Posttranscriptional regulation of plasminogen activator inhibitor-1 in human lung carcinoma cells in vitro

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Shetty, Sreerama, and Steven Idell. Posttranscriptional regulation of plasminogen activator inhibitor-1 in human lung carcinoma cells in vitro. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L148–L156, 2000.—Plasminogen activator inhibitor-1 (PAI-1), the major circulating inhibitor of urokinase [urokinase-type plasminogen activator (uPA)], has been linked to the pathogenesis of lung cancer. PAI-1 belongs to the serpin family of inhibitors and inhibits both free urokinase (uPA) and receptor-bound urokinase (uPA receptor). Although PAI-1 has been related to a poor prognosis in lung carcinoma, mechanisms that regulate its expression in human lung cancer cells are not well understood. We used cultured human small cell and non-small cell lung carcinoma cell lines as model systems to elucidate the regulatory mechanisms that control expression of PAI-1. Levels of PAI-1 protein were significantly increased in selected lung carcinoma cells compared with those in normal small-airway epithelial cells. Corresponding steady-state levels of PAI-1 mRNA were similarly increased in these cells. The half-life of PAI-1 mRNA was prolonged in these lung carcinoma cell lines after transcriptional or translational blockade. We identified a 60-kDa protein that binds the 3′-untranslated region of PAI-1 mRNA, and complex formation of this binding protein with PAI-1 mRNA reciprocally correlates with mRNA stability. The findings demonstrate that expression of PAI-1 is regulated at the posttranscriptional level in small cell- and non-small cell-derived human lung carcinoma cell lines. Altered regulation of PAI-1 at the posttranscriptional level may contribute to relative overexpression by malignant lung epithelial cells. A newly identified regulatory protein that binds to the 3′-untranslated region of PAI-1 mRNA appears to be involved in the posttranscriptional regulation of PAI-1 gene expression by human lung carcinoma cells in vitro.

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EXTRACELLULAR PROTEOLYTIC ENZYMES such as serine proteinases and metalloproteinases have been implicated in the pathogenesis of cancer growth and metastasis. It is currently believed that the balance between the release of proteolytic enzymes and their inhibitors by solid tumors facilitates cancer cell invasion into the surrounding normal tissues via the breakdown of basement membranes and the extracellular matrix (5, 15, 20). Metastasis is a sequential process in which malignant cells are released from the primary tumor and disseminate to distant sites where they proliferate and invade host tissues, in part due to their proteolytic capacity (28). Interactions between cellular proteases and their inhibitors are therefore potentially important determinants of tumor growth and invasiveness.

Plasminogen activator inhibitor-1 (PAI-1) and urokinase-type plasminogen activator (uPA) appear to play a cooperative role in the propagation and spread of neoplasms. uPA localizes plasmin-dependent fibrinolytic activity at the cell surfaces by binding uPA receptor (uPAR) (5, 20), and PAI-1 inhibits expression of this proteolytic activity. PAI-1 can inhibit uPA bound to uPAR at the cell surface and can therefore modulate processes that influence neoplastic growth, including mitogenesis, cellular adhesion, chemotaxis, and cellular migration (40). Complexes between uPAR, uPA, and PAI-1 are also internalized into the cellular interior. These complexes are then degraded with recycling of the receptor to the cell surface. This PAI-1-dependent mechanism of uPAR recycling may serve to maintain the proteolytic, proliferative, and invasive potential of malignant cells.

During the last decade, evidence for involvement of PAI-1 in cancer growth and metastasis has steadily increased. Several studies of different human cancers have consistently shown that high levels of PAI-1 in tumors correlate with poor patient outcome (reviewed in Ref. 5). In particular, increased expression of PAI-1 in tumor tissue appears to be associated with a poor prognosis in carcinomas of the lung (5). These observations underscore the potential importance of pathways that govern PAI-1 expression to the clinical outcome in lung cancer.

Current understanding of the regulatory mechanisms that control the expression of PAI-1 in lung cancer cells is incomplete. To elucidate these mechanisms, we used cultured cell lines derived from small cell and non-small cell lung tumors as model systems to determine whether there was a differential expression of PAI-1 and PAI-1 mRNA in the different cell types. Having found that this was the case, we proceeded to develop evidence that PAI-1 is regulated at the posttranscriptional level in cultured human lung carcinoma-derived cell lines. To our knowledge, this is the first demonstration of this type of regulation of PAI-1 in human epithelial cell lines, particularly those derived from small cell and non-small cell lung carcinomas. The regulatory mechanism involves the interaction of PAI-1 mRNA with a newly identified PAI-1 mRNA binding
protein (mRNABP) that appears to influence PAI-1 mRNA stability.

METHODS

Materials. Culture media, penicillin, streptomycin, and FCS were purchased from Gibco BRL (Life Technologies, Grand Island, NY). Tissue culture plastics were from Becton Dickinson Labware (Lincoln Park, NJ). BSA, ovalbumin, Tris base, apotinin, dithiotreitol, phenylmethylsulfonyl fluoride, silver nitrate, and ammonium persulfate were from Sigma (St. Louis, MO). Acrylamide, bis-acrylamide, and nitrocellulose were from Bio-Rad Laboratories (Richmond, CA), and XAR X-ray film was from Eastman Kodak (Rochester, NY). In vitro transcription and RNase protection assay kits were purchased from Ambion (Austin, TX). Restriction enzymes were from New England BioLabs (Beverly, MA), and [32P]UTP was from DuPont (Wilmington, DE).

Cell cultures. Human small-airway lung epithelial cells (SAECs) were obtained from Clonetics (San Diego, CA). These cells or lung epithelial tumor cells, including H1395 and A549 human lung adenocarcinoma cells, H157 human lung squamous cells, H460 large cell lung carcinoma cells, and H146 human lung small cell lung carcinoma cells, were obtained from the American Type Culture Collection. These cells were maintained in RPMI 1640 medium containing 10% heat-inactivated FCS, 1% glutamine, and 1% antibiotics and characterized as previously described (34, 38).

Western blotting. We used SDS-PAGE and Western blotting to measure the antigenic PAI-1 level of cells and conditioned medium. Cell lysates and conditioned medium, prepared as described earlier (38) from SAECs or human lung carcinoma-derived cells, were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 1% BSA for 1 h at room temperature, hybridized overnight with PAI-1 monoclonal antibodies, washed, and developed with a horseradish peroxidase-conjugated secondary antibody.

Plasmid construction. Human PAI-1 cDNA was obtained by PCR amplification of a human lung cDNA library and subcloned to PCR II-Topo vector (Invitrogen). The orientation and sequence of the clones were confirmed by sequencing. The human PAI-1 cDNA containing complete coding and a 3' untranslated sequence in PCR II-Topo vector was linearized by XhoI or SacI, purified on 1% agarose gels, extracted with phenol-chloroform, and used as a template for in vitro transcription.

In vitro transcription. The full-length template or deletion products of PAI-1 were linearized with Hind III or Xba I, purified on 1% agarose gels, and transcribed in vitro with SP6 or T polymerase for sense or antisense mRNA according to the supplier's protocol except that 50 µCi (800 Ci/mmol) of [32P]UTP were substituted for unlabeled UTP in the reaction mixture with an Ambion in vitro transcription kit. Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activity of the product was 4.9–5.2 × 10^6 counts·min⁻¹·µg⁻¹.

RNase protection assay. Total RNA was isolated from SAECs or lung carcinoma-derived cells grown to confluence in T75 flasks with TRI reagent. We incubated total RNA (20 µg) with 32P-labeled cRNA probes in hybridization buffer, heated the material for 3 min at 95°C, and hybridized it at 42°C overnight. We then digested the material with RNase A/T1 mixture for 30 min at 37°C. RNase-resistant fragments were separated by electrophoresis on Tris-borate-EDTA (TBE)-polyacrylamide-urea gels, which were then dried and autoradiographed. The intensity of the bands was measured densitometrically and normalized against that of β-actin loading controls.

Preparation of cytosolic extracts. Cytosolic extracts of carcinoma-derived cells and SAECs were prepared by suspending the cell pellet in a buffer containing 25 mM Tris-HCl (pH 7.9), 0.5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, apotinin, leupeptin, and cystatin. The cells were lysed by four cycles of freezing and thawing as previously described (36) and centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was collected and used as the cytosolic fraction. The protein contents of the cytosolic extracts were measured with a Bio-Rad protein assay kit with serum albumin standards.

RNA-protein binding (gel mobility shift) assays. Binding assays were performed as previously described by Shetty et al. (36) with a uniformly 32P-labeled transcript of PAI-1. Reactions were performed at 30°C by incubating these transcripts (20,000 counts/min) with cytosolic extracts (20 µg) in 15 mM KCl, 5 mM MgCl₂, 0.25 mM EDTA, 0.25 mM dithiothreitol, 12 mM HEPES, pH 7.9, 10% glycerol, and Escherichia coli tRNA (200 ng/µl) in a total volume of 20 µl at 30°C for 30 min. The reaction mixtures were treated with 50 U of RNase T1 and incubated for an additional 30 min at 37°C. To avoid nonspecific protein binding, 5 mg/ml of heparin were added, and the mixture was incubated at room temperature for an additional 10 min. The samples were then separated by electrophoresis on 5% native polyacrylamide gels with 0.25× TBE running buffer. The gels were dried and autoradiographed at −70°C with Kodak XAR film.

Ultraviolet cross-linking assay. In these assays, RNA-protein binding reactions were done as described in RNA-protein binding (gel mobility shift) assays and then processed in a different manner. After the addition of heparin, the reaction mixtures were transferred to a 96-well microtiter plate and irradiated at 4°C at 2,500 µJ for 10 min with an ultraviolet (UV) Stratalinker chamber apparatus (Stratagene). The samples were then boiled for 5 min and separated on a 10% SDS-polyacrylamide gel under nonreducing conditions. The gels were dried, and 32P-labeled proteins were visualized by autoradiography. We also preincubated SAEC cytosolic extracts containing PAI-1 mRNA with tumor cell cytosolic extracts before PAI-1 mRNA binding to determine whether the latter extracts interfere with the PAI-1 mRNA-PAI-1 mRNA interaction.

Separate experiments were designed to test the specificity of the PAI-1 mRNA-PAI-1 mRNA interaction. In these experiments, cytosolic extracts were preincubated with a 400-fold molar excess of either unlabeled 3'UTR or coding region sense transcripts for PAI-1, uPA, or uPAR followed by a 32P-labeled PAI-1 3'-UTR sense transcript, and the mixtures were then analyzed by gel mobility shift and UV cross-linking assays.

RESULTS

Expression of PAI-1 in nonmalignant SAECs and lung carcinoma-derived cells. The expression of PAI-1 was determined by Western blot analysis in nonmalignant SAECs versus malignant lung carcinoma-derived cells. Malignant H460 cells secreted large amounts of PAI-1 because the conditioned medium contained relatively increased concentrations of PAI-1 compared with any of the lung carcinoma-derived cells (Fig. 1A). Among lung carcinoma-derived cells, detectable levels of PAI-1 were observed in A549, H460, and H157 cell-conditioned media, whereas the conditioned media of H1395 and H146 cells did not contain detectable...
amounts of PAI-1 protein. PAI-1 protein was detected only in A549, H157, and H460 cell lysates (Fig. 1B). These data confirm that H460 cells express relatively large amounts of PAI-1 and that malignant lung epithelial cells express variable amounts of PAI-1 that is dependent on the cell type. Malignant cells derived from large cell (H460) and squamous cell tumors (H157) and adenocarcinomas (A549) were found to express PAI-1.

PAI-1 mRNA expression. Having determined that PAI-1 expression was variably increased by malignant epithelial cells, we next asked whether the increased tumor cell expression of PAI-1 versus that of nonmalignant SAECs was due to relatively increased levels of PAI-1 mRNA. To address this question, we measured the levels of PAI-1 mRNA in normal and tumor cells by RNase protection assay with a PAI-1 antisense probe and densitometric scanning. As shown in Fig. 2, PAI-1 mRNA is elevated in the H460 and A549 tumor-derived cells compared with that in the nonmalignant SAECs. The disparity between the observed levels of protein and the steady-state levels of PAI-1 mRNA suggested to us that posttranscriptional as well as posttranslational regulatory mechanisms could be operative.

Effects of translational inhibitors on PAI-1 mRNA in SAECs and lung carcinoma-derived epithelial cells. Confluent cells were treated with buffer alone or with translational inhibitors including cycloheximide (10 µg/ml), anisomycin (250 ng/ml), and emitine (10 µg/ml) for 8 h. The levels of PAI-1 mRNA in each of the cell lines were analyzed by RNase protection assay and densitometric scanning. Cycloheximide, anisomycin, and emitine all increased levels of PAI-1 mRNA in SAECs and selected carcinoma-derived cells (Fig. 3). The increment in PAI-1 mRNA was greater in SAECs and H157 cells, which had relatively decreased basal PAI-1 mRNA levels versus those in H460 and A549 cells. These data suggest that regulation of PAI-1 mRNA stability involves the participation of a protein, raising the possibility that a PAI-1 mRNA-binding protein (mRNABP) could be involved in the process.

Stability of PAI-1 mRNA. We next performed experiments to determine whether the observed increments in PAI-1 mRNA in the malignant cell lines related, in part, to increased stability. The lung carcinoma-derived cells and SAECs were first treated with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole to inhibit ongoing transcription. Total RNA was next isolated from the cells at different intervals, and PAI-1 mRNA levels at these intervals were then analyzed by RNase protection assay.
extracts of SAECs showed that a 60-kDa radioactive protein band complexed with the PAI-1 3'-UTR (Fig. 5C) but not with the PAI-1 mRNA coding region (Fig. 5D). Expression of the PAI-1 mRNABP was undetectable in the lung carcinoma-derived cell lines except for H1395 cells, and the pattern was consistent with that identified by the gel mobility shift assays described in RNA-protein binding (gel mobility shift) assays.

Addition of a molar excess of homologous unlabeled RNA to the binding mixture diminished the complex formation in a concentration-dependent manner. However, incubation with the same amount of PAI-1 coding region transcript or uPA or uPAR 3'-UTR or coding region transcripts did not inhibit complex formation (Fig. 6). Predigestion of cell lysates with proteinase K abolished the formation of the complex, and addition of SDS before the binding reaction inhibited the RNA-protein interaction. The results suggest that a cellular protein(s) forms a specific complex with the PAI-1 mRNA 3'-UTR.

Preincubation of SEAC cytoplasmic extracts with cytosolic extracts of H460 or A549 cells but not of H157 cells inhibited the interaction between SAEC PAI-1-mRNABP binding and the PAI-1 mRNA 3'-UTR (Fig. 7).

**DISCUSSION**

The activation of plasminogen by either uPA or tissue-type plasminogen activator generates the protease plasmin, which provides an important means by which cellular proteolytic activity can be localized at the cell surface (40). Fibrinolysis initiated by uPA-mediated plasminogen activation contributes to remodeling of the stroma of solid tumors (5). uPA also influences a range of pathophysiological functions germane to neoplastic spread. These include cell migration (9, 40) and tumor invasion and metastasis (24, 25). The local generation of plasmin by uPA is one mechanism by which neoplastic cells degrade extracellular matrices to relocate from their original location to more distant sites (2, 40). The uPAR is essential for uPA-dependent pericellular proteolysis and is localized at the leading edge of migrating cells (40). uPAR is a high-affinity receptor for uPA. Precise regulation of plasminogen activator activity is thus a critical feature of many biological processes related to the propagation and spread of various tumors including carcinoma of the lung. The regulation of uPA may occur at various levels, including the synthesis and secretion of uPA, transcriptional or posttranscriptional control of uPA mRNA expression, and the interactions between uPA and its receptor (uPAR) or its major circulating inhibitor (PAI-1).

PAI-1 is an inhibitor of the serpin superfamily of protease inhibitors and is able to inhibit the activation of plasminogen by either uPA or tissue-type plasminogen activator (2, 40). The balance between the expression of PAI-1 and uPA appears to be an important determinant of remodeling of the tumor stroma. Because PAI-1 can inhibit extracellular matrix degradation initiated by uPA bound to uPAR, the interaction of PAI-1 with receptor-bound uPA appears to be an espe-
cially critical event in the pathogenesis of neoplastic growth (5, 30). In addition, tumor cell invasiveness is facilitated by saturation of uPAR with either exogenously supplemented uPA or overexpressed uPA (1, 24) so that expression of PAI-1 likely modulates the process. PAI-1 may also influence cellular proliferation because the binding of functionally active uPA to uPAR has been shown to be required to mediate cell proliferation in several cell types including fibroblasts and normal and malignant mesothelioma cells (37, 38). More recently, Shetty and Idell (35) also found that uPA is mitogenic for SAECs and lung carcinoma-derived cells and that these cells also express readily detectable amounts of uPA that might act as an autocrine mitogen through association with uPAR at the cell surface. Relative levels of PAI-1, uPA, and uPAR expressed by cells are interdependent because the trimeric complex of uPA, uPAR, and PAI-1 that may form at the cell surface (37) triggers the internalization and turnover of each of these proteins.

Several tumor types such as breast, ovarian, lung, prostate, and renal cell tumors express increased amounts of uPA, its receptor uPAR, and PAI-1 (4, 10–12, 22, 32). In general, the more aggressively the tumor cells behave, the more uPA and its receptor are expressed (5). Interestingly, PAI-1 was also found to be a marker of prognosis in cancer patients. Tumor levels of PAI-1 were found to be related to aggressive disease in patients with both node-positive and node-negative breast cancer (12). PAI-1, like uPA, was also a strong prognostic marker in patients with node-negative disease. The prognostic value of PAI-1 in patients with breast cancer was likewise confirmed in other studies (5, 8). In other cancers, including carcinoma of the stomach and ovarian carcinoma, increased expression of PAI-1 is similarly related to a poor prognosis. In patients with adenocarcinoma of the lung, increased levels of PAI-1 were associated with a poor prognosis, whereas there was no association between levels of uPA and survival (27). In patients with squamous cell
Fig. 5. Identification of PAI-1 mRNA binding proteins by gel mobility shift and ultraviolet (UV) cross-linking assays. A: cytoplasmic extracts prepared from lung carcinoma cells and normal nonmalignant SAECs were incubated with a $^{32}$P-labeled PAI-1 3'-untranslated region (UTR) mRNA sense transcript and digested with RNase T1 and heparin. Lanes 1-6, cytosolic SAECs and H1395, H157, H146, H460, and A549 cells, respectively; lane 7, free probe (Fp) alone. B: lanes 1-7, same as in A except that cytosolic extracts were hybridized with $^{32}$P-labeled PAI-1 coding region sense transcripts. RNA-protein complexes were analyzed by gel mobility shift assay. C: lanes 1-7, corresponding cytosolic extracts as in A were subjected to UV cross-linking and SDS-PAGE. D: lanes 1-7, same as in C except that cytosolic extracts were hybridized with $^{32}$P-labeled PAI-1 coding region sense transcripts. Arrows, RNA-protein complex.

Fig. 6. Demonstration of a specific interaction between PAI-1 mRNA 3'-UTR and SAEC cytoplasmic extracts. $^{32}$P-labeled PAI-1 mRNA 3'-UTR sense transcript was incubated with cytoplasmic lysate alone (lane 1) or with a 400-fold molar excess of unlabeled competitor RNA corresponding to PAI-1 3'-UTR (lane 2), PAI-1 coding region (lane 3), uPA 3'-UTR (lane 4), uPA coding region (lane 5), uPA receptor (uPAR) 3'-UTR (lane 6), and uPAR coding region (lane 7). Samples were analyzed by gel mobility shift assay (A) or UV cross-linking assay (B). Cytosolic extracts were treated with 2.5 mg/ml of protease K (lane 8) or 0.1% SDS (lane 9). Lane 10, probe alone. Arrows, RNA-protein complex.
carcinoma of the lung, high tumor tissue levels of PAI-1 combined with increased levels of uPAR were associated with a poor prognosis (26).

The results from experimental in vivo model systems of tumor metastasis also support the findings that high tissue levels of PAI-1, uPA, and uPAR in many tumor types predict a poor patient prognosis and strongly suggest an important role for the uPA system in cancer growth and metastasis (5). These observations provide additional support for a role of the fibrinolytic system in the pathogenesis of neoplastic spread. Regulation of the components of this system therefore assumes potential clinical importance. In previous studies, Shetty and colleagues elucidated the mechanisms that influence posttranscriptional control of uPAR mRNA in malignant mesothelioma cells (36), lung carcinoma cells (35), rabbit pleural mesothelial cells, and lung fibroblasts (34). Shetty and Idell (35) recently found that uPAR is overexpressed in cultured malignant mesothelioma and lung carcinoma cells and that uPAR mRNA is upregulated in these cells at a posttranscriptional level. In this study, we sought to determine whether PAI-1 expression in cultured human malignant lung carcinoma cells similarly involved posttranscriptional control. We found that cultured lung squamous cell carcinoma, large cell carcinoma, and adenocarcinoma cells expressed large amounts PAI-1 compared with normal SAECS and that PAI-1 gene expression in these cells was likewise subject to posttranscriptional regulation.

It has previously been reported (5, 7, 13, 16, 17, 31, 39) that the increased expression of PAI-1 induced by cytokines, endotoxin, glucocorticoids, or insulin occurs via transcriptional activation. We are aware of only a single prior report (6) of the posttranscriptional regulation of PAI-1, which was described in HepG2 cells. In this study, we confirm that PAI-1 is likewise regulated at the posttranscriptional level by lung carcinoma cells in vitro.

We also sought to test whether PAI-1 mRNAs are differentially regulated in lung carcinoma-derived cells versus nonmalignant SAECS. We found that PAI-1 mRNA has a short half-life of 1–2 h in SAECS. Conversely, PAI-1 mRNA is stabilized in selected lung tumor cells. In these cell lines, PAI-1 mRNA has a half-life of 8–12 h. The increased PAI-1 mRNA stability in these lung carcinoma-derived cells is consistent with the increased steady-state PAI-1 mRNA levels. In addition, increased expression of PAI-1 mRNA in these cells after transcriptional blockade confirms that PAI-1 is regulated at the posttranscriptional level.

In cultured SAECS and lung carcinoma-derived cells, a potential link between PAI-1 and translation emerged from in vitro studies showing that the protein synthesis inhibitors such as cycloheximide, emetine, and anisomycin superinduced PAI-1 mRNA. These observations suggest the likelihood that the posttranscriptional regulatory mechanism involves a protein. The induction of c-fos, c-myc, or granulocyte-macrophage colony-stimulating factor transcripts have been shown to involve similar posttranscriptional regulatory mechanisms (14, 21, 33).

The 60-kDa protein that interacts with PAI-1 mRNA is unlike the uPAR mRNA BP previously described by Shetty et al. (36) in that it binds to a 3'-UTR sequence rather than to the coding region. Occasionally, we also observed a radiolabeled band of lower molecular mass (Figs. 6B and 7B); however, this varies with the sample preparation and probably is a proteolytic degradation product. We now provide circumstantial evidence that supports the possibility that the PAI-1 mRNA interaction with the PAI-1 mRNA BP could play a regulatory role at the posttranscriptional level. First, translational inhibitors increased the stability of PAI-1 mRNA. These data suggest that posttranscriptional regulation of PAI-1 gene expression involves a protein that destabilizes PAI-1 mRNA. Second, the gel mobility shift and UV cross-linking experiments confirm an interaction of

Fig. 7. A: effect of tumor cell cytosolic extracts on PAI-1 mRNA-PAI-1 mRNA binding protein interaction. $^{32}$P-labeled PAI-1 mRNA 3'-UTR sense transcript was incubated with cytoplasmic lysate alone or with an equal amount of cytoplasmic extracts from H157, A549, and H460 cells. Samples were analyzed by UV cross-linking assay. Lane 1, Fp; lanes 2–5, cytoplasmic extract from SAECS, H157, A549, and H460 cells, respectively; lanes 6–8, SAECS cytosolic extracts mixed with cytosolic extracts of H157, A549, and H460 cells. Stable RNA-protein complexes were separated on SDS-polyacrylamide gel under nonreducing conditions. B: $^{32}$P-labeled PAI-1 mRNA 3'-UTR sense transcript was incubated with varying amounts (0, 5, 10, 25, 50, 75, 100, and 150 μg of protein; lanes 1–8, respectively) of SAECS cytoplasmic lysate. Samples were analyzed by UV cross-linking assay. Stable RNA-protein complexes were separated on SDS-polyacrylamide gel under nonreducing conditions.
PAI-1 mRNA with a distinct binding protein, suggesting that this PAI-1 mRNA-PAI-1-mRNABP complex formation could regulate message stability. Last, the PAI-1 mRNABP interaction with its corresponding mRNA was inversely correlated with increased stability of PAI-1 mRNAs. In lung carcinoma-derived cells overexpressing PAI-1 mRNA, interaction of the binding proteins with PAI-1 mRNA was reciprocally decreased compared with the complex formation identified in SAECs. These observations in aggregate suggest that the PAI-1 mRNABP-PAI-1 mRNA interaction may serve to destabilize PAI-1 mRNA. The specificity of the PAI-1 mRNABP was assessed by competition experiments in which an unlabeled PAI-1 3′-UTR but not the PAI-1 mRNA coding region, uPAR 3′-UTR, or uPA 3′-UTR sense probe was effectively competed by its labeled PAI-1 3′-UTR analog. The involvement of a specific protein factor is indicated by the finding that pretreatment with either SDS or proteinase K completely destroyed the complex. Mixing of SAEC cytosolic extracts containing PAI-1 mRNABP with cytosolic extracts from H460 or A549 cells that lack the binding protein inhibited the PAI-1 mRNABP-PAI-1 mRNA interaction. However, cytosolic extracts of H157 cells failed to alter the mRNA-protein interaction. These data suggest that some but not all tumor cells contain certain factors that can inhibit the PAI-1 mRNA-protein interaction. The mechanism(s) by which this inhibitory regulation occurs needs further study.

Several intriguing observations support the concept that there is a coordinated role for uPA, uPAR, and PAI-1 in tumor growth and that the expression of these proteins is regulated at the posttranscriptional level (3, 6, 18, 19, 23, 34–36). To reiterate, we found that the interaction of a destabilizing determinant in the coding of uPAR mRNA and a 50-kDa cytoplasmic uPAR mRNABP determines the abundance of uPAR mRNA in the cytoplasm (36). Overexpression of this coding region determinant results in increased cell surface uPAR and uPA-uPAR-mediated cellular functions relevant to tumor growth (35). Other mRNA destabilizing elements have likewise been linked to the pathogenesis of neoplastic growth. For example, it has also been reported that removal of another mRNA destabilizing element correlates with the increased oncogenicity of the protooncogene fos (29).

In summary, we found that PAI-1 mRNA was overexpressed by lung carcinoma-derived tumor cells compared with nonmalignant control SAECs. PAI-1 protein levels were elevated in some of the lung tumor cells compared with those in nonmalignant cells, and the variability of PAI-1 expression suggested that translational regulation is also involved in the differential expression of PAI-1. Increased PAI-1 mRNA levels in the lung carcinoma-derived cells are, in part, attributable to increased PAI-1 mRNA stability, confirming the involvement of posttranscriptional regulation. A newly identified 60-kDa PAI-1 mRNABP is present in the cytoplasm of SAECs and H1395 cells. This PAI-1 mRNABP is decreased in the cytoplasm of lung carcinoma-derived cells that express increased levels of PAI-1 mRNA. The PAI-1 mRNABP interacts with the 3′-UTR of PAI-1 mRNA, and formation of the complex may destabilize PAI-1 mRNA.

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