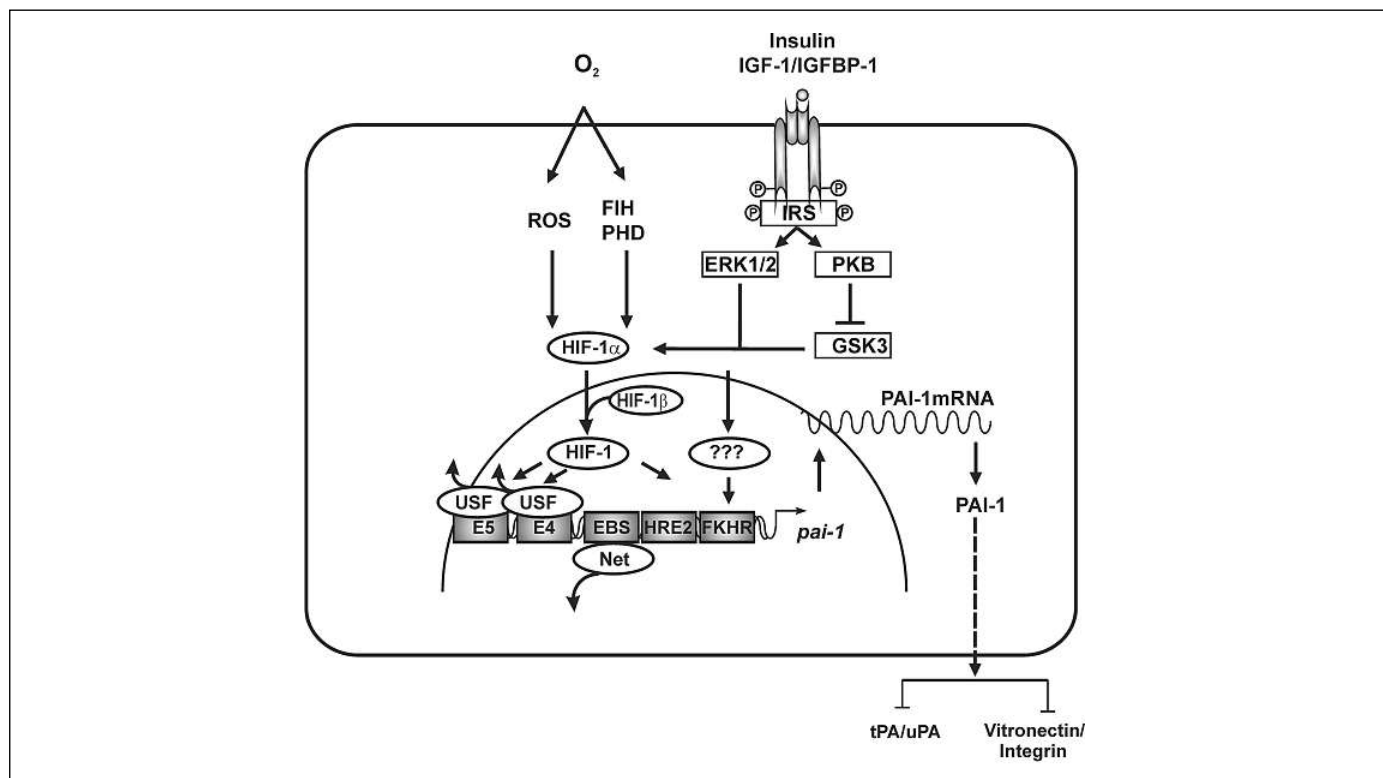
Insulin signaling involves second messengers including members of the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades (91). Thereby the PI3K, which generates phosphatidylinositol-3,4,5-phosphate (PI(3,4,5)P<sub>3</sub>), has a key role in the metabolic actions of insulin while the MAPK pathway is more involved in the growth promoting actions of insulin. PI(3,4,5)P<sub>3</sub> regulates the activity or subcellular localization of a variety of signaling molecules such as phosphatidylinositol-dependent kinase (PDK) and protein ki-

nase B (PKB) known as Akt, which are also involved in the transmission of the insulin signal (92, 93).

It has long been suggested that the effects of insulin are mediated through a common insulin-responsive element (IRE) and a transcription factor that binds to an IRE (94–96). However, up to now at least eight distinct IREs have been characterized (97), suggesting that there is no single consensus IRE.

Insulin has been shown to increase the endogenous PAI-1 gene expression in HepG2 cells (98), primary rat hepatocytes (99), and the transcription of a human PAI-1 promoter Luc construct in human umbilical vein endothelial cells (100, 101).

Insulin acted as an inducer of PAI-1 gene expression in all different model systems studied, but the search for the insulin responsive elements displayed some differences. The insulin response of the human PAI-1 promoter was first suggested to involve the regions -93/-62, -157/-128, and -777/-741 and the PKC/MAPK pathway when HepG2 cells were used (102). In another study with HepG2 cells, the involvement of the MAPK pathway was confirmed, and it was additionally shown that the insulin effect was associated with activation of the transcription factor hypoxia-inducible factor-1 (HIF-1) (103). At first glance this may



**Figure 4: Regulation of PAI-1 expression in response to insulin, IGF-I and hypoxia.** Insulin signaling involves activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB) and mitogen-activated protein kinase (MAPK) cascades via insulin receptor substrates (IRS). The activation of the MAPK pathway and the PI3K/PKB pathway, via inhibition of glycogen synthase-3 (GSK3), stabilizes the transcription factor hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ). HIF-1 $\alpha$  then becomes transported to the nucleus where it then recruits its partner HIF-1 $\beta$ , thus forming HIF-1. HIF-1 acts primarily via its high-affinity binding site HRE2 in the human PAI-1 promoter, while the low-affinity HIF-1 but high affinity upstream stimulatory factor (USF) binding E-boxes, E4 and E5 as well

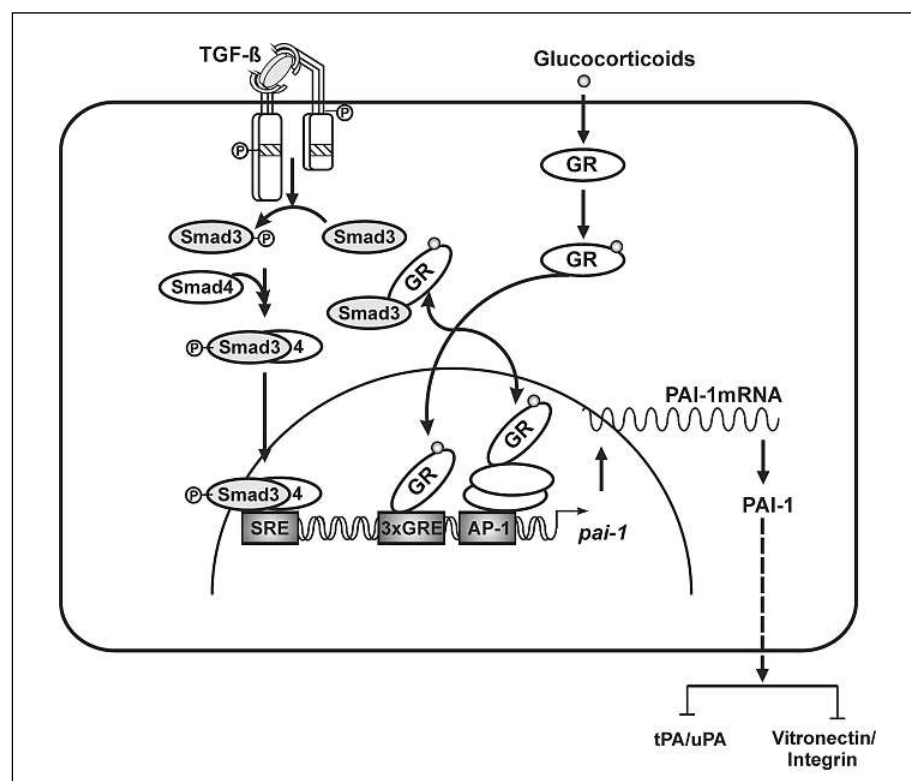
as a putative FoxO1/FKHR-like binding site affect the insulin-dependent induction. The nature of the protein binding to the FoxO1/FKHR-like binding site has not been identified yet. Under hypoxia, HIF-1 $\alpha$  becomes stabilized due to the inhibition of HIF-prolylhydroxylases (PHD) and the factor-inhibiting HIF (FIH) as well as due to the modulated formation of reactive oxygen species. Once stabilized HIF-1 $\alpha$  gets into the nucleus, forms HIF-1 and acts via the HRE2 and E4 and E5. Thereby it outcompetes USF transcription factors. In addition, hypoxia downregulates Net levels; Thereby Net binding to the Ets-binding sites (EBS) is lost, however, the role of Net in liver and for hypoxia-mediated PAI-1 gene regulation in human cells has not been determined yet.

be surprising since this factor normally induces expression of genes under hypoxic conditions by binding hypoxia responsive elements (HREs), but this activation can also occur in the presence of insulin and other growth factors (104–109). HIF-1 appears to act primarily via the HRE2 in the human PAI-1 promoter which constitutes a high-affinity HIF-1 binding site. In addition, the low-affinity HIF-1 but high affinity upstream stimulatory factor (USF) binding E-boxes, E4 and E5 (110) as well as a putative FoxO1/FKHR-like (111) binding site (-52/-45) (Fig. 4), affected the insulin-dependent induction but only under normoxia (103). This indicates that the insulin effects on PAI-1 may be partially mediated via USFs which would be in line with findings from the fatty acid synthase promoter (112, 113). Although the E4- and E5-sites are not present in the rat PAI-1 promoter (Fig. 5), the data with respect to HIF-1 are in line with the results for the rat PAI-1 promoter in primary rat hepatocytes where the insulin effects were also mediated via HIF-1 binding to the HRE (99). Although the response to insulin in HepG2 cells was mediated via the MAPK signal transduction pathway (103) and that in primary rat hepatocytes was mediated via the PI3K/PKB pathway (99), this does not necessarily need to be conflicting. This can be explained by the fact that the tumor-derived HepG2 hepatoma cells would mainly require the growth promoting actions of insulin which are mediated via the MAPK pathway while the primary rat hepatocytes would resemble the more physiological stationary hepatocyte as apparent *in vivo* where the PI3K/PKB pathway has a key role in the metabolic actions of insulin (114). Thus, the insulin-dependent PAI-1 induction may be mediated via the MAPK or the PI3K/PKB pathway depending on the cell type.

While the involvement of HIF-1 and USF transcription factors in the insulin-dependent induction of PAI-1 expression

seems to be plausible, the involvement of FoxO transcription factors would be in contrast to the so far described action of FoxOs in response to insulin. Upon insulin treatment, the FoxO proteins become phosphorylated, which results in their nuclear export and cytoplasmic retention and subsequently in inhibition of target gene expression (115–119). Thus, a mutation of the FoxO1-like binding site in the human PAI-1 promoter should reduce basal promoter activity and should lead to a loss of the inhibitory effect of insulin. However, mutation of the FoxO1-like binding site within the human PAI-1 promoter did not reduce basal promoter activity. Further, if FoxO proteins would bind to that site, insulin should have mediated an inhibition of promoter activity, but this was not the case. Instead, insulin induced promoter activity and mutation of this site abolished the insulin effect only under normoxia (103). Thus, it seems likely that this element binds another or atypical FoxO protein which has not yet been identified. In addition, the insulin effect under hypoxia was not abolished, and thus this factor may not be the major regulator of insulin-dependent PAI-1 expression.

Although the results concerning the up-regulation of PAI-1 expression by insulin in different cell culture models show similar effects, the data concerning the up-regulation of human PAI-1 by insulin *in vivo* are somewhat contradictory. Some studies *in vivo* investigating the effects of insulin infusion found no effect on the levels of PAI-1 in blood or even a decrease of PAI-1 levels and activity (120–122). On the other hand, induction of PAI-1 by insulin was found when the perfused forearm model was used to study the local effects of insulin infusion (123). Furthermore, in combination with hypertriglyceridemia and hyperglycemia, hyperinsulinemia was shown to increase PAI-1 plasma levels (80). Thus, further investigations are still necessary to elucidate the



**Figure 5: Regulation of PAI-1 expression in response to glucocorticoids.** Glucocorticoids bind to their cytoplasmic glucocorticoid receptors (GRs) and the glucocorticoid-receptor-complex is translocated to the nucleus where the activated GR can directly bind to the three glucocorticoid response elements (GREs) identified by sequence similarity or other DNA bound transcription factors. Glucocorticoids can also repress the TGF- $\beta$ -dependent human PAI-1 induction through direct binding of ligand-bound glucocorticoid receptor (GR) to Smad3 which acts in concert with Smad4. By contrast, an increase of TGF- $\beta$ -induced PAI-1 expression can be mediated via the interaction of the GR with another protein like AP-1. The AP-1 activity is also supposed to be a result of a directly TGF- $\beta$ -induced MAPK signalling. In addition, cooperation between GR and other factors like Sp1 is also possible to modulate PAI-1 expression.

complete physiological role of PAI-1 activation by insulin and its molecular mechanisms.

Similar to insulin it was found that IGF-1-dependent induction of PAI-1 gene expression occurred via a transcriptional mechanism involving HIF-1. IGF-1 enhanced HIF-1 $\alpha$  protein levels and HIF-1 DNA-binding to HRE2, and the classical E-boxes E4 and E5, and again the HRE played the major role while E4 and E5 had a supportive role. Inhibitor studies and expression of dominant-negative PDK1, dominant-negative Raf and the PKB inhibitor tribbles-3 (TRB3) then revealed that the PI3K and the MAPK (ERK) pathway but not PKB mediate the enhancement of HIF-1 $\alpha$  and PAI-1 by IGF-1 in HepG2 cells (124).

The enhancement of PAI-1 by IGF-1 may have consequences for several physiological and pathophysiological situations which require cell proliferation and angiogenesis. In conjunction with a variety of other molecules IGF-1-induced PAI-1 levels may promote vascularization of tumor tissue by contributing to prevent excessive degradation of cellular matrix which then enables endothelial cell sprouting. However, clear differences between certain types of tumors appear to exist and further knowledge of these variations may help to better understand the role of PAI-1 in these processes.

## Glucocorticoids

Glucocorticoids regulate crucial functions in hepatocytes and the genes mainly controlled by glucocorticoid receptors (GR) are involved in increasing blood glucose levels via gluconeogenesis and mobilization of amino and fatty acids. The phosphoenolpyruvate carboxykinase (PEPCK) and tyrosine aminotransferase (TAT) are two prototypic genes regulated by the GR. Once bound to their cytoplasmic GRs the glucocorticoid-receptor-complex is translocated to the nucleus via the microtubule network. Within the nucleus, the activated GR can directly bind to DNA elements termed glucocorticoid response elements (GREs) or it can indirectly bind to DNA through its interaction with other DNA bound transcription factors. The GREs usually consist of two six base pair "half-sites" separated by a three-base-pair spacer and upon binding to the GRE as a homodimer, GR serves also as a scaffold for the recruitment of coactivators, chromatin remodeling factors and other factors that modulate the activity of the transcriptional machinery (125, 126).

A number of studies have shown that glucocorticoids can enhance PAI-1 expression in different cell types, among them liver and hepatoma cells (127–129). The first incidence that the human PAI-1 promoter could be activated by glucocorticoids came from a study in which it was shown that a fragment spanning 805 nucleotides of the 5' flanking and 72 of the 5' untranslated region contained all necessary information to respond to glucocorticoids and a GRE consensus site at -800/-549/-100 could be identified by sequence similarity (130). The next identification of promoter elements involved in transcriptional regulation by glucocorticoids came from a study with HTC rat hepatoma cells. Here it was shown by electrophoretic mobility shift assays, DNase-I protection assays and mutation analysis that the region at -1212/-1196 of the rat PAI-1 promoter bound a glucocorticoid receptor which could transactivate the PAI-1 promoter (129).

In addition to the activation of transcription, GRs have also been shown to reduce transcription. This occurs via binding to DNA sequences distinct from positive GREs or the interaction with other DNA-bound transcription factors such as AP-1, NF- $\kappa$ B or Smads. All this may mainly account for the anti-inflammatory actions of glucocorticoids.

Interestingly, this mechanism seems to be important for the regulation of PAI-1 expression by other inducers like TGF- $\beta$ . Although PAI-1 is considered to be a prototypical TGF- $\beta$  induced gene, this induction may be at variance in response to glucocorticoids in the various types of liver cells. In the hepatocyte derived human tumor cell line Hep3B it was shown that the TGF- $\beta$ -dependent human PAI-1 induction can be repressed by glucocorticoids through direct binding of liganded glucocorticoid receptor (GR) to the carboxyl terminal transactivation domain of Smad3 (131, 132).

Smad3 acted in concert with Smad4 which bound via their MH1 domain to a novel TGF- $\beta$  response element (-732/-721) in the human PAI-1 gene (133). By contrast, in human keratinocytes it was shown that exposure to TGF- $\beta$  induced human PAI-1 expression via E4 -566/-559 (134) whereas in the rat PAI-1 promoter it acted via HRE2 (-165/-160) (135).

Furthermore, glucocorticoid mediated repression was enhanced upon overexpression of steroid receptor coactivator-1 (SRC-1) and GR-interacting protein-1 (GRIP-1) whereas SRC-1 and GRIP-1 in the absence of glucocorticoids alone enhanced TGF- $\beta$ -induced activation (136).

The latter effect may depend on the interaction of SRC-1 with the transcriptional co-activators p300/CBP indicating that SRC-1 may facilitate a functional link between Smad3 and p300/CBP (137). This suggests that, depending on the stimulus and the cell type, different PAI-1 promoter activation or repression models may be achieved by the GR coactivators, SRC-1 and GRIP-1. Similarly, studies from primary hepatic stellate cells and cirrhotic fat storing cells also showed that glucocorticoids decreased TGF- $\beta$ -dependent induction (138).

By contrast, in primary rat hepatocytes dexamethasone significantly enhanced TGF- $\beta$ -induced PAI-1 expression. Likewise, an increase of TGF- $\beta$ -induced PAI-1 expression through dexamethasone was also observed in HTR-8 SV neo cells (139).

These findings implicate that the enhancement of TGF- $\beta$ -mediated PAI-1 expression by dexamethasone is due to an indirect transcriptional mechanism like the interaction of the GR with another protein. It is possible that the positive cooperation between Smads and activator protein-1 (AP-1) contributes to this effect. The human and the rat PAI-1 promoter were shown to contain an AP-1 binding site (140–142); the presence of this site makes it likely that conditions leading to modified interactions between the GR and AP-1 proteins (Jun/Fos/Fra) regulate PAI-1 transcription via AP-1 complexes. The AP-1 activity is also supposed to be a result of a directly TGF- $\beta$ -induced MAPK signaling (142). In line, pharmacological inhibition of the MEK/ERK pathway by PD98059 clearly suppressed TGF-induced PAI-1 expression in primary hepatocytes (138), which points to cooperative action between TGF- $\beta$  and ERK signaling pathway. Thereby, this response seems to be mediated via ERK1 since overexpression of ERK2 in primary hepatocytes and HepG2 cells had almost no effect on PAI-1 expression (143). Further,

ERK1 seems then to act not only via AP-1 but may require also the co-activation of Sp1 which was shown to bind two Sp1 sites (Sp1a, -76/-71 and Sp1b, -46/-41) and also to be involved in the response to glucose and angiotensin II (144).

Together, the action of glucocorticoids on PAI-1 expression represents a new level of control since PAI-1 expression depends on the cell-type, the interactions between the GR with its DNA binding elements and the coactivators as well as GR interacting transcription factors.

## Cytokines

In addition to its role in metabolism, the liver is a center of defence and largely responds to injury with the so called acute phase response. Thereby the liver synthesizes and secretes a large number of proteins, the so called acute phase reactants. PAI-1 was shown to be an important component of the acute phase response in humans since its levels were increased in patients with sepsis, surgery or trauma (145, 146). By contrast, the contribution of PAI-1 to the acute phase response was questioned in rats (147). The major mediators of the AP response have been shown to be cytokines, including IL-1, IL-6 and TNF $\alpha$ .

Although IL-1 $\beta$  and TNF $\alpha$  appeared not to stimulate PAI-1 production in human primary hepatocytes (16), PAI-1 expression was strongly regulated by IL-1 and TNF $\alpha$  in HepG2 cells (148), whereas IL-6 alone had only a modest effect on PAI-1 levels (149, 150). However, IL-6 in combination with IL-1 caused a synergistic induction of PAI-1 expression (149–151). Although the cytokine-dependent regulation of PAI-1 expression occurred at transcriptional level, the data obtained for the corresponding DNA response elements seem to be different. While no STAT3 binding element participating in the IL-6 response could be mapped so far, it was shown in HepG2 cells that IL-6 increased hepatic PAI-1 expression via the -232- to -210-bp region of the promoter containing a C/EBPdelta binding site (152). One might speculate that a similar mechanism possibly applies also for other IL-6 type cytokines like oncostatin M (OSM). However, it was shown that the AP-1 element of the PAI-1 promoter mediated activation by OSM and also IL-1 in astrocytes, thus indicating that the response to OSM may be cell type-specific. Overexpression of dominant-negative STAT1, STAT3, STAT5 and an inhibitor of nuclear factor- $\kappa$ B (I $\kappa$ B) suppressed the OSM- and IL-1-induced expression of the PAI-1 reporter construct (153). This would suggest the possibility of a direct activation of PAI-1 expression by C/EBPdelta and an indirect activation of PAI-1 expression by the STAT pathway as well as an additional involvement of the NF- $\kappa$ B pathway.

The IL-1 response as well as the TNF $\alpha$  response may in principle also be mediated via the so called NF- $\kappa$ B-like sites (86). Importantly, a comprehensive analysis in a recent study identified a 5' distal TNF $\alpha$ -responsive enhancer of the human and mouse PAI-1 gene. This enhancer located 15 kb upstream of the transcription start site contains a conserved NF- $\kappa$ B-binding site (5'-TGGAATTTCT-3') at -14889/-14880 that was able to bind the NF- $\kappa$ B subunits p50 and p65 as well as mediated the response to TNF $\alpha$  (85); whether this element could mediate also the response to IL-1 was not studied. Interestingly, the fact that this newly identified TNF response element was only conserved

in human and mouse PAI-1 genes but not in the rat PAI-1 gene is in line with an earlier study showing that PAI-1 is not an acute phase reactant in rat liver (147). Interestingly, another study investigating the TNF $\alpha$  response of PAI-1 in HUVECs found that direct binding of Nur77/NAK-1 to the PAI-1 promoter was necessary to mediate the TNF $\alpha$ -induced PAI-1 expression (154). Together, these results indicate that induction of PAI-1 by cytokines may be cell-type and/or species specific and may involve different molecular mechanisms.

## Hypoxia

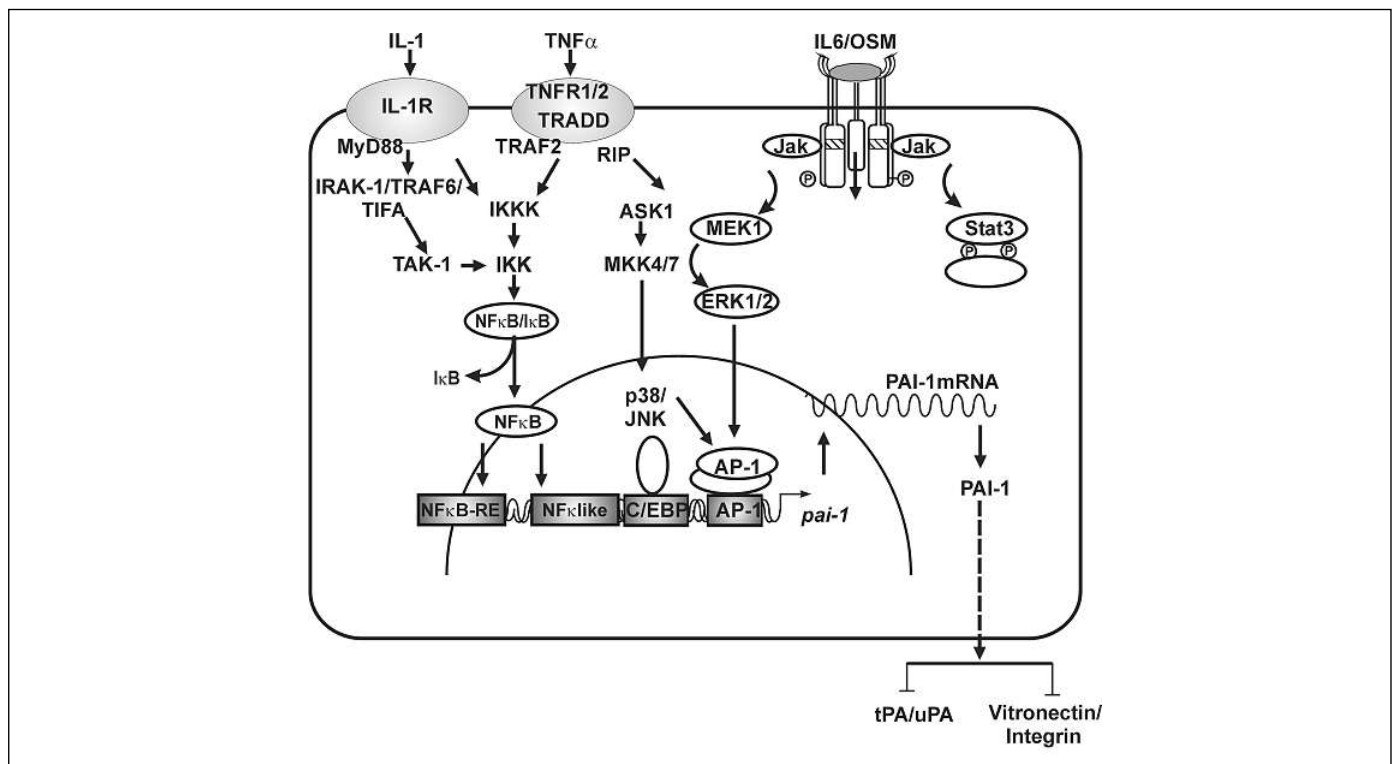
Several diseases characterized by reduced delivery of oxygen to the liver lead to perivenous hypoxia and can be associated with perivenous damage. This occurs especially during heart failure (ischemic hepatitis) (155), obstructive lung dysfunction (sleep apnea / Pickwickian syndrome) (156), gut ischemia (157, 158) or cases of drug hepatotoxicity as observed with many xenobiotics like the industrial chemical carbon tetrachloride, the pharmacological agent acetaminophen or the "cultural poison" ethanol. The toxic metabolites are formed in the liver and cause perivenous damage (159, 160) which is associated with perivenous hypoxia (161–163).

A first incidence that hypoxia induces PAI-1 expression *in vivo* came from studies with mice placed in a hypoxic environment (5–6% O<sub>2</sub>). Those mice which were exposed to hypoxia displayed enhanced PAI-1 plasma levels and PAI-1 mRNA as well as protein in the lungs when compared to the normoxic controls (164). The induction of PAI-1 expression by hypoxia was then confirmed in rat primary hepatocytes (165, 166) and in four human liver cell lines Chang, Hep3B, HuH7 and HepG2 (167). It was shown that the transcription factor responsible for the hypoxia-dependent PAI-1 activation was hypoxia-inducible factor-1 (HIF-1) acting via HREs within the rat and human PAI-1 promoter (165–167). Further, studies with the murine PAI-1 promoter have also shown that the hypoxic response is mediated via HREs [HRE-1 (-182/-178) and HRE-2 (-171/-167)]. In these studies with the macrophage-derived RAW cell line it was also shown that the hypoxia-dependent PAI-1 gene expression could be augmented by C/EBPalpha and early growth response gene-1 (EGR-1) binding the mouse PAI-1 promoter regions -209/-200 and -137/-129, respectively (168). Thus, although the involvement of HIF-1 appears to dominate the hypoxic response of the PAI-1 gene in hepatocytes and hepatoma cells, other factors like EGR-1 and C/EBPalpha may contribute to the hypoxia-dependent PAI-1 enhancement in other cell types. This appears to be even more complex since it was shown in the HTR-8/SVneo human trophoblast cell line (169) and in renal clear cell carcinoma cells (170) that in addition to HIF-1 also HIF-2 played an important and similar role in hypoxia-dependent PAI-1 expression.

The regulation of PAI-1 expression by HIFs appears not only to be important under conditions of hypoxia but also under conditions leading to the activation of HIF-1 $\alpha$  under normoxic conditions. This can be achieved by the action of hormones, growth- and coagulation factors, cytokines and conditions of mechanical-, physical- or chemical stress (for review see [171]). In liver, these events may be also of importance for situations associated with growth factors and cytokines which stimulate liver re-

generation and promote hepatoprotection. Thereby, both PKB and MAP kinases including p38 MAP kinase can contribute to the activation of the HIF pathway. While ERK-1 was also able to phosphorylate HIF-1 $\alpha$  and to promote its nuclear accumulation (172, 173), overexpression of PKB and the p38 upstream kinases MKK3 and MKK6 resulted in enhanced HIF-1 $\alpha$  levels and stimulated HIF-1-dependent PAI-1 expression (143). Although PKB induces HIF-1 $\alpha$  stabilization, HIF-1 $\alpha$  is not a direct substrate for PKB/Akt. However, inhibition and depletion of the PKB target glycogen synthase kinase-3 (GSK3) induced HIF-1 $\alpha$ , whereas overexpression reduced HIF-1 $\alpha$  levels. This regulation occurred in a VHL-independent fashion via phosphorylation of HIF-1 $\alpha$  within the oxygen-dependent degradation domain (174). Furthermore, GSK3 contributes to PAI-1 expression by phosphorylating and stabilizing Rev-erb alpha which is involved in regulating circadian gene expression. Interestingly, expression of a Rev-erb alpha variant mimicking a GSK3 phosphorylated variant negatively influenced circadian PAI-1 oscillations (175). Thus, GSK3 turned out to be a major determinant for the hypoxia-dependent and the circadian PAI-1 expression.

In addition to its prominent post-translational regulation, HIF-1 $\alpha$  can serve also as a bridge for inflammatory mediators and growth factors which transcriptionally regulate HIF-1 $\alpha$  via NF- $\kappa$ B. Interestingly, in pulmonary artery smooth muscle cells it could be shown that hypoxia activated HIF-1 $\alpha$  transcription via PI3K signaling and binding of NF- $\kappa$ B to a functional element in the HIF-1 $\alpha$  promoter (176, 177). These cell culture data were then corroborated also for the liver by an approach *in vivo* using mice lacking IKK- $\beta$ , an upstream regulator of NF- $\kappa$ B. These studies show that NF- $\kappa$ B activity is required for HIF-1 $\alpha$  protein accumulation under hypoxia in the liver and brain of hypoxic animals (178). In addition, hepatocyte growth factor (HGF) and its receptor the oncogene Met appear also to be important in PAI-1 regulation. While HGF has been shown to enhance HIF-1 $\alpha$  via NF- $\kappa$ B (179) it was also shown that hypoxia can enhance the levels of Met via HIF-1 $\alpha$  (180). When Met expressing lentiviral vectors were transduced into mice Met signalling in turn induces venous thrombosis due to enhanced PAI-1 expression and a tumorigenic process in hepatocytes (181). Noticeably, PAI-1(-/-) mice showed accelerated liver regeneration and higher levels of



**Figure 6: Regulation of PAI-1 expression in response to cytokines.** PAI-1 is an important component of the acute phase response in humans the cytokines, interleukin-1 (IL-1), IL-6 and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) induce human PAI-1 expression. IL-1 binds to its receptor and IL-1R signalling recruits specific adaptor proteins like MyD88. Binding of MyD88 allows recruitment of IL-1R associated kinase (IRAK)-1 and IRAK-4. Active IRAK-1 interacts with TRAF6 and TIFA and the IRAK-1/TRAF6/TIFA complex interacts with the kinase TAK1 and the adaptor molecules TAB1 and TAB2. After phosphorylation of TAK1 the TRAF6-TAB2/3-TAK1-TAB1 complex migrates to the cytosol. Active TAK1 then leads to the downstream activation of I $\kappa$ B kinases (IKK) and JNK or p38 MAPKs. IKKs activate NF- $\kappa$ B by phosphorylating the NF- $\kappa$ B inhibitory protein I $\kappa$ B $\alpha$ , leading to its ubiquitination and proteasome-de-

pendent degradation, whereas JNK and p38 phosphorylate several other transcription factors. The IL-1 response as well as the TNF $\alpha$  response may in principle be mediated via the NF- $\kappa$ B-like site and the NF- $\kappa$ B-binding site in the far upstream enhancer. IL-6 and OSM-induced receptor clustering activates Janus kinases, mainly Jak1 which phosphorylate tyrosines within the gp130 receptor subunit leading to recruitment of other signaling proteins with matching SH2 domains such as signal transducers and activators of transcription (STATs) or adapter proteins for the mitogen activated protein kinases Erk1/2 and p38. While no STAT3 binding element could be mapped IL-6 increased hepatic PAI-1 expression via a C/EBPdelta binding site. The AP-1 element of the PAI-1 promoter may mediate activation by OSM.

mature HGF (78). Thus, agents that modulate the activity of the NF- $\kappa$ B pathway may simultaneously contribute to the hypoxic response as well as to inflammatory processes and immunity.

Comparison between human and rat PAI-1 genes revealed strict conservation of the intron-exon structure (182, 183). In addition, two regions of the promoter showed a high degree of similarity: a 60 bp region – from –90 to the TATA box (90% identical), and the sequence located at –753 and –510 (> 80% identical) (Fig. 6) (183). Although partially conserved, a number of differences between the human, mouse and rat promoter exist and might account for some different regulatory patterns between these genes. Especially, the conservation for HIF-1 binding to the HRE was observed, whereas the USF-2-binding rHRE1 was absent from the human promoter. Instead, two classical E-boxes E4 and E5 (–566/–559 and –681/–674) were found which might function as putative USF-binding sites. Thus, due to these differences between the rat and human as well as the mouse PAI-1 promoter, it is conceivable that USF-2 might have a distinct effect on human PAI-1 gene transcription. In fact, it was shown that USF-2 could downregulate PAI-1 expression in primary rat hepatocytes via binding to the low-affinity HIF-1 but high-affinity USF-2 site HRE1 and to a lesser extent via the high-affinity HIF-1 and low-affinity USF-2 site HRE2 implicating a competition between HIF-1 and USF-2 (166). By contrast, in human HepG2 hepatoma cells USF-2 induced human PAI-1 expression. This occurred via binding of USF-2 to E4 and E5 within the promoter. In addition, the HRE contributed to the USF effect without binding it (110). These data suggested that PAI-1 expression depends on either the promoter context or USF activity which might be cell type-specific. Indeed, cotransfection of human or rat PAI-1 promoter luciferase constructs with expression vectors for wild-type USF-2 or USF-2 mutants in human HepG2 and rat H4IIE cells as well as in primary rat hepatocytes revealed that the effects of USF on PAI-1 expression depend on the cell type rather than the promoter context (110). This mode of action may be important during carcinogenesis when high levels of PAI-1 are found. This may be caused in part by defective USF proteins which can no longer downregulate PAI-1. Indeed, cell culture experiments with rat embryonic fibroblasts (REFs) showed that transfection of either USF-1 or USF-2 inhibited cellular transformation induced by c-Myc or activated Ras. In addition, USF-2 also inhibited transformation in REFs induced by the adenovirus oncoprotein E1A, while USF-1 did not, thus highlighting the broader inhibitory function of USF-2 (184). In line, many cancer cells including the prostate cancer cell line PC-3 (185, 186) displayed a loss of USF-2 transcriptional activity while it was active in non-tumorigenic cells. These findings imply that USFs and especially USF-2 may be important also as a suppressor of liver cell transformation and tumor progression.

## References

1. Loskutoff DJ, Quigley JP. PAI-1, fibrosis, and the elusive provisional fibrin matrix. *J Clin Invest* 2000; 106: 1441–1443.
2. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993; 143: 949–958.
3. Wigler M, Ford JP, Weinstein IB. Glucocorticoid inhibition of the fibrinolytic activity of tumor cells. In: *Proteases and biological control*. New York: Cold Spring Harbor, 1975: 849–856.
4. Seifert SC, Gelehrter TD. Mechanism of dexamethasone inhibition of plasminogen activator in rat hepatoma cells. *Proc Natl Acad Sci USA* 1978; 75: 6130–6133.
5. Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thromb Haemost* 2005; 93: 631–640.
6. Andreasen PA, Riccio A, Welinder KG, et al. Plasminogen activator inhibitor type-1: reactive center and amino-terminal heterogeneity determined by protein and cDNA sequencing. *FEBS Lett* 1986; 209: 213–218.

In addition to USF-2, Net which is together with Elk-1 and Sap-1 a member of the ternary complex factor family of Ets transcription factors, seems to be of importance for the regulation of PAI-1 by hypoxia and also for a number of other genes (187). In mouse embryonic fibroblasts, hypoxia downregulated Net protein levels by ubiquitination and proteasomal degradation. Thereby Net binding to the three Ets-binding sites located between –519 and –319 of the mouse PAI-1 promoter (188) was modulated and PAI-1 was downregulated. These findings seem to be very important for mouse fibroblasts and the regulation of HIF-1 and will most likely have more general impact; however, the role of Net in liver and for hypoxia-mediated PAI-1 gene regulation in human cells has not been determined yet.

## Conclusion

At the moment it appears that a number of hormonal, metabolic and environmental stimuli exert an increase in PAI-1 expression in hepatocytes and hepatocyte-derived cell lines as well as in the liver *in vivo*.

Although a number of mechanistical details appear to be valid also for the situation *in vivo*, a careful interpretation should be made from study to study and between studies with cell lines and intact animals, since the mechanisms involved in PAI-1 expression may vary depending on the species, the cell type and the animals used. Often a number of cell types do not maintain the phenotype of the parent cell-type which is part of an entire organ or tissue.

Elevated PAI-1 levels and hypofibrinolysis are common during the development of alcoholic liver disease. Further, PAI-1 plays a critical role in both acute and chronic hepatic inflammation. These findings indicate a role of PAI-1 as a useful target for therapy to halt or blunt disease progression.

It is tempting to speculate that various stimuli induce PAI-1 expression via activation of different kinase signaling pathways. The transcription factors involved in positively transferring these responses are primarily NF- $\kappa$ B, HIF-1, Sp1, AP-1 and Smad2/3. Thereby the hypoxia and PKB-MAPK signaling can merge at HIF-1 $\alpha$ . The negative regulation of PAI-1 expression can be exerted partially by GR antagonizing Smad3, USF-2, Rev-erb alpha and the new factor Net. Although quite a lot of progress has been achieved over the recent years a complete model for the regulation of PAI-1 gene expression in liver cannot be made, and numbers of issues have to be resolved in the future.

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7. Ginsburg D, Zeheb R, Yang AY, et al. cDNA cloning of human plasminogen activator-inhibitor from endothelial cells. *J Clin Invest* 1986; 78: 1673–1680.
8. Ny T, Sawdey M, Lawrence D, et al. Cloning and sequence of a cDNA coding for the human beta-migrating endothelial-cell-type plasminogen activator inhibitor. *Proc Natl Acad Sci USA* 1986; 83: 6776–6780.
9. Pannkoek H, Veerman H, Lambers H, et al. Endothelial plasminogen activator inhibitor (PAI): a new member of the Serpin gene family. *EMBO J* 1986; 5: 2539–2544.
10. Wun TC, Kretzmer KK. cDNA cloning and expression in *E. coli* of a plasminogen activator inhibitor (PAI) related to a PAI produced by Hep G2 hepatoma cell. *FEBS Lett* 1987; 210: 11–16.
11. Philips M, Juul AG, Thorsen S, et al. Immunological relationship between the fast-acting plasminogen activator inhibitors from plasma, blood platelets and endothelial cells demonstrated with a monoclonal antibody against an inhibitor from placenta. *Thromb Haemost* 1986; 55: 213–217.
12. Collen D. Report of the Meeting of the Subcommittee on Fibrinolysis, Jerusalem, Israel, June 8. *Thromb Haemost* 1986; 56: 415–416.
13. Erickson LA, Schleaf RR, Ny T, et al. The fibrinolytic system of the vascular wall. *Clin Haematol* 1985; 14: 513–530.
14. Reilly CF, McFall RC. Platelet-derived growth factor and transforming growth factor-beta regulate plasminogen activator inhibitor-1 synthesis in vascular smooth muscle cells. *J Biol Chem* 1991; 266: 9419–9427.
15. Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *J Clin Invest* 1991; 88: 1346–1353.
16. Busso N, Nicodeme E, Chesne C, et al. Urokinase and type I plasminogen activator inhibitor production by normal human hepatocytes: modulation by inflammatory agents. *Hepatology* 1994; 20: 186–190.
17. Alessi MC, Peiretti F, Morange P, et al. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 1997; 46: 860–867.
18. Seiffert D, Smith JW. The cell adhesion domain in plasma vitronectin is cryptic. *J Biol Chem* 1997; 272: 13705–13710.
19. Mimuro J, Loskutoff DJ. Purification of a protein from bovine plasma that binds to type 1 plasminogen activator inhibitor and prevents its interaction with extracellular matrix. Evidence that the protein is vitronectin. *J Biol Chem* 1989; 264: 936–939.
20. Cubellis MV, Andreassen P, Ragno P, et al. Accessibility of receptor-bound urokinase to type-1 plasminogen activator inhibitor. *Proc Natl Acad Sci USA* 1989; 86: 4828–4832.
21. 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.
22. Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thromb Haemost* 2005; 93: 631–640.
23. Juhan VI, Roul C, Alessi MC, et al. Increased plasminogen activator inhibitor activity in non insulin dependent diabetic patients--relationship with plasma insulin. *Thromb Haemost* 1989; 61: 370–373.
24. Schneider DJ, Nordt TK, Sobel BE. Attenuated fibrinolysis and accelerated atherogenesis in type II diabetic patients. *Diabetes* 1993; 42: 1–7.
25. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414: 813–820.
26. Kim JK, Gavrilova O, Chen Y, et al. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* 2000; 275: 8456–8460.
27. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. *Cell* 1996; 87: 377–389.
28. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B et al. Life without white fat: a transgenic mouse. *Genes Dev* 1998; 12: 3168–3181.
29. Eddy AA. Plasminogen activator inhibitor-1 and the kidney. *Am J Physiol Renal Physiol* 2002; 283: F209–F220.
30. Lagoa CE, Vodovotz Y, Stolz DB, et al. The role of hepatic type 1 plasminogen activator inhibitor (PAI-1) during murine hemorrhagic shock. *Hepatology* 2005; 42: 390–399.
31. Wang H, Vohra BP, Zhang Y, et al. Transcriptional profiling after bile duct ligation identifies PAI-1 as a contributor to cholestatic injury in mice. *Hepatology* 2005; 42: 1099–1108.
32. Bergheim I, Guo L, Davis MA, et al. Critical role of plasminogen activator inhibitor-1 in cholestatic liver injury and fibrosis. *J Pharmacol Exp Ther* 2006; 316: 592–600.
33. Reilly TP, Bourdi M, Brady JN, et al. Expression profiling of acetaminophen liver toxicity in mice using microarray technology. *Biochem Biophys Res Commun* 2001; 282: 321–328.
34. Ganey PE, Luyendyk JP, Newport SW, et al. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. *Hepatology* 2007; 46: 1177–1186.
35. Bergheim I, Guo L, Davis MA, et al. Metformin prevents alcohol-induced liver injury in the mouse: Critical role of plasminogen activator inhibitor-1. *Gastroenterology* 2006; 130: 2099–2112.
36. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* 2001; 414: 821–827.
37. Jungermann K, Kietzmann T. Zonation of parenchymal and nonparenchymal metabolism in liver. *Annu Rev Nutr* 1996; 16: 179–203.
38. Jungermann K, Kietzmann T. Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* 2000; 31: 255–260.
39. Kietzmann T. Oxygen-dependent regulation of hepatic glucose metabolism. *Methods Enzymol* 2004; 381: 357–376.
40. Kuster J, Beuers U, Jungermann K. Modulation of the sympathetic nerve action on carbohydrate and ketone body metabolism by fatty acids, glucagon and insulin in perfused rat liver. *Biol Chem Hoppe Seyler* 1989; 370: 1035–1044.
41. Jungermann K. Role of intralobular compartmentation in hepatic metabolism. *Diabetes Metab* 1992; 18: 81–86.
42. Jelinek LJ, Lok S, Rosenberg GB, et al. Expression cloning and signaling properties of the rat glucagon receptor. *Science* 1993; 259: 1614–1616.
43. Mayo KE, Miller LJ, Bataille D, et al. International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 2003; 55: 167–194.
44. Lee CQ, Yun YD, Hoeffler JP, et al. Cyclic-AMP-responsive transcriptional activation of CREB-327 involves interdependent phosphorylated subdomains. *EMBO J* 1990; 9: 4455–4465.
45. Gonzalez GA, Montminy MR. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 1989; 59: 675–680.
46. Montminy M, Koo SH, Zhang X. The CREB family: key regulators of hepatic metabolism. *Ann Endocrinol (Paris)* 2004; 65: 73–75.
47. Heaton JH, Nebes VL, O'Dell LG, et al. Glucocorticoid and cyclic nucleotide regulation of plasminogen activator and plasminogen activator-inhibitor gene expression in primary cultures of rat hepatocytes. *Mol Endocrinol* 1989; 3: 185–192.
48. 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.
49. Heaton JH, Gelehrter TD. Cyclic nucleotide regulation of plasminogen activator and plasminogen activator-inhibitor messenger RNAs in rat hepatoma cells. *Mol Endocrinol* 1990; 4: 171–178.
50. Heaton JH, Tillmann BM, Leff NS, et al. Cyclic nucleotide regulation of type-1 plasminogen activator-inhibitor mRNA stability in rat hepatoma cells. Identification of cis-acting sequences. *J Biol Chem* 1998; 273: 14261–14268.
51. Tillmann-Bogush M, Heaton JH, Gelehrter TD. Cyclic nucleotide regulation of PAI-1 mRNA stability. Identification of cytosolic proteins that interact with an a-rich sequence. *J Biol Chem* 1999; 274: 1172–1179.
52. Heaton JH, Dlakic WM, Dlakic M, et al. Identification and cDNA cloning of a novel RNA-binding protein that interacts with the cyclic nucleotide-responsive sequence in the Type-1 plasminogen activator inhibitor mRNA. *J Biol Chem* 2001; 276: 3341–3347.
53. Thoresen GH, Sand TE, Refsnes M, et al. Dual effects of glucagon and cyclic AMP on DNA synthesis in cultured rat hepatocytes: stimulatory regulation in early G1 and inhibition shortly before the S phase entry. *J Cell Physiol* 1990; 144: 523–530.
54. Diehl AM, Yang SQ, Wolfgang D, et al. Differential expression of guanine nucleotide-binding proteins enhances cAMP synthesis in regenerating rat liver. *J Clin Invest* 1992; 89: 1706–1712.
55. Uno S, Nakamura M, Seki T, et al. Induction of tissue-type plasminogen activator (tPA) and type-1 plasminogen activator inhibitor (PAI-1) as early growth responses in rat hepatocytes in primary culture. *Biochem Biophys Res Commun* 1997; 239: 123–128.
56. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993; 143: 949–958.
57. Yee JA, Yan L, Dominguez JC, et al. Plasminogen-dependent activation of latent transforming growth factor beta (TGF beta) by growing cultures of osteoblast-like cells. *J Cell Physiol* 1993; 157: 528–534.
58. Fanelli CG, Porcellati F, Rossetti P, et al. Glucagon: the effects of its excess and deficiency on insulin action. *Nutr Metab Cardiovasc Dis* 2006; 16 (Suppl 1): S28–S34.
59. Grant MB, Ellis EA, Caballero S, et al. Plasminogen activator inhibitor-1 overexpression in non-proliferative diabetic retinopathy. *Exp Eye Res* 1996; 63: 233–244.
60. Minneman KP, Esbenshade TA. Alpha 1-adrenergic receptor subtypes. *Annu Rev Pharmacol Toxicol* 1994; 34: 117–133.
61. Bylund DB, Regan JW, Faber JE, et al. Vascular alpha-adrenoceptors: from the gene to the human. *Can J Physiol Pharmacol* 1995; 73: 533–543.
62. Hieble JP, Bondinell WE, Ruffolo RR, Jr. Alpha- and beta-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. *J Med Chem* 1995; 38: 3415–3444.
63. Xing M, Post S, Ostrom RS, et al. Inhibition of phospholipase A2-mediated arachidonic acid release by cyclic AMP defines a negative feedback loop for P2Y receptor activation in Madin-Darby canine kidney D1 cells. *J Biol Chem* 1999; 274: 10035–10038.
64. Ruan Y, Kan H, Parmentier JH, et al. Alpha-1A adrenergic receptor stimulation with phenylephrine promotes arachidonic acid release by activation of

- phospholipase D in rat-1 fibroblasts: inhibition by protein kinase A. *J Pharmacol Exp Ther* 1998; 284: 576–585.
65. Williams NG, Zhong H, Minneman KP. Differential coupling of  $\alpha$ 1-,  $\alpha$ 2-, and  $\beta$ -adrenergic receptors to mitogen-activated protein kinase pathways and differentiation in transfected PC12 cells. *J Biol Chem* 1998; 273: 24624–24632.
66. Xiao L, Pimentel DR, Wang J, et al. Role of reactive oxygen species and NAD(P)H oxidase in  $\alpha$ (1)-adrenoceptor signaling in adult rat cardiac myocytes. *Am J Physiol Cell Physiol* 2002; 282: C926–C934.
67. Brown NJ, Bradford J, Wang Z, et al. Modulation of angiotensin II and norepinephrine-induced plasminogen activator inhibitor-1 expression by AT1a receptor deficiency. *Kidney Int* 2007; 72: 72–81.
68. Garcia-Caballero A, Olivares-Reyes JA, Catt KJ, et al. Angiotensin AT(1) receptor phosphorylation and desensitization in a hepatic cell line. Roles of protein kinase c and phosphoinositide 3-kinase. *Mol Pharmacol* 2001; 59: 576–585.
69. Vaughan DE. Angiotensin and vascular fibrinolytic balance. *Am J Hypertens* 2002; 15: 3S–8S.
70. Wilms H, Rosenstiel P, Unger T, et al. Neuroprotection with angiotensin receptor antagonists: a review of the evidence and potential mechanisms. *Am J Cardiovasc Drugs* 2005; 5: 245–253.
71. Rockey DC. Vascular mediators in the injured liver. *Hepatology* 2003; 37: 4–12.
72. Garcia-Sainz JA, Macias-Silva M. Angiotensin II stimulates phosphoinositide turnover and phosphorylase through AII-1 receptors in isolated rat hepatocytes. *Biochem Biophys Res Commun* 1990; 172: 780–785.
73. Nakamura S, Nakamura I, Ma L, et al. Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo. *Kidney Int* 2000; 58: 251–259.
74. 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.
75. Chen HC, Feener EP. MEK1,2 response element mediates angiotensin II-stimulated plasminogen activator inhibitor-1 promoter activation. *Blood* 2004; 103: 2636–2644.
76. Naldini L, Tamagnone L, Vigna E, et al. Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. *EMBO J* 1992; 11: 4825–4833.
77. Bueno M, Salgado S, Beas-Zarate C, et al. Urokinase-type plasminogen activator gene therapy in liver cirrhosis is mediated by collagens gene expression down-regulation and up-regulation of MMPs, HGF and VEGF. *J Gene Med* 2006; 8: 1291–1299.
78. Shimizu M, Hara A, Okuno M, et al. Mechanism of retarded liver regeneration in plasminogen activator-deficient mice: Impaired activation of hepatocyte growth factor after Fas-mediated massive hepatic apoptosis. *Hepatology* 2001; 33: 569–576.
79. Bataller R, Sancho-Bru P, Gines P, et al. Liver fibrogenesis: a new role for the renin-angiotensin system. *Antioxid Redox Signal* 2005; 7: 1346–1355.
80. Calles EJ, Mirza SA, Sobel BE, et al. Induction of hyperinsulinemia combined with hyperglycemia and hypertriglyceridemia increases plasminogen activator inhibitor 1 in blood in normal human subjects. *Diabetes* 1998; 47: 290–293.
81. Iwasaki Y, Kambayashi M, Asai M, et al. High glucose alone, as well as in combination with proinflammatory cytokines, stimulates nuclear factor  $\kappa$ B-mediated transcription in hepatocytes in vitro. *J Diabetes Complications* 2007; 21: 56–62.
82. Li NX, Karin M. Is NF- $\kappa$ B the sensor of oxidative stress? *FASEB J* 1999; 13: 1137–1143.
83. Bowie AG, O'Neill LAJ. Vitamin C inhibits NF- $\kappa$ B activation by TNF via the activation of p38 mitogen-activated protein kinase. *Journal of Immunology* 2000; 165: 7180–7188.
84. Hayakawa M, Miyashita H, Sakamoto I, et al. Evidence that reactive oxygen species do not mediate NF- $\kappa$ B activation. *EMBO J* 2003; 22: 3356–3366.
85. Hou B, Eren M, Painter CA, et al. Tumor necrosis factor  $\alpha$  activates the human plasminogen activator inhibitor-1 gene through a distal nuclear factor  $\kappa$ B site. *J Biol Chem* 2004; 279: 18127–18136.
86. Dawson SJ, Wiman B, Hamsten A, et al. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem* 1993; 268: 10739–10745.
87. Chen YQ, Su M, Walia RR, et al. Sp1 sites mediate activation of the plasminogen activator inhibitor-1 promoter by glucose in vascular smooth muscle cells. *J Biol Chem* 1998; 273: 8225–8231.
88. 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 USA* 2000; 97: 12222–12226.
89. Ahn JD, Morishita R, Kaneda Y, et al. Transcription factor decoy for activator protein-1 (AP-1) inhibits high glucose- and angiotensin II-induced type 1 plasminogen activator inhibitor (PAI-1) gene expression in cultured human vascular smooth muscle cells. *Diabetologia* 2001; 44: 713–720.
90. Suzuki M, Akimoto K, Hattori Y. Glucose upregulates plasminogen activator inhibitor-1 gene expression in vascular smooth muscle cells. *Life Sci* 2002; 72: 59–66.
91. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001; 414: 799–806.
92. Lietzke SE, Bose S, Cronin T, et al. Structural basis of 3-phosphoinositide recognition by pleckstrin homology domains. *Mol Cell* 2000; 6: 385–394.
93. Alessi DR, James SR, Downes CP, et al. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B $\alpha$ . *Curr Biol* 1997; 7: 261–269.
94. Alexander-Bridges M, Mukhopadhyay NK, Jhala U, et al. Growth factor-activated kinases phosphorylate IRE-ABP. *Biochem Soc Trans* 1992; 20: 691–693.
95. O'Brien RM, Granner DK. Regulation of gene expression by insulin. *Biochem J* 1991; 278: 609–619.
96. O'Brien RM, Bonovich MT, Forest CD, et al. Signal transduction convergence: phorbol esters and insulin inhibit phosphoenolpyruvate carboxykinase gene transcription through the same 10-base-pair sequence. *Proc Natl Acad Sci USA* 1991; 88: 6580–6584.
97. O'Brien RM, Streeper RS, Ayala JE, et al. Insulin-regulated gene expression. *Biochem Soc Trans* 2001; 29: 552–558.
98. Schneider DJ, Sobel BE. Augmentation of synthesis of plasminogen activator inhibitor type 1 by insulin and insulin-like growth factor type 1: implications for vascular disease in hyperinsulinemic states [published erratum appears in *Proc Natl Acad Sci USA* 1992; 89: 1148]. *Proc Natl Acad Sci USA* 1991; 88: 9959–9963.
99. 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.
100. Li XN, Grenett HE, Benza RL, et al. Genotype-specific transcriptional regulation of PAI-1 expression by hypertriglyceridemic VLDL and Lp(a) in cultured human endothelial cells. *Arterioscler Thromb Vasc Biol* 1997; 17: 3215–3223.
101. Grenett HE, Benza RL, Li XN, et al. Expression of plasminogen activator inhibitor type I in genotyped human endothelial cell cultures: genotype-specific regulation by insulin. *Thromb Haemost* 1999; 82: 1504–1509.
102. Banfi C, Eriksson P, Giandomenico G, et al. Transcriptional regulation of plasminogen activator inhibitor type 1 gene by insulin: insights into the signaling pathway. *Diabetes* 2001; 50: 1522–1530.
103. 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 NY Acad Sci* 2006; 1090: 355–367.
104. Zelzer E, Levy Y, Kahana C, et al. Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1 $\alpha$ /ARNT. *EMBO J* 1998; 17: 5085–5094.
105. Jiang BH, Jiang G, Zheng JZ, et al. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. *Cell Growth Differ* 2001; 12: 363–369.
106. Stiehl DP, Jekmann W, Wenger RH, et al. Normoxic induction of the hypoxia-inducible factor 1 $\alpha$  by insulin and interleukin-1 $\beta$  involves the phosphatidylinositol 3-kinase pathway. *FEBS Lett* 2002; 512: 157–162.
107. Treins C, Giorgetti-Peraldi S, Murdaca J, et al. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. *J Biol Chem* 2002; 277: 27975–27981.
108. Richard DE, Berra E, Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1 $\alpha$  in vascular smooth muscle cells. *J Biol Chem* 2000; 275: 26765–26771.
109. Grolach A, Diebold I, Schini-Kerth VB, et al. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. *Circ Res* 2001; 89: 47–54.
110. Dimova EY, Kietzmann T. Cell type-dependent regulation of the hypoxia-responsive plasminogen activator inhibitor-1 gene by upstream stimulatory factor-2. *J Biol Chem* 2006; 281: 2999–3005.
111. Vulin AI, Stanley FM. A Forkhead/winged helix-related transcription factor mediates insulin-increased plasminogen activator inhibitor-1 gene transcription. *J Biol Chem* 2002; 277: 20169–20176.
112. Wang D, Sul HS. Upstream stimulatory factors bind to insulin response sequence of the fatty acid synthase promoter. USF1 is regulated. *J Biol Chem* 1995; 270: 28716–28722.
113. Wang D, Sul HS. Upstream stimulatory factor binding to the E-box at –65 is required for insulin regulation of the fatty acid synthase promoter. *J Biol Chem* 1997; 272: 26367–26374.
114. Shepherd PR, Nave BT, Rincon J, et al. Involvement of phosphoinositide 3-kinase in insulin stimulation of MAP-kinase and phosphorylation of protein kinase-B in human skeletal muscle: implications for glucose metabolism. *Diabetologia* 1997; 40: 1172–1177.
115. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 1999; 96: 857–868.
116. Kops GJ, de Ruiter ND, Vries-Smits AM, et al. Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 1999; 398: 630–634.
117. Rena G, Guo S, Cichy SC, et al. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J Biol Chem* 1999; 274: 17179–17183.

118. Biggs WH, III, Meisenhelder J, Hunter T, et al. Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* 1999; 96: 7421–7426.
119. Takaishi H, Konishi H, Matsuzaki H, et al. Regulation of nuclear translocation of forkhead transcription factor AFX by protein kinase B. *Proc Natl Acad Sci USA* 1999; 96: 11836–11841.
120. Grant PJ, Kruihof EK, Felley CP, et al. Short-term infusions of insulin, triacylglycerol and glucose do not cause acute increases in plasminogen activator inhibitor-1 concentrations in man. *Clin Sci (Lond)* 1990; 79: 513–516.
121. Landin K, Tengborn L, Chmielewska J, et al. The acute effect of insulin on tissue plasminogen activator and plasminogen activator inhibitor in man. *Thromb Haemost* 1991; 65: 130–133.
122. Vuorinen-Markkola H, Puhakainen I, Yki-Jarvinen H. No evidence for short-term regulation of plasminogen activator inhibitor activity by insulin in man. *Thromb Haemost* 1992; 67: 117–120.
123. Carmassi F, Morale M, Ferrini L, et al. Local insulin infusion stimulates expression of plasminogen activator inhibitor-1 and tissue-type plasminogen activator in normal subjects. *Am J Med* 1999; 107: 344–350.
124. 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.
125. Dostert A, Heinzel T. Negative glucocorticoid receptor response elements and their role in glucocorticoid action. *Curr Pharm Des* 2004; 10: 2807–2816.
126. Gottlicher M, Heck S, Herrlich P. Transcriptional cross-talk, the second mode of steroid hormone receptor action. *J Mol Med* 1998; 76: 480–489.
127. Coombs RJ, Jenkins N. Characterization of a plasminogen activator inhibitor induced by glucocorticoids in immature bovine Sertoli cell-enriched cultures. *J Endocrinol* 1988; 117: 69–74.
128. Loskutoff DJ, Roegner K, Erickson LA, et al. The dexamethasone-induced inhibitor of plasminogen activator in hepatoma cells is antigenically-related to an inhibitor produced by bovine aortic endothelial cells. *Thromb Haemost* 1986; 55: 8–11.
129. Brudzinski CJ, Johnson MR, Goble CA, et al. Mechanism of glucocorticoid induction of the rat plasminogen activator inhibitor-1 gene in HTC rat hepatoma cells: identification of cis-acting regulatory elements. *Mol Endocrinol* 1993; 7: 1169–1177.
130. Riccio A, Lund LR, Sartorio R, et al. The regulatory region of the human plasminogen activator inhibitor type-1 (PAI-1) gene. *Nucleic Acids Res* 1988; 16: 2805–2824.
131. 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.
132. Li G, Wang S, Gelehrter TD. Identification of glucocorticoid receptor domains involved in transrepression of transforming growth factor-beta action. *J Biol Chem* 2003; 278: 41779–41788.
133. 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.
134. Allen RR, Qi L, Higgins PJ. Upstream stimulatory factor regulates E box-dependent PAI-1 transcription in human epidermal keratinocytes. *J Cell Physiol* 2005; 203: 156–165.
135. Kutz SM, Higgins CE, Samarakoon R, et al. TGF-beta 1-induced PAI-1 expression is E box/USF-dependent and requires EGFR signaling. *Exp Cell Res* 2006; 312: 1093–1105.
136. 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.
137. Dennler S, Pendaries V, Tacheau C, et al. The steroid receptor co-activator-1 (SRC-1) potentiates TGF-beta/Smad signaling: role of p300/CBP. *Oncogene* 2005; 24: 1936–1945.
138. Wickert L, Chatain N, Kruschinsky K, et al. Glucocorticoids activate TGF-beta induced PAI-1 and CTGF expression in rat hepatocytes. *Comp Hepatol* 2007; 6: 5.
139. 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.
140. 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.
141. 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.
142. Guo B, Inoki K, Isono M, et al. MAPK/AP-1-dependent regulation of PAI-1 gene expression by TGF-beta in rat mesangial cells. *Kidney Int* 2005; 68: 972–984.
143. Kietzmann T, Jungermann K, Gorchak A. Regulation of the hypoxia-dependent plasminogen activator inhibitor 1 expression by MAP kinases. *Thromb Haemost* 2003; 89: 666–673.
144. Chen YQ, Su M, Walia RR, et al. Sp1 sites mediate activation of the plasminogen activator inhibitor-1 promoter by glucose in vascular smooth muscle cells. *J Biol Chem* 1998; 273: 8225–8231.
145. Leithauser B, Matthias FR, Nicolai U, et al. Hemostatic abnormalities and the severity of illness in patients at the onset of clinically defined sepsis. Possible indication of the degree of endothelial cell activation? *Intensive Care Med* 1996; 22: 631–636.
146. Woodhouse PR, Meade TW, Khaw KT. Plasminogen activator inhibitor-1, the acute phase response and vitamin C. *Atherosclerosis* 1997; 133: 71–76.
147. Podor TJ, Hirsh J, Gelehrter TD, et al. Type 1 plasminogen activator inhibitor is not an acute phase reactant in rats. Lack of IL-6- and hepatocyte-dependent synthesis. *J Immunol* 1993; 150: 225–235.
148. de Boer JP, Abbink JJ, Brouwer MC, et al. PAI-1 synthesis in the human hepatoma cell line HepG2 is increased by cytokines—evidence that the liver contributes to acute phase behaviour of PAI-1. *Thromb Haemost* 1991; 65: 181–185.
149. 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.
150. Seki T, Gelehrter TD. Interleukin-1 induction of type-1 plasminogen activator inhibitor (PAI-1) gene expression in the mouse hepatocyte line, AML 12. *J Cell Physiol* 1996; 168: 648–656.
151. Seki T, Healy AM, Fletcher DS, et al. IL-1beta mediates induction of hepatic type 1 plasminogen activator inhibitor in response to local tissue injury. *Am J Physiol* 1999; 277: G801–G809.
152. Dong J, Fujii S, Li H, et al. Interleukin-6 and mevastatin regulate plasminogen activator inhibitor-1 through CCAAT/enhancer-binding protein-delta. *Arterioscler Thromb Vasc Biol* 2005; 25: 1078–1084.
153. 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.
154. Gruber F, Hufnagl P, Hofer-Warbinek R, et al. Direct binding of Nur77/NAK-1 to the plasminogen activator inhibitor 1 (PAI-1) promoter regulates TNF alpha-induced PAI-1 expression. *Blood* 2003; 101: 3042–3048.
155. Mastrapasqua G, Scapinello A, Madia D, et al. Ischemic hepatitis. Description of 4 cases and review of the literature. *Minerva-Gastroenterol-Dietol* 1993; 39: 93–97.
156. Mathurin P, Durand F, Ganne N, et al. Ischemic hepatitis due to obstructive sleep apnea. *Gastroenterology* 1995; 109: 1682–1684.
157. Horie Y, Wolf R, Anderson DC, et al. Hepatic leukostasis and hypoxic stress in adhesion molecule-deficient mice after gut ischemia/reperfusion. *J Clin Invest* 1997; 99: 781–788.
158. Horie Y, Wolf R, Miyasaka M, et al. Leukocyte adhesion and hepatic microvascular responses to intestinal ischemia/reperfusion in rats. *Gastroenterology* 1996; 111: 666–673.
159. Zimmerman HJ. *Hepatotoxicity*. New York: Appleton-Century-Crafts, 1978.
160. Israel Y, Orrego H. Hepatocyte demand and substrate supply as factors in the susceptibility to alcoholic liver injury: pathogenesis and prevention. *Clin Gastroenterol* 1981; 10: 355–373.
161. Lieber CS. Biochemical factors in alcoholic liver disease. *Semin Liver Dis* 1993; 13: 136–153.
162. Jauhanon P, Baraona E, Miyakawa H, et al. Mechanism for selective perivenular hepatotoxicity of ethanol. *Alcohol Clin Exp Res* 1982; 6: 350–357.
163. Baraona E, Jauhanon P, Miyakawa H, et al. Zonal redox changes as a cause of selective perivenular hepatotoxicity of alcohol. *Pharmacol Biochem Behav* 1983; 18 (Suppl 1): 449–454.
164. Pinsky DJ, Liao H, Lawson CA, et al. Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. *J Clin Invest* 1998; 102: 919–928.
165. 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.
166. 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.
167. Fink T, Ebbesen P, Zachar V. Quantitative gene expression profiles of human liver-derived cell lines exposed to moderate hypoxia. *Cell Physiol Biochem* 2001; 11: 105–114.
168. Liao H, Hyman MC, Lawrence DA, et al. Molecular regulation of the PAI-1 gene by hypoxia: contributions of Egr-1, HIF-1alpha, and C/EBPalpha. *FASEB J* 2007; 21: 935–949.
169. Meade ES, Ma YY, Guller S. Role of hypoxia-inducible transcription factors 1alpha and 2alpha in the regulation of plasminogen activator inhibitor-1 expression in a human trophoblast cell line. *Placenta* 2007; 28: 1012–1019.
170. Carroll VA, Ashcroft M. Role of hypoxia-inducible factor (HIF)-1alpha versus HIF-2alpha in the regulation of HIF target genes in response to hypoxia,

insulin-like growth factor-I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. *Cancer Res* 2006; 66: 6264–6270.

171. Kietzmann T, Gorchach A. Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. *Semin Cell Dev Biol* 2005; 16: 474–486.

172. Richard DE, Berra E, Gothie E, et al. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. *J Biol Chem* 1999; 274: 32631–32637.

173. Mylonis I, Chachami G, Samiotaki M, et al. Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. *J Biol Chem* 2006; 281: 33095–33106.

174. Flugel D, Gorchach A, Michiels C, et al. Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. *Mol Cell Biol* 2007; 27: 3253–3265.

175. 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.

176. BelAiba RS, Bonello S, Zahringer C, et al. Hypoxia up-regulates hypoxia-inducible factor-1{alpha}

transcription by involving phosphatidylinositol 3-kinase and nuclear factor {kappa}B in pulmonary artery smooth muscle cells. *Mol Biol Cell* 2007; 18: 4691–4697.

177. Bonello S, Zahringer C, BelAiba RS, et al. Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. *Arterioscler Thromb Vasc Biol* 2007; 27: 755–761.

178. Rius J, Guma M, Schachtrup C, et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature* 2008; 453: 807–811.

179. Tacchini L, De Ponti C, Matteucci E, et al. Hepatocyte growth factor-activated NF-kappaB regulates HIF-1 activity and ODC expression, implicated in survival, differently in different carcinoma cell lines. *Carcinogenesis* 2004; 25: 2089–2100.

180. Eckerich C, Zapf S, Fillbrandt R, et al. Hypoxia can induce c-Met expression in glioma cells and enhance SF/HGF-induced cell migration. *Int J Cancer* 2007; 121: 276–283.

181. Boccaccio C, Sabatino G, Medico E, et al. The MET oncogene drives a genetic programme linking cancer to haemostasis. *Nature* 2005; 434: 396–400.

182. Bosma PJ, van-den BE, Kooistra T, et al. Human plasminogen activator inhibitor-1 gene. Promoter and

structural gene nucleotide sequences. *J Biol Chem* 1988; 263: 9129–9141.

183. Bruzdinski CJ, Riordan JM, Nordby EC, et al. Isolation and characterization of the rat plasminogen activator inhibitor-1 gene. *J Biol Chem* 1990; 265: 2078–2085.

184. Luo X, Sawadogo M. Antiproliferative properties of the USF family of helix-loop-helix transcription factors. *Proc Natl Acad Sci USA* 1996; 93: 1308–1313.

185. Ismail PM, Lu T, Sawadogo M. Loss of USF transcriptional activity in breast cancer cell lines. *Oncogene* 1999; 18: 5582–5591.

186. Qyang Y, Luo X, Lu T, et al. Cell-type-dependent activity of the ubiquitous transcription factor USF in cellular proliferation and transcriptional activation. *Mol Cell Biol* 1999; 19: 1508–1517.

187. Gross C, Dubois-Pot H, Wasylyk B. The ternary complex factor Net/Elk-3 participates in the transcriptional response to hypoxia and regulates HIF-1 alpha. *Oncogene* 2008; 27: 1333–1341.

188. Buchwalter G, Gross C, Wasylyk B. The ternary complex factor Net regulates cell migration through inhibition of PAI-1 expression. *Mol Cell Biol* 2005; 25: 10853–10862.