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Plasminogen-activator inhibitor type 1 is a potent natural inhibitor of extracellular matrix degradation by fibrosarcoma and colon carcinoma cells

(proteolysis/cancer invasion/cDNA transfection)

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ABSTRACT **We have analyzed the role of plasminogen-activator inhibitor type 1 (PAI-1) in the regulation of tumor cell-mediated extracellular matrix degradation. Immunocytochemical analysis revealed PAI-1 associated with microgranular and fibrillar material of the extracellular matrix and demonstrated the presence of PAI-1 as a cell surface-associated antigen. Transforming growth factor β significantly reduced matrix degradation mediated by HT-1080 human fibrosarcoma cells. This inhibition was correlated with an increase in PAI-1 antigen expression, whereas urinary-type plasminogen activator (u-PA) secretion was unaffected. In this experimental system, PAI-1 regulated extracellular matrix breakdown, as added PAI-1 inhibited matrix solubilization, whereas monoclonal antibodies to PAI-1 increased it. A cell line (L_{PAI}) producing high levels of biologically active PAI-1 was established by transfection of a human PAI-1 cDNA clone into mouse L cells. Coculture experiments demonstrated that L_{PAI} cells prevented matrix degradation by $L_{u\text{-}PA}$ cells (L cells expressing high levels of u-PA) or Co-115 human colon carcinoma cells (expressing tissue-type plasminogen activator). These results indicate that PAI-1 may play a critical role in the regulation of extracellular matrix degradation during tumor cell invasion.**

Plasminogen-activator inhibitor type 1 (PAI-1) is a member of the serpin family of protease inhibitors and reacts specifically and rapidly with both tissue-type (t-PA) and urinary-type (u-PA) plasminogen activator (1). PAI-1 is involved in the control of fibrinolysis and may also have a regulatory function in angiogenesis (2) and malignancy (3). Tumor invasion requires a complex interaction of events that include recognition and attachment of tumor cells to extracellular matrix (ECM) binding sites, proteolytic dissolution of matrix proteins, and tumor cell migration within the surrounding tissue (4). In this process, a proteolytic cascade is triggered by the u-PA-mediated conversion of plasminogen to plasmin and the subsequent activation of latent collagenase(s) (5). PAI-1, the primary inhibitor of u-PA, may participate in the control of tumor cell-mediated stroma breakdown. In support of this hypothesis is the observation that PAI-1 is a major component of the ECM and is stabilized by the ECM (6). PAI-1 is expressed at high levels by various types of cells, including endothelial cells (7), fibroblasts (8), and smooth muscle cells (9). Accumulation of PAI-1 within the tissue environment may play a critical role in the control of stroma invasion and/or in the process of intra- and extravasation by tumor cells. A variety of tumor cells also express u-PA in addition to PAI-1 (8, 10). The degree of tumor cell-mediated proteolysis may therefore result from a fine regulation between tumor cell-secreted proteases and protease inhibitors. The present study was undertaken to analyze the role of PAI-1 in tumor cell-mediated ECM degradation.

MATERIALS AND METHODS

Materials. Human transforming growth factor β1 (TGFβ1) was obtained from R & D Systems (Minneapolis), human plasminogen from KabiVitrum (Stockholm), and human high molecular weight u-PA (>95% two-chain form) from Serono (Coinsins, Switzerland). Monoclonal antibodies to PAI-1, used for affinity-chromatography purification of PAI-1 (11) and for the neutralization of PAI-1 activity in matrix degradation assays (12), were kindly provided by D. Collen (Center for Thrombosis and Vascular Research, Leuven, Belgium) and by L. Lund (Rigshospitalet, Copenhagen). Immunopurified rabbit polyclonal antibody to PAI-1 was obtained as described (13). Polyclonal antibodies against human u-PA and t-PA were prepared as described (10). Early-passage bovine aortic endothelial cells (BAECs) were kindly provided by P. Mignatti (Universita di Pavia, Pavia, Italy). All other materials were as previously described (11, 14, 15) or of the best reagent grade available.

Purification of PAI-1. Human Hep G2 hepatoma cells (16) were cultured to confluency in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum, washed, and maintained for 4 days in DMEM/1% fetal bovine serum. Five-liter batches were concentrated 50-fold by ultrafiltration and passed over a 2-ml Sepharose column to which 2 mg of monoclonal antibody to PAI-1 (MA-7D4) had been coupled. PAI-1 was eluted using 4 M potassium thiocyanate, dialyzed, and reactivated using 12 M urea (11). The specific activity of the different batches was between 125,000 and 250,000 units/mg, indicating that 20–40% of the product was in the active form [1 unit of inhibitor is defined as the amount of PAI-1 capable of inhibiting 1 international unit (= 2 ng) of t-PA]. Reactivated PAI-1 displayed an *in vitro* activity half-life at 37°C of ≈2 hr (data not shown).

Assays of PA and PAI Expressed by Cell Cultures. HT-1080 human fibrosarcoma cells were cultured as described (10), and conditioned media derived from nontreated and TGFβ-treated cells were harvested as indicated (see *Results*). Cell viability was always >90% as indicated by trypan blue exclusion. PAI-1 and u-PA antigens were measured by radioimmunoassay (RIA) (13) and PA activity was assayed by the ^{125}I-fibrin plate assay (14). Zymographic analysis of PAs, reverse fibrin zymography of PAI, and Northern blot analysis

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Abbreviations: BAEC, bovine aortic endothelial cell; ECM, extracellular matrix; PA, plasminogen activator; PAI-1, PA inhibitor type 1; TGFβ, transforming growth factor β; t-PA, tissue-type PA; u-PA, urinary type PA (urokinase).

were performed as described (17–19). The cDNA inserts of human PAI-1, u-PA, and t-PA used for the hybridization procedure were as reported (20, 21).

ECM Degradation Assay. Radiolabeled ECMs derived from R22 rat smooth muscle cells were prepared and assays of matrix degradation were performed as reported (15, 22). In some experiments, the effect of antibodies on matrix degradation was analyzed by incubating tumor cells with anti-human t-PA, u-PA, or PAI-1 antibodies or with nonimmune antibodies.

Light and Electron Microscopy Preparation. Cultures were fixed with phosphate-buffered 2% paraformaldehyde/0.5% glutaraldehyde and incubated with affinity-purified rabbit polyclonal anti-PAI-1 or nonimmune antibodies (2 μg/ml), followed by biotinylated goat anti-rabbit IgG (12.5 μg/ml) and horseradish peroxidase-avidin D (50 μg/ml; Vector Laboratories). Peroxidase activity was revealed using diaminobenzidine (Sigma) as described (23). Preparations were postfixed with 2% osmium tetroxide, dehydrated, and embedded in Epon 812, and sections were contrasted with uranyl acetate.

Expression of Human PAI-1 in Mouse L Cells. A plasmid construct (pSV2PAlex) carrying the entire coding region of human PAI-1 cDNA (24) under the control of the simian virus 40 promoter was cotransfected together with a pSV2neo selectable marker gene into recipient mouse L cells by the calcium phosphate precipitation technique (25). Control transfections were performed with pSV2neo DNA alone.

RESULTS

Immunocytochemical Localization of PAI-1. To localize PAI-1 in the ECM invaded by HT-1080 cells, we performed indirect immunoperoxidase staining using immunoaffinity-purified polyclonal anti-human PAI-1 IgG. Light microscopy demonstrated PAI-1 reactivity as evenly distributed within the ECM and associated with the pericellular space (data not shown). Electron microscopy revealed PAI-1 associated with both microgranular and fibrillar material (Fig. 1). In addition, cell surface-associated PAI-1 was observed in some areas as lining the cell membrane. PAI-1 reactivity was also observed as focal patches, often associated with plasma membrane pseudopodial protrusions as well as with cell surface pits (as shown in Fig. 1 *Upper*).

Effect of Anti-PAI-1 Antibodies and Purified PAI-1 on HT-1080 Cell-Mediated ECM Breakdown. The influence of PAI-1 bound to the ECM or produced by the HT-1080 cells was investigated by preincubating (3 hr at 37°C) the ^{3}H-labeled ECM with a monoclonal antibody against human PAI-1 (clone 1) or with a control monoclonal antibody of unrelated (anti-trinitrophenyl) specificity. HT-1080 cells were then plated (10^5 cells per well) and matrix solubilization was monitored after 24 hr (Fig. 2*A*). A 4-fold higher degradation was observed in the presence of neutralizing anti-PAI-1 IgG compared to the degradation observed either in the absence of antibody or in the presence of control monoclonal antibody. In a separate experiment, we analyzed the effect of purified human PAI-1 on HT-1080 cell-mediated matrix degradation (Fig. 2*B*). Tumor cells were plated on the ECM (10^5 cells per well) and cultured overnight. The following day, fresh medium containing PAI-1 (250 units/ml) was added. Furthermore, as purified and denaturant-reactivated PAI-1 displays a short half-life *in vitro* (1–2 hr), the inhibitor was repeatedly added in this experiment (at a final concentration of 250 units/ml) every 2 hr. Under these conditions, 75% inhibition of matrix breakdown was observed after 2 hr and 45% after 8 hr of incubation.

Modulation of ECM Breakdown by TGFβ. The intrinsic instability of exogenous PAI-1 precluded the analysis of its long-term effect. We therefore performed experiments with

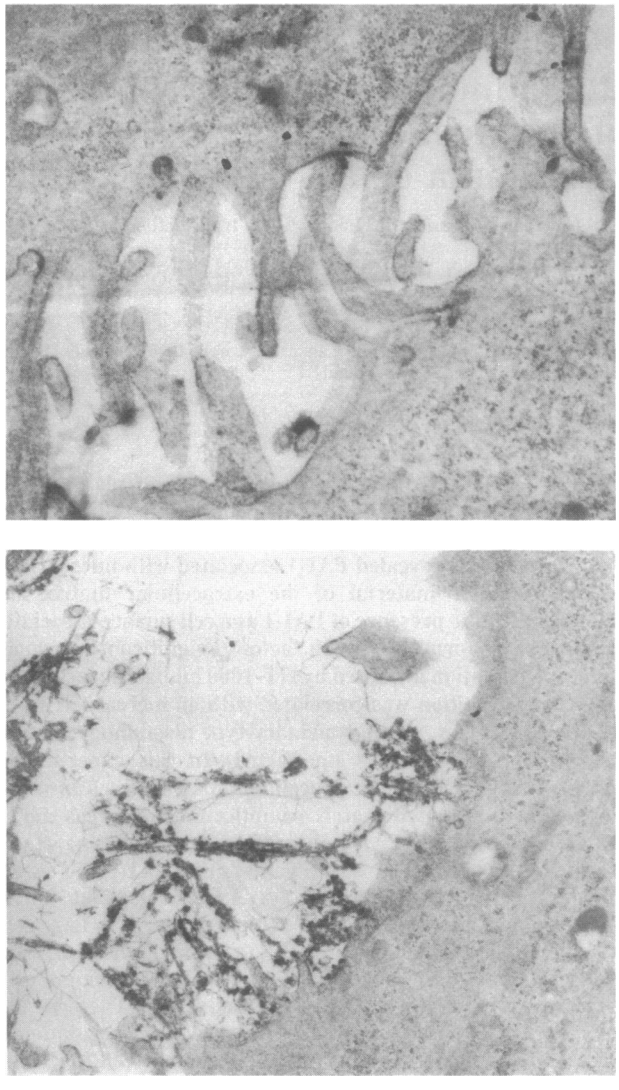

FIG. 1. PAI-1 distribution on R22 smooth muscle cell ECM invaded by HT-1080 fibrosarcoma cells. Immunocytochemical analysis using affinity-purified polyclonal antibodies against PAI-1 was used to visualize the HT-1080 cell surface (*Upper*) and the interaction between the HT-1080 cell and the R22 ECM (*Lower*) by electron microscopy. (*Upper*, ×21,500; *Lower*, ×10,000.)

HT-1080 cells treated with TGFβ, a potent inducer of PAI-1 (26). HT-1080 cells were cultured on ECMs over a 4-day incubation period with or without 200 pM TGFβ. Approximately 70% inhibition of HT-1080-mediated matrix degradation by TGFβ was observed (data not shown). The effect of TGFβ on the levels of PAI-1 and u-PA antigens was also investigated in this assay. TGFβ induced a 5-fold increase in PAI-1 release, whereas only slight, nonsignificant stimulation of u-PA was observed. These effects were not due to differences in cell proliferation, as daily cell counts did not show any detectable effect of TGFβ on the HT-1080 growth rate.

Expression of Human PAI-1 in Mouse L Cells. The experiments performed with TGFβ do not establish a direct role of PAI-1 in the regulation of ECM degradation, since this growth factor may also have influenced cell behavior in other ways. We therefore transfected the PAI-1 gene into mouse L cells to continuously express biologically active human PAI-1 and analyze its long-term effect. Mouse L cells were used because they do not constitutively express PA activity and are not capable of degrading the ECM *in vitro* (15) and they express very little endogenous PAI (24). Transfection of a human full-length PAI-1 cDNA clone together with a neomycin-resistance (*neo*) gene or of the *neo* gene alone allowed

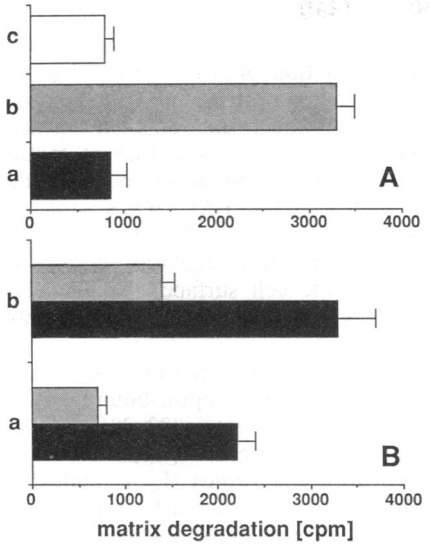

FIG. 2. Influence of anti-PAI-1 antibodies (*A*) and purified PAI-1 (*B*) on HT-1080 cell-mediated ECM degradation. (*A*) Matrix solubilization in the absence of antibodies (bar a) and in the presence of monoclonal antibodies against PAI-1 (bar b) or control antibodies (bar c). (*B*) Matrix solubilization after 2 and 8 hr of incubation (bars a and b, respectively) with purified human PAI-1 (gray bars) or medium alone (black bars).

the isolation of several L_{PAI} transfectants (L_{PAIC4} and L_{PAIC6}), as well as a control L_{neo} transfectant. RIA of PAI-1 released by these cells gave the following results: L_{PAI} clone 6, 250 ng/ml per 24 hr; L_{PAI} clone 4, 50 ng/ml per 24 hr; L_{neo} or parental L cells, 2–5 ng/ml per 24 hr.

Reverse fibrin zymography of 24-hr serum-free cell-conditioned media (Fig. 3*A*) showed that L_{PAI} cells expressed a 55-kDa lysis-resistant band that comigrated with BAEC-derived PAI. Conversely, L_{neo} cells exhibited a weak PAI-related band. R22 rat smooth muscle cells also released a 55-kDa PAI that may represent the rat counterpart of human PAI-1.

Northern blot analysis (Fig. 3*B*) demonstrated the presence of human PAI-1 mRNA in L_{PAI} cells, whereas no PAI-1 mRNA could be detected in L_{neo} cells. It is known that, in most cells, two distinct transcripts of 2.2 and 3.2 kilobases (kb) exist for PAI mRNA (20), which are thought to result from alternative polyadenylylation sites (27). As expected, HT-1080 cells displayed both mRNA species, whereas L_{PAI} cells stably transfected with the PAI-1 cDNA displayed a predominant 2.2-kb transcript and a weak 1.8-kb transcript. The absence of the larger (3-kb) PAI-1 transcript and the presence of an abundant 2.2-kb PAI-1 transcript were expected due to the size of the PAI-1 cDNA used for transfection (2.2 kb), which is lacking the complete 3′ untranslated region (24). The presence of the weak 1.8-kb signal may be due to aberrant rearrangement of the PAI-1 cDNA during integration into the genome or to alternative posttranscriptional processing of the transcript.

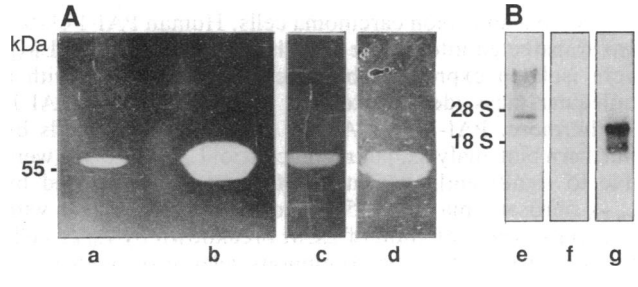

FIG. 3. Expression of PAIs by cell cultures. (*A*) Reverse fibrin zymography of cell culture supernatants. Lanes a and b, L_{neo} and L_{PAI} cells, respectively; lane c, R22 cells; lane d, TGFβ-stimulated BAECs. (*B*) Northern blot analysis of PAI-1 mRNA expression. Lane e, HT-1080 cells; lane f, L_{neo} cells; lane g, L_{PAI} cells. Positions of 28S and 18S rRNA are indicated as size markers.

Coculture of L_{PAI} Cells with $L_{u\text{-}PA}$ Transfectants or Co-115 Colon Carcinoma Cells: Effect on ECM Degradation. To analyze the effect of PAI-1 release on $L_{u\text{-}PA}$-mediated matrix degradation, coculture experiments of L_{PAI} or control L_{neo} cells with $L_{u\text{-}PA}$ cells were performed. $L_{u\text{-}PA}$ cells, which express high levels of u-PA, were established in our laboratory by transfection of the human urokinase (u-PA) gene into mouse L cells (15). A constant number of $L_{u\text{-}PA}$ cells was plated together with various concentrations of L_{PAI} (clones 4 and 6) or L_{neo} cells. The cumulative plot of the radioactivity solubilized after a 3-day matrix degradation assay showed (Fig. 4*A*) that L_{PAI} cells were able to prevent $L_{u\text{-}PA}$-mediated matrix solubilization, the maximum inhibitory effect (75%) being obtained with the highest density of L_{PAI} clone 6 cells. L_{PAI} clone 4 cells also prevented matrix degradation, but to a lesser degree (maximal inhibition of 35%), and control L_{neo} cells had no effect. L_{neo} or L_{PAI} cells alone did not induce significant matrix degradation (<10% compared to $L_{u\text{-}PA}$-mediated matrix breakdown). PA activity, measured in 24-hr serum free supernatants of $L_{PAI}/L_{u\text{-}PA}$ cocultures, gradually decreased with increasing L_{PAI} cell density and was no longer detectable in the presence of 4×10^5 L_{PAI} cells (data not shown). In comparison, only a partial inhibitory effect (20%) was observed with 4×10^5 L_{neo} cells. Zymographic analysis indicated that all u-PA activity (54-kDa lytic band) was

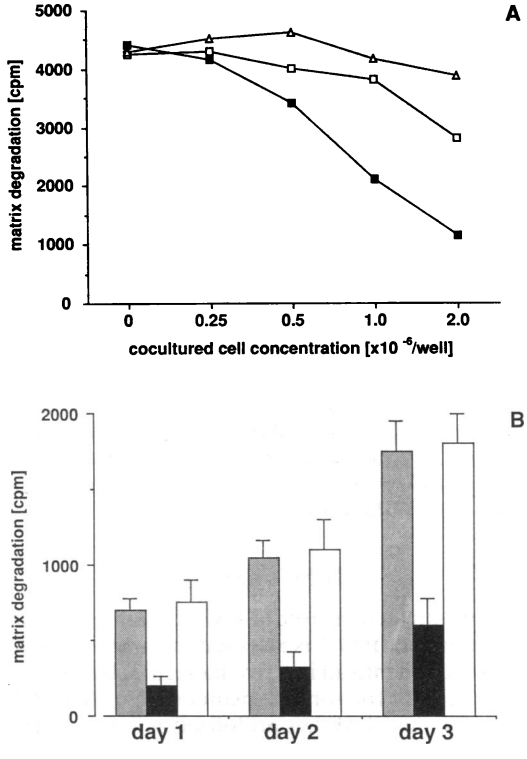

FIG. 4. Matrix degradation by cocultures of $L_{u\text{-}PA}$ (*A*) or Co-115 (*B*) cells with L_{PAI} and L_{neo} cells. (*A*) $L_{u\text{-}PA}$ cells (10^5 per well) were plated on the ECM together with various concentrations of control L_{neo} cells (△), L_{PAI} clone 4 cells (□), or L_{PAI} clone 6 cells (■). Data represent the mean of six determinations (SDs were <10% of the mean). (*B*) Co-115 human colon carcinoma cells (2×10^5 per well) were cultured on the ECM for 24 hr. L_{PAI} (clone 6) or L_{neo} cells were then added to these cultures (10^5 cells per well) and matrix solubilization was analyzed for 3 days thereafter. Gray bars, Co-115 cells alone; black bars, Co-115/L_{PAI} coculture; white bars, Co-115/L_{neo} coculture.

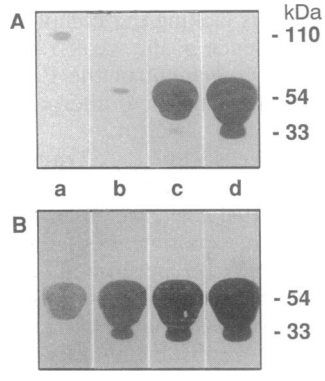

FIG. 5. Zymographic analysis of PA expressed by cocultures of $L_{u\text{-}PA}$ and L_{PAI} or L_{neo} cells. $L_{u\text{-}PA}$ cells (10^5 per well) were plated together with various concentrations of L_{PAI} clone 6 (*A*) or L_{neo} cells (*B*) and grown 24 hr in DMEM/10% fetal bovine serum, followed by 24 hr in serum-free DMEM/0.1% bovine serum albumin containing purified plasminogen at 5 μg/ml. Supernatants were then collected for zymographic analysis. Lanes a–c, 4, 1, and 0.25×10^5 L_{PAI} or L_{neo} cells per well, respectively, cocultured with $L_{u\text{-}PA}$ cells; lane d, $L_{u\text{-}PA}$ cells alone.

blocked in cocultures of $L_{u\text{-}PA}$ cells with L_{PAI} cells, but not with L_{neo} cells (Fig. 5). The inhibition of the 54-kDa u-PA-related band was accompanied by the appearance of a 110-kDa lytic band, which represents a complex formed between u-PA and PAI-1 (14).

Coculture experiments were also performed using Co-115 human colon carcinoma cells and L_{PAI} cells. We previously reported that Co-115 cells express high levels of t-PA (10). Northern blot analysis of Co-115 cell extracts confirmed the presence of t-PA mRNA, whereas no u-PA mRNA transcript could be detected (data not shown). Matrix degradation was inhibited to a large extent when Co-115 cells were cocultured with L_{PAI} cells, whereas control L_{neo} cells had no effect (Fig. 4*B*). The effect of PAI-1 on Co-115-associated PA activity was analyzed by zymography of supernatants of L_{PAI}/Co-115 and control L_{neo}/Co-115 cocultures. As shown for $L_{PAI}/L_{u\text{-}PA}$ cocultures, increasing numbers of L_{PAI} cells inhibited the 68-kDa t-PA-related lytic zone, with the concomitant appearance of a 120-kDa PA/PAI-related lytic band. Co-115 cells cocultured with L_{neo} cells induced only limited inhibition of the t-PA lytic band and no complex formation. To exclude the possibility that u-PA activity was involved in Co-115 cell-mediated ECM breakdown, we also performed the matrix degradation assay with antibodies directed against human u-PA and t-PA. Anti-t-PA antibodies prevented matrix degradation by 80%, whereas anti-u-PA or nonimmune antibodies had no effect (data not shown).

DISCUSSION

We have investigated the role of PAI-1 in tumor cell-mediated ECM degradation. PAI-1 is released in an active form and is rapidly converted into an inactive molecule. However, PAI-1 has a high affinity for some constituent(s) of the ECM and, once bound to the ECM, has prolonged activity (6). Immunocytochemical analysis performed in this study confirmed that PAI-1 is bound to ECM constituents, as previously reported with HT-1080 human fibrosarcoma cells (28) and human endothelial cells (29). Analysis by electron microscopy furthermore demonstrated the presence of PAI-1 in close association with the cell membrane, suggesting the possible existence of a membrane receptor for PAI-1 and/or the pericellular deposition of PAI-1 bound to an intermediate adhesion protein. In this context, vitronectin has been characterized as the ECM binding protein capable of interacting with PAI-1 (30). Our finding of PAI-1 reactivity associated with cell surface pits suggests the existence of an internalization process. Pöllänen *et al.* (28), using HT-1080 cells, showed the association of u-PA as focal patches on cell extension sites. These findings of u-PA and our own on PAI-1 localization suggest the formation of a surface-associated complex. Sakata *et al.* (31) showed that interactions between t-PA and PAI-1 with endothelial cells occurred mainly on the cell surface, indicating that PAI-1 plays a role in the regulation of pericellular fibrinolysis. Taken together, these observations suggest that a fine regulation of proteolysis could occur directly at the cell surface, in particular at critical contact sites between matrix and plasma membrane extensions.

These findings are of particular importance in view of the recent demonstration that receptor-bound u-PA plays a role in tumor invasion and metastasis (32, 33). In this context, we have shown that PAI-1 can regulate matrix degradation mediated by various cell lines ($L_{u\text{-}PA}$ and HT-1080 cells) expressing high levels of human u-PA. As human u-PA does not bind to the mouse receptor (34), we may conclude that PAI-1 blocks degradation of $L_{u\text{-}PA}$ cells by inhibiting cell-secreted u-PA. Conversely, the observation that PAI-1 prevents HT-1080 cell-mediated degradation suggests that it may also regulate receptor-bound u-PA (35).

TGFβ significantly inhibited ECM degradation by HT-1080 cells. This inhibition was correlated with an increase in PAI-1 expression, whereas u-PA secretion was not significantly affected. Mignatti *et al.* (2) reported that TGFβ inhibited bovine capillary endothelial cell invasion of the human amnion membrane. TGFβ can stabilize the ECM by various mechanisms (36), including stimulation of the biosynthesis of ECM components such as fibronectin and collagen, increase in protease inhibitor secretion, and decrease in protease expression. Our results, which indicate that PAI-1 may be involved in the prevention of matrix degradation by TGFβ, do not exclude the possibility that an increase in ECM protein synthesis may also contribute to this effect. Direct evidence for the role of PAI-1 in the regulation of ECM degradation by HT-1080 cells was obtained by addition of purified, active PAI-1, which, despite its short half-life, efficiently prevented matrix breakdown, and by addition of neutralizing anti-PAI-1 monoclonal antibodies, which resulted in a 4-fold increase in matrix solubilization. Stephens *et al.* (35) provided evidence that surface-bound u-PA on HT-1080 cells was accessible to inhibition by PAI-1, which blocked the plasmin-mediated activation of pro-urokinase. Such a mechanism is likely to be involved in the prevention of matrix degradation by PAI-1 described in this study.

Protease inhibitors may represent a natural control mechanism in tumor cell invasion. To mimic the possible interaction(s) likely to occur between tumor cells and their microenvironment, we investigated the degradation of ECM by cocultures of PAI-1-expressing cells and PA-expressing fibrosarcoma and colon carcinoma cells. Human PAI-1 cDNA was transfected into mouse L cells and transfectants (L_{PAI}) were isolated expressing biologically active PAI-1 with a molecular mass identical to that of natural human PAI-1. Furthermore, PAI-1 mRNA was detected in L_{PAI} cells by Northern blot analysis, but not in control L_{neo} cells. We were able to significantly inhibit ECM degradation induced by $L_{u\text{-}PA}$ fibrosarcoma cells (15) by coculturing these cells with L_{PAI} cells. The inhibition of ECM breakdown by $L_{u\text{-}PA}$ cells may be attributed to the continuous release of biologically active PAI-1 by L_{PAI} cells, as control L_{neo} cells had no such effect. This was further substantiated by the total inhibition of PA activity measured in $L_{PAI}/L_{u\text{-}PA}$ cocultures and by zymographic analysis, which demonstrated the abolition of the u-PA-related lytic band in parallel with the appearance of a complex formed between u-PA and PAI.

To verify that these conclusions were also valid for tumor cells expressing t-PA, we performed similar coculture experiments with Co-115 human colon carcinoma cells and L_{PAI} or L_{neo} cells. L_{PAI} gave a pronounced inhibitory effect on Co-115-mediated matrix degradation, whereas L_{neo} cells were unable to do so. To analyze the contribution of t-PA in Co-115-mediated matrix degradation, we performed immunoinhibition studies, which demonstrated that antibodies against t-PA inhibited matrix degradation by 80%, whereas nonimmune or anti-u-PA antibodies had no effect. These results demonstrate that t-PA plays an essential role in ECM degradation by Co-115 colon carcinoma cells and indicate that PAI-1 also prevents t-PA-mediated ECM proteolysis.

Previous reports indicate that natural protease inhibitors can contribute to the stability of the ECM. Several groups have shown that inhibitors such as protease nexin (a broad-spectrum serine protease inhibitor) or tissue inhibitor of metalloprotease (TIMP, a collagenase-specific inhibitor) can efficiently prevent the degradation and invasion of the ECM (5, 37) as well as metastasis formation in animals (38). Moreover, smooth muscle cells secrete a PAI that may influence the invasive potential of tumor cells (9). We have demonstrated the expression of a PAI by R22 rat smooth muscle cells, suggesting that this inhibitor may help stabilize the R22 cell-derived ECM.

The expression of PAI-1 by a variety of cell types (endothelial cells, fibroblasts, smooth muscle cells) and its accumulation within the ECM suggest that this inhibitor may represent an additional and natural matrix factor in tissue remodeling events such as inflammation or tumor invasion. This study reports the efficient inhibition of matrix breakdown by human PAI-1, a potent and specific inhibitor of both u-PA and t-PA. Our results indicate that this inhibitor may be critically involved in the modulation of ECM invasion by a variety of tumor cells, including mouse fibrosarcoma and human colon carcinoma cells, and suggest a potential therapeutic benefit in the regulation of tumor invasion.

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