Our previous studies showed that thymosin \( \beta 4 \) (T\( \beta 4 \)) promotes skin and corneal wound healing through its effects on cell migration, angiogenesis and possibly cell survival (1–3). However, the precise molecular mechanism through which it functions and its potential role in solid organ wound healing remains unknown. In our studies of the biological activity of T\( \beta 4 \) we found that this 43-amino acid peptide strongly activates endothelial cells to express and release plasminogen activator inhibitor type 1 (PAI-1). It occurs via the mechanism involving the signaling pathway leading to activation of the MAPK cascade and enhanced c-Fos/c-Jun DNA binding activity (4). Such a role of AP-1 in activation of transcriptional events by T\( \beta 4 \) was confirmed by independent studies (5). Recently, T\( \beta 4 \) was reported to stimulate migration of cardiomyocytes and endothelial cells and promote survival of cardiomyocytes by formation of a functional complex with PINCH and integrin-linked kinase, resulting in activation of the survival kinase Akt (6). These observations revealed that T\( \beta 4 \) affects cellular functions by activation of several signaling pathways and induces changes in the cell properties necessary for both migration and survival. Here, we show that T\( \beta 4 \) up-regulates expression and induces the surface accumulation of \( \alpha 1 \)-acid glycoprotein (AGP) known to bind PAI-1 and prolong its inhibitory activity (7). AGP is expressed by activated endothelial cells and exerts its effect by interacting with components of the endothelial glycocalyx (8). In this study, we identify a previously unrecognized function of AGP, a direct and primary role in plasminogen activation. We provide evidence that the AGP-PAI-1 complex is assembled on the cellular surface, and formation of such a complex inhibits generation of plasmin. Interestingly all major components tested in these studies, namely PAI-1, AGP, and T\( \beta 4 \) are up-regulated during inflammation. Therefore, the present results may indicate that a normally endogenous protein such as T\( \beta 4 \), expressed in all types of cells, may be re-deployed to protect endothelium, for example, in the setting of acute inflammation events.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Human PAI-1, \( \alpha 1 \)-acid glycoprotein, Glu-plasminogen, and plasminogen-free fibrinogen were purchased from Calbiochem-Novabiochem Co. The stable PAI-1 mutant (14-1B) previously characterized by Berkenpas et al. (9) was purchased from Calbiochem-Novabiochem Co. Recombinant PAI-1, active and substrate forms, and MA-5F4C12 were kindly donated by Dr. P. J. Declerck (Louvain, Belgium). Antibodies to AGP, mouse monoclonal antibody AAG2, and rabbit polyclonal antibodies were purchased from ICN Biomedicals, Inc. (Aurora, OH). Monoclonal antibody to uPAR (#3936) was from American Diagnostica. Mouse monoclonal antibodies to PAI-1, MAI-12, were purchased from Biopool (Umea, Sweden). Anti-rabbit IgG horseradish peroxidase (HRP) conjugate, 5-bromo-4-chloro-3-indolyl-\( \beta \)-d-galactopyranoside, and Wizard Miniprep and Maxiprep kits for isolation of plasmid DNA were purchased from Promega Corp. (Madison, WI). All standard tissue culture reagents including DMEM, fetal bovine serum (FBS), and Lipofectamine Plus reagent were from Invitrogen (Eggenstein, Germany).

**Cell Cultures—**Human umbilical vein endothelial cells (HUVECs) were isolated from freshly collected umbilical cords by collagenase treatment (10), and cell cultures were maintained as described previously (11). For the experiments, confluent cultures were used at the third passage. Human endothelial cell line EA.hy926, derived by fusion of human umbilical vein endothelial cells with continuous human lung carcinoma cell line A549 (12) was obtained as a gift from Professor Cora-Jean S. Edgell (Pathology Department, University of North Carolina at Chapel Hill). This endothelial hybrid cell line is presently the best characterized macrovascular EC line (for review, see Bouis et al. (13)).
The cells were cultured in DMEM with high glucose, supplemented with 10% FBS, hypoxanthine/aminopterin/thymidine (100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine), and antibiotics in a 90–95% humidified atmosphere of 5% CO2 at 37 °C. Monocytic cells (U937) cells were from American Type Culture Collection (Rockville, MD) and cultured as described by the supplier.

**Measurements of PAI-1 and AGP mRNA**—For this purpose, EA.hy926 cells were stimulated for 4 and 24 h with different concentrations of Tβ4 (0–160 nM), then total cellular RNA was extracted by the TRIzol reagent method (Invitrogen) using a single-step purification protocol (14). RNA pellets were dissolved in water, and their concentrations and purity were determined by spectrophotometer readings at 260 and 280 nm. The relative amounts of specific mRNAs were quantified by RT PCR as described previously (5). The following primers, 5′-CTGCGCAACCTGCTGAAACA-3′ and 5′-GAGCATTTCGGATCCTTGCTGATC-3′, specific for mRNA of PAI-1 and AGP, respectively, were used. In the same samples, β-actin mRNA was amplified with primers 5′-GTGGGCGCCCAGGCACCA-3′ and 5′-CTCCTTAATGTCACGATTCCC-3′, used as an intrinsic control of mRNA quantity PCR amplification. The final products were separated by electrophoresis in 7% polyacrylamide gels in TAE buffer (40 mM Tris acetate, 2 mM EDTA, pH 8.0) using the genetic size marker 100 bp DNA Ladder (Promega). Bands were visualized by UV, the results were recorded photographically and analyzed densitometrically using an LKB Ultrascan XL Enhanced Laser Densitometer.

**Measurements of PAI-1, AGP, and Vimentin by ELISA**—HUVECs or U937 cells were plated in 48-well plates at the density of 2 × 10^5 cells/ml. On the next day, they were starved overnight in medium containing 0.1% FBS, and then stimulated for 24 or 48 h with different concentrations of Tβ4 or Tβ10 (0–80 nM). Afterward, the supernatants were collected and assayed for PAI-1 antigen by enzyme-linked immunosorbent assay using the Elisa-Imulyse PAI-1 kit. To measure AGP concentration in the conditioned media, a competitive inhibition ELISA was employed. For this purpose, the wells of 96-well microtiter plates were coated overnight at 4 °C with recombinant AGP at 1.0 μg/ml in PBS. Unbound proteins were washed from the wells, and nonspecific binding sites were blocked by incubation with TBS, pH 7.5, containing 1% BSA and 0.01% Tween 20 for an hour at room temperature. In competitive inhibition experiments, 50-μl aliquots of anti-AGP polyclonal antibodies, producing 60% of the binding to the immobilized AGP, were preincubated overnight at 4 °C with 50 μl of the serial dilutions of the conditioned media. Then, 100-μl aliquots of the incubation mixtures were added to the wells coated with AGP and incubated overnight at 4 °C. Plates were then extensively washed with TBS and incubated with goat anti-rabbit secondary antibodies conjugated with horseradish peroxidase. The reaction was developed using ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) from Sigma at 1 mg/ml in 0.1 M sodium citrate, pH 4.5, and the change in color was determined at 405 nm. To detect nonspecific binding, all assays were simultaneously done on plates coated with BSA alone and processed as
described above. The background binding to BSA was subtracted from all samples before data analysis. Vitronectin was analyzed by a sandwich-type immunoassay using the ELISA assay kit HVNKT (Innovative Research Inc., Southfield, MI).

Immunoprecipitation Experiments—The conditioned media were diluted 2-fold with TBS and incubated for 1 h with 10 μg/ml anti-AGP polyclonal antibodies and then precipitated with protein G-Sepharose beads (Amersham Biosciences) for 1 h at room temperature. The beads were washed with TBS and used for Western blot analysis with anti-AGP, anti-PAI-1 or anti-vitronectin antibodies, respectively. For the detection of AGP-PAI-1 complex in endothelial cells, EA.hy926 control cells, unstimulated and stimulated with T4 (40 nM) or transfected with pEGFP-N1-AGP, were washed three times with Hank’s balanced salt solution and treated with 200 nM AGP for 1 h at 37 °C in serum-free medium or left untreated as controls. After addition of EGTA to a final concentration 10 mM, extraction of membrane-bound proteins was performed at room temperature for 20 min. Protease inhibitor mixture (Roche Applied Science) was added to extracts and they were centrifuged to remove cell debris. Supernatants were used for immunoprecipitation with anti-AGP polyclonal antibodies followed by Western blot analysis.

Flow Cytometry—Subconfluent HUVECs and U937s were harvested and washed with serum-free DMEM. To evaluate PAI-1 bound to the cellular surface, cells were stimulated for 24 h with different concentrations of T4 (0–80 nM), then incubated with FITC conjugated antibodies to PAI-1 (MAI-12) at the concentration of 50 μg/ml. In other experiments, T4 stimulated cells were incubated in the serum-supplemented medium containing AGP (200 nM) and PAI-1 (100 nM) or PAI-1 (100 nM) alone at 4 °C for 45 min. Then after washing, cells (2 × 10^5) suspended in DMEM containing 1% BSA were incubated in the dark at 4 °C for 30 min with FITC-conjugated monoclonal antibodies against PAI-1 or AGP. After double washing with 1% BSA/PBS, the cells were fixed by mixing the sedimented cells with 1% paraformaldehyde in PBS and resuspended in appropriate fluid for flow cytometry analysis. Fluorescence was measured with a FACScan flow cytometer (BD Biosciences), and the data were analyzed with CELLQuest software (BD Biosciences).

Solid Phase Binding Assays—Binding of PAI-1 forms, active, latent, and substrate, to immobilized AGP was measured by enzyme-linked immunosorbent assay. The wells of 96-well microtiter plates were coated with AGP at 4 μg/ml in PBS. Nonspecific binding sites were blocked by incubation with 1% BSA in PBS for 2 h at room temperature. Direct binding assays were performed by adding increasing concentrations of PAI-1 molecular forms in PBS containing 0.1% BSA and 0.002% Tween 80, pH 7.4. The plates were incubated overnight at 4 °C followed by detection of bound PAI-1 using MA-55F4C12-HRP. The background binding to BSA was subtracted from all samples before data analysis.

Construction of EGFP-AGP Fusion Protein—Human AGP sequence was amplified from pGAD10 plasmid containing human cDNA library by PCR using primers 5’-CCTCCTGGTCTCGAGATGGCGCTGTCCTGGGTTCTTACAG-3’ and 5’-CCAAGGCTGTGTCCGGATCCGATTCCCCCTCCTCC-3’. Subcloned into XhoI and BamHI sites in pEGFP-N1. The obtained pEGFP-N1-AGP was propagated in Escherichia coli, purified with Wizard Midiprep (Promega), and sequenced to confirm the open reading frame. Plasmids containing the EGFP-AGP fusion construct were transfected into EA.hy926 cells with Lipofectamine. Briefly, 1.0 μg of plasmid DNA and 5 μl of Lipofectamine solution were incubated for 45 min in 200 μl of Opti-MEM (Invitrogen) and then diluted with 800 μl Opti-MEM. This solution was added to growing EA.hy926 cells in 20% FBS medium. Expression of the fusion construct was evaluated by confocal microscopy after 24 and 48 h.

FIGURE 3. Binding of PAI-1 to AGP on cell membranes. HUVECs, before (A) and after incubation (B) with T4 (40 nM) for 24 h were analyzed for intrinsic PAI-1 expression using monoclonal antibody MAI-12. Similarly, EA.hy926 cells (C) and U937 cells (D) were tested. Then PAI-1 expression was analyzed after preincubation of T4-activated cells with PAI-1 (100 nM) or first with AGP (200 nM) and then with PAI-1 (100 nM). Afterward, cells were washed, briefly fixed with paraformaldehyde, and then incubated with antibodies directed against PAI-1 (MAI-12) and normal, preimmune sheep IgG prior to their analysis by fluorescence flow cytometry.
Confocal Microscopy—For microscopic examination, cells (5 × 10⁶ cells/ml) were plated on Permanox coverslips in 8-well tissue culture chamber slides (Nunc) with detachable chambered upper structures. After 24-h incubation, they were fixed with ice cold 3% formaldehyde in PBS for 20 min, washed three times with PBS, and incubated with blocking buffer (PBS containing 3% BSA). After washing with PBS, the cells were incubated with biotinylated PAI-1 (100 nM) in the absence or presence of AGP (200 nM). After washing, the biotinylated PAI-1 was detected by incubation for 30 min with avidin-conjugated Texas Red rhodamine. The cells were then visualized using a helium/neon ion laser (543 nm excitation) and analyzed with MultiScan v.8.08 software. For intracellular probe visualization the confocal laser scanning microscope CLSM Phoibos 1000 (Amersham Biosciences) was used, which is based on a Nikon Optiphot microscope (equipped with Nikon oil immersion objectives 60 × NA 1.4 and 100 × NA 1.4 and with an Argon laser with fluorescence filter, line selection 488 nm, dichroic mirror 510, emission 510 nm).

Plasminogen Activation on the Cellular Surface—EA.hy926 and U937 cells were grown in the presence of different concentrations of Tβ4 (0–80 nM) for 24 h, and subconfluent cells were harvested, washed with serum-free DMEM, and suspended in 0.01 M Tris-HCl buffer, pH 7.4, containing 0.14 M NaCl and 0.1% BSA. After addition of uPA to a concentration of 1.4 nM, the cells were incubated for 20 min at 37 °C, followed by three washes in 0.01 M Tris-HCl buffer, pH 7.4, containing 0.14 mM NaCl and 0.1% BSA. The cells were incubated at a final concentration of 1 × 10⁶ cells/ml in 0.01 M Tris-HCl, 0.14 M NaCl with plasminogen (0.175 μM), and 0.2 mM H-D-Val-Leu-Lys-AMC (Bachem), a plasmin-specific fluorogenic peptide substrate. These incubations were made in 1-cm fluorometer cuvettes, which were maintained at 37 °C and gently stirred in a PerkinElmer Life Sciences LS-50 spectrofluorometer equipped with a micromagnetic stirrer. The fluorescence was measured continuously at an excitation wavelength of 380 nm and an emission wavelength of 480 nm. These data were converted to plasmin concentrations by calculating the rate of change in fluorescence between 1-min intervals and comparison with a calibration curve constructed using a standard plasmin.

RESULTS

AGP Expression in Tβ4-stimulated Endothelial Cells—AGP, one of the major acute phase proteins, can interact with PAI-1 and stabilize its inhibitory activity toward plasminogen activators (7). Since AGP is synthesized by activated endothelial cells (8), in preliminary experiments we tested whether its expression is influenced by Tβ4. Fig. 1A shows that treatment of endothelial cells with Tβ4 for 24 h resulted in up-regulation of AGP mRNA, even to a higher extent than PAI-1 mRNA. When used at 80 nM, Tβ4 increased the concentrations of PAI-1 and AGP mRNA by 8- and 10-fold, respectively. The specificity of this effect was evidenced by the fact that there was no increase in mRNA of t-PA, vWF or actin analyzed in the same samples. The specificity of the Tβ4 enhancing effect on expression of PAI-1 and other endothelial cells was well documented in our recent work (7). The same effect of Tβ4 on endothelial cells (HUVECs) and U937 cells could be observed at the
level of AGP synthesis and secretion. In this experiment, endothelial cells were incubated with increasing concentrations of Tβ4, and aliquots of the conditioned media were collected at different time points and analyzed by ELISA. Fig. 1B shows that Tβ4 significantly increased the expression of AGP secreted from HUVECs and U937 cells in the culture medium in a concentration-dependent manner. The maximum of AGP release was approached after treatment of the cells for 24 h with extracellular Tβ4 concentrations as low as 80 nM. To determine whether endogenous AGP was expressed on the surface of endothelial cells (HUVECs and EA.hy926) and U937 cells, each type was stained with anti-AGP monoclonal antibody AAG2 labeled with fluorescein. Unstimulated cells did not show the presence of AGP to be associated with their membranes (data not shown). However, treatment of the cells with increasing concentrations of Tβ4 (10–80 nM) for 24 h resulted in a marked increase of the relative fluorescence intensity of the FITC immunolabeling for AGP, indicating association of this protein with the activated cells (Fig. 2A–C).

Formation of the AGP-PAI-1 Complex on Endothelial Cells—Since PAI-1 14–1B showed the same binding characteristics as the active form of wild PAI-1 when its interaction with AGP was analyzed (15), it was used in all remaining experiments to maintain PAI-1 functional activity during the extended incubations required for different assays. The next experiments were designed to detect the PAI-1-AGP complex formation on HUVECs, EA.hy926 cells, and U937 cells. Immunolocalization by fluorescence-activated cell sorting and confocal microscopy were used to confirm the cell surface colocalization of PAI-1 and AGP. Similar to AGP, PAI-1 was not detectable on unstimulated HUVECs (Fig. 3A). However, incubation of Tβ4 stimulated HUVEC, EA.hy926, and U937 cells with anti-PAI-1 monoclonal antibody MAI-12 showed that these cells express substantial amounts of endogenous PAI-1 bound to their membranes (Fig. 3, B–D). Preincubation of both types of cells with stable PAI-1 mutant 14–1B (100 nM) resulted in a marked increase of the surface associated PAI-1 expression. When cells were first preincubated with AGP (200 nM) and then with PAI-1 (100 nM), the increase in PAI-1 expression on these cells was much more profound (Fig. 3, B–D). Since the presence of anti-AGP IgG virtually eliminated this additional increase in the binding of MAI-12 IgG to the cellular surface (data not shown), it thereby indicated that the binding of PAI-1 to endothelial cells was mediated by AGP. These experiments show that there are two pools of AGP and PAI-1 on Tβ4 activated cells: (a) the first one results from the enhanced endogenous expression of AGP upon activation of the cells with Tβ4 at the mRNA and protein level followed by their secretion, and (b) the second one caused by binding of added AGP and then PAI-1 to the membrane.

We next turned to confocal immunofluorescence microscopy to explain which molecular forms of PAI-1 bind to endothelial cells and search for colocalization of AGP and PAI-1 at the cellular surfaces. EA.hy926 cells were transfected with pEGFP-N1-AGP expressing EGFP-AGP and then incubated with either active or latent PAI-1. Specifically bound PAI-1 was identified by staining with avidin-conjugated Texas Red rhodamine. Unstimulated endothelial cells did not show any binding of active PAI-1 (Fig. 4A, panels a–c) or latent PAI-1 (data not shown). EA.hy926 cells transfected with pEGFP-N1-AGP showed increased expression of AGP and bound exclusively the active form of PAI-1 which well colocalized with AGP (Fig. 4A, panels d–f). Since these cells were not stimulated it indicates that overexpression of AGP led to its secretion and thus the released AGP at least partly becomes associated with the cells. When such cells were stimulated with Tβ4, they showed higher expression of EGFP-AGP and bound significantly more PAI-1 (data not shown). There was actually no interaction of latent PAI-1 with the endothelial cells overexpressing AGP (Fig. 4A, panels g–i). This is clearly seen when fluorescence intensity was quantitated using Image program (Fig. 4, B–D). To further support this observation, we analyzed the interactions of active, latent, and substrate PAI-1 with isolated AGP. For this purpose, increasing concentrations of PAI-1 were incubated with AGP immobilized on ELISA plate wells, and the bound PAI-1 was monitored with anti PAI-1 monoclonal antibody MA-55F4C12. Fig. 4E shows that AGP binds exclusively to the active PAI-1 molecule and does not interact either with substrate or latent PAI-1.

Fig. 5A shows that when cells overexpressing AGP were activated by Tβ4, their morphology was strikingly changed. The cells containing the AGP-PAI-1 complexes were flattened and spread. In some cells, AGP was focally enriched in regions located subjacent to the plasma membrane (Fig. 5A, panels a–c), while in others AGP was diffusely present throughout the cytoplasm and was concentrated at the basal surface of the cell (Fig. 5A, panels d–f). Interestingly, PAI-1 is also enriched within these same regions (Fig. 5A, panels c and f) supporting our flow cytom-
Binding of PAI-1 to Endothelial Cells

The AGP-PAI-1 Complex and Plasmin Generation on Endothelial Cells—Accumulation of active PAI-1 in the complex with AGP on Tβ4-treated cells raised the possibility that this interaction might influence plasmin activation. To test this hypothesis, we measured plasmin generation by Tβ4 activated endothelial cells using the fluorogenic plasmin-specific peptide substrate, and conditions were established under which no plasmin was detected unless both plasminogen and sc-uPA were added to the cells. Substrate hydrolysis was subsequently monitored at 480 nm. Fig. 6A shows that when the concentration of Tβ4 added to EA.hy926 cells was increased from 0 to 80 nM, the rates of plasmin generation significantly decreased. The same direction of changes was observed when U937 cells treated with Tβ4 were used as a control (Fig. 6B), indicating that the released PAI-1 modified the fibrinolytic potential on both types of cells. The inhibition was abolished to a large extent by antibodies to AGP and PAI-1 in both types of cells (Fig. 6C). Similarly, a function blocking monoclonal antibody to uPAR neutralized the inhibitory effect of Tβ4 on U937 cells and EA.hy926 cells, while anti-αVβ3 antibodies used as a control did not show any effect. These observations support the role of AGP in accumulation of active PAI-1 on Tβ4 activated cells and its contribution for optimizing inhibition of plasmin formation on the cellular surface.

DISCUSSION

In the present study, we have shown that activation of endothelial cells with Tβ4 results in accumulation of active PAI-1 on their cellular membranes complexed with AGP and, thereby, influences their fibrinolytic response. Specifically, we have provided evidence that (a) PAI-1, in contrast to AGP, does not bind to quiescent cells but only to the Tβ4 activated endothelial cells. The cell surface accumulation of PAI-1 on EA.hy926 cells is dependent on the synthesis of AGP induced by Tβ4. (b) AGP colocalizes with PAI-1 on activated endothelial cells, (c) cells enriched in such complexes showed a changed morphology by becoming larger and spread with the AGP-PAI-1 complex mostly located on their basal surface, (d) they have a reduced ability to generate plasmin on their surface in an AGP-dependent manner, and (e) the mechanism underlying this involvement depends upon the direct and simultaneous binding of active PAI-1 released by Tβ4. These interactions were demonstrable both with purified AGP in vitro (7) and on the surface of endothelial cells. The capacity of PAI-1 and AGP to interact on intact endothelial cells was detected by flow cytometry and confocal microscopy and further supported by contribution of both components in inhibition of plasminogen activation on the surfaces of both endothelial and U937 cells.

A number of previous studies showed that components of the plasminogen system such as plasminogen, plasminogen activators, and their inhibitor, PAI-1, are localized or accumulated at the cell surface through specific receptors and hence are able to regulate pericellular proteolysis-related events (16, 17). They are differentially expressed on activated and motile cells including invading tumor cells, leukocytes, and migrating endothelial cells. However, there is little or no expression of these proteins on most normal, quiescent cells (18). PAI-1 has the ability to interact, reversibly and irreversibly, with several proteins. In addition to plasminogen activators and other proteolytic enzymes, it is known to interact with the cell surface through (a) the uPA-receptor, thus inducing the internalization of the resulting PAI-1-uPAuPAR complex. Such binding also leads to the deactivation and internalization of integrins that are bound to uPAR (19, 20) and inhibition of uPA-induced cell migration (21). In addition, (b) PAI-1, both free and in a complex with uPA, binds to the low density lipoprotein receptor-related protein (LRP). All free forms of PAI-1, active, latent, and cleaved, can interact with plasminogen activators and other proteolytic enzymes, it is known to interact with the cell surface through (a) the uPA-receptor, thus inducing the internalization of the resulting PAI-1-uPAuPAR complex. Such binding also leads to the deactivation and internalization of integrins that are bound to uPAR (19, 20) and inhibition of uPA-induced cell migration (21). In addition, (b) PAI-1, both free and in a complex with uPA, binds to the low density lipoprotein receptor-related protein (LRP). All free forms of PAI-1, active, latent, and cleaved, can interact with LRP with a relatively low affinity and promote cell migration (22). PAI-1 in a complex with u-PA or t-PA interacts with LRP with high affinity and is efficiently endocytosed by cells. In this work we provide...
evidence that PAI-1 can also interact with AGP associated with cellular membranes. Recently, by screening the human liver cDNA library to search for proteins that bind PAI-1, we discovered AGP to have such an ability (7). Since AGP is known to localize at inflammation foci, it may provide the basic molecular framework for the concentration of PAI-1. Interestingly, T84 is also accumulated in such areas, and particularly in wound fluid (3, 23, 24). It suggests that AGP may serve as a receptor for PAI-1 on cells that naturally express low concentrations of membrane PAI-1 receptors. This may be the case with endothelial cells, which do not contain LRP (25, 26) and show low expression of uPAR.

In summary, the major observation of this work shows that vascular cells produce appreciable quantities of AGP, which may serve as a bridge for active PAI-1 on cells and in extracellular matrix, thereby concentrating and redistributing PAI-1 related biological activities. These cross-regulatory interactions provide additional points of control for cellular processes pertinent to cell adhesion and migration.

REFERENCES
1. Malinda, K. M., Sidhu, G. S., Mani, H., Banaudha, K., Maheshwari, R. K., Goldstein, A. L., and Kleinman, H. K. (1999) *J. Invest. Dermatol.* 113, 364–368
2. Sosne, G., Szliter, E. A., Barrett, R., Kernacki, K. A., Kleinman, H., and Hazlett, L. D. (2002) *Exp. Eye Res.* 74, 293–299
3. Grant, D. S., Rose, W., Yaen, C., Goldstein, A., Martinez, J., and Kleinman, H. (1999) *Angiogenesis* 3, 125–135
4. Al-Nedawi, K. N., Czyz, M., Bednarek, R., Szemraj, J., Swiatkowska, M., Cierniowska-Cieslak, A., Wyczolkowska, J., and Cierniewski, C. S. (2004) *Blood* 103, 1319–1324
5. Marinissen, M. J., Chiariello, M., Tanom, T., Bernard, O., Narumiya, S., and Gutkind, J. S. (2004) *Mol. Cell* 14, 29–41
6. Bock-Marquette, A., Saxena, M., White, M. D., DiMaio, J. M., and Srivastava, D. (2004) *Nature* 432, 466–472