Urokinase plasminogen activator (uPA)1 is a serine protease that catalyzes the conversion of plasminogen to plasmin. The plasminogen/plasmin system includes the uPA, its receptor, and its inhibitor (plasminogen activator inhibitor-1). Interactions between these molecules regulate cellular proteolysis as well as adhesion, cellular proliferation, and migration, processes germane to the pathogenesis of lung injury and neoplasia. In previous studies, we found that uPA regulates cell surface fibrinolysis by regulating its own expression as well as that of the uPA receptor and plasminogen activator inhibitor-1. In this study, we found that uPA alters expression of the tumor suppressor protein p53 in Beas2B airway epithelial cells in both a time- and concentration-dependent manner. These effects do not require uPA catalytic activity because the amino-terminal fragment of uPA lacking catalytic activity was as potent as two chain active uPA. Single chain uPA also enhanced p53 expression to the same extent as intact two chain active uPA and the amino-terminal fragment. Pretreatment of cells with anti-β1 integrin antibody blocked uPA-induced p53 expression. uPA-induced p53 expression occurs without increased p53 mRNA expression. However, uPA induced oncprotein MDM2 in a concentration-dependent manner. uPA-induced p53 expression does not require activation of tyrosine kinases. Inactivation of protein-tyrosine phosphatase SHP-2 inhibits both basal and uPA-induced p53 expression. Plasminogen activator inhibitor-1 (PAI-1) inhibits both basal and uPA-induced p53 expression. Plasminogen activator inhibitor-1 also blocked uPA-induced p53 expression. The induction of p53 expression by exposure of lung epithelial cells to uPA is a newly recognized pathway by which urokinase may influence the proliferation of lung epithelial cells. This pathway could regulate pathophysiological alterations of p53 expression in the setting of lung inflammation or neoplasia.

Urokinase plasminogen activator (uPA)1 is a serine protease that catalyzes the conversion of plasminogen to plasmin (1–3).

The proteolytic enzyme uPA has been implicated in the pathogenesis of lung inflammation and the growth of lung tumors. uPA facilitates remodeling of the transitional stroma via the breakdown of basement membranes and extracellular matrix proteins, including fibrin (1–3), and is implicated in stromal remodeling that may occur in either lung inflammation or neoplasia.

Pathologic overexpression of uPA has been linked to a wide range of inflammatory and neoplastic lung diseases (4, 5). For example, high levels of uPA as well as PAI-1 and uPAR in lung tumor tissue correlate with poor prognosis (6, 7). In addition, increasingly compelling evidence for the involvement of the uPA system in cell proliferation, migration, tumor invasion, and metastasis (8–17) has positioned plasminogen activation as central to the propagation of neoplasms (5). In certain tumors, neoplastic growth can be effectively attenuated by PAI-1, presumably through its interaction with uPA (18).

In addition to its proteolytic properties, recent data from our laboratory has demonstrated that uPA enhanced expression of uPA, uPAR, and PAI-1 in Beas2B cells (19–21). Elaboration of these molecules influences two major features associated with lung neoplasia, including excessive proliferation of neoplastic cells and their capacity to invade normal tissues. It is now clear that the pathogenesis of lung neoplasia prominently involves expression of tumor suppressor protein p53 (22–24) as well as extracellular matrix degradation by uPA-mediated plasminogen activation (6, 7, 24). Among the plethora of potentially useful markers of tumor virulence that have been identified to date, both uPA and p53 appear to be promising (24, 25). The prognostic value of uPA and p53 accumulation has been reported in primary breast cancer patients (8–16), supporting their role in tumor progression and overall survival. However, no direct relationship between tumor suppression and extracellular matrix degradation has been established to date.

Stimulation of the lung epithelium by uPA elicits proliferative responses via signaling mechanisms that are incompletely characterized at present. Expression of growth factor activity has been attributed to uPA stimulation and suggests activation of such signaling processes (8–16). uPA bound to its receptor activates intracellular signaling through protein intermediates that are currently not well understood. Overexpression of either uPA, uPAR, or PAI-1 by tumor cells (19, 25–28) as well as potent growth factor activity of uPA in epithelial cells prompted us to test the possibility that uPA might regulate p53 expression in lung epithelial cells, a possibility that has not been explored previously, to our knowledge. We now report a new paradigm by which p53 expression by lung epithelial cells is regulated by uPA. We also characterize the responsible mechanism. This pathway could influence alveolar p53 expression and thereby modulate uPA-mediated responses of lung epithelial cells in lung injury or neoplasia.

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Urokinase plasminogen activator (uPA)1 is a serine protease that catalyzes the conversion of plasminogen to plasmin (1–3).
EXPERIMENTAL PROCEDURES

Materials—Culture media, penicillin, streptomycin, and fetal calf serum were purchased from Invitrogen; tissue culture plastics were from Discovery Labware, Herbymycin A, genistein, bovine serum albumin (BSA), ovalbumin, Tris base, aprotinin, dithiothreitol, phenylmethylsulfonyl fluoride, ammonium persulfate, 12-nitrylate 13-acetate were from Sigma. Acrylamide, bisacrylamide, and nitrocellulose were from Bio-Rad. Recombinant high molecular weight two chain (HMW) uPA was a generous gift from Drs. Jack Henkin from Abbott. The low molecular weight (LMW) amino-terminal fragment (ATF), anti-uPA, and anti-uPAR antibodies were obtained from American Diagnostics (Greenwich, CT). Anti-integrin antibodies were generated by co-transfection of 293 cells with pACCMV.pLpA and pM17 cDNA. Anti-phosphoserine 15 and serine 20-p53 antibodies were from Cell Signaling Technology (Beverly, MA). A5 and B428 compounds were from Angstrom Pharmaceuticals (San Diego, CA). XAR-x-ray film was purchased from Eastman Kodak Co.

Cell Cultures—Human bronchial epithelial cells (Beas2B) were obtained from the ATCC. These cells were maintained in L15C-9 or RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 1% glutamine, and 1% antibiotics as described previously (19). Primary cultures of human small airway epithelial (SAE) cells were obtained from Clonetics (San Diego) and cultured in the same media, as described previously (19).

Total Protein Extraction and Western Blotting—Cells were grown to confluence and were serum-starved overnight with RPMI 1640 media. The cells were treated with or without recombinant human high molecular weight chain uPA or other agents for selected times in serum-free media supplemented with 0.5% BSA. Following these treatments, the cells were lysed in lysis buffer (10 mM Tris-Cl, pH 7.4, containing 150 mM NaCl, 1% Triton X-100, 15% glycerol, 1 mM NaVO₄, 1 mM NaF, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 3–10 μg of aprotinin per 100 ml). The cell lysates were prepared using three cycles of freezing and thawing. Proteins from Beas2B cell lysates (50 μg) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 1% BSA in wash buffer for 1 h at room temperature followed by overnight hybridization with anti-p53 monoclonal antibody in the same buffer at 4 °C and washed, and p53-immunoreactive proteins were detected by enhanced chemiluminescence. The intensity of bands was measured by densitometry and normalized against that of β-actin.

Induction of p53 by Urokinase

SHP-2 cDNA was cloned into a eukaryotic expression vector pcDNA3.1. The Beas2B cells were transfected with vector cDNA or vector DNA containing SHP-2 cDNA using Lipofectamine, and stable cell lines were created by treating Beas2B cells with neomycin over 3 months as described above. The effect of SHP-2 overexpression on p53 induction was confirmed by Western blot.

Asessment of Apoptosis—In order to determine whether uPA induces apoptosis, we treated Beas2B cells with varying concentrations (0–10 ng/ml) of uPA. The cells were assayed for internucleosomal DNA fragmentation and labeled with [32P]dCTP using a Rediprime labeling kit from Promega (Madison, WI). Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activity of the product was 6–7 × 10⁶ cpm/μg. p53 mRNA Assessment by Northern Blotting—Northern blotting was used to assess the level of p53 mRNA. Confluent Beas2B cells were serum-starved overnight in RPMI/BSA media and treated with two chain human recombinant uPA for varying times (0–24 h) in the same media. Total RNA was isolated using TriReagent, and RNA (20 μg) was hybridized with [32P]dCTP using a Rediprime labeling kit from Promega (Madison, WI) followed by hybridizations and washes for 15 min at 65 °C in 1× SSC, 1% SDS, and 1× SSC, 1% SDS and 0.1% SSC, 1% SDS. The RNA was transferred to Hybond N+ according to the instructions of the manufacturer. Prehybridization and hybridization were done at 65 °C in NaCl (1 mM), SDS (1%) and 100 μg/ml salmon sperm DNA. Hybridization was performed with p53 cDNA probes (1 ng/ml) labeled to ~6–7 × 10⁶ cpm/μg of DNA overnight. After hybridization, the filters were washed twice for 15 min at 65 °C with 2× SSC, 1% SDS, 1× SSC, 1% SDS, and 0.1% SSC, 1% SDS. The membranes were exposed to x-ray film at ~70 °C overnight.

RESULTS

Time- and Concentration-dependent Induction of p53 by uPA—We found previously that lung carcinoma-derived cells differentially express uPA in vitro (25). Because uPA induces its own expression in Beas2B cells (20), we initially sought to determine whether uPA regulates p53 expression in Beas2B cells, a nonmalignant lung epithelial cell line. We therefore treated Beas2B cells with varying concentrations (0–1000 ng/ml) of uPA. The cells were assayed for internucleosomal DNA fragmentation and labeled with [32P]dCTP using a Rediprime labeling kit from Promega (Madison, WI). Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activity of the product was 6–7 × 10⁶ cpm/μg. p53 mRNA Assessment by Northern Blotting—Northern blotting was used to assess the level of p53 mRNA. Confluent Beas2B cells were serum-starved overnight in RPMI/BSA media and treated with two chain human recombinant uPA for varying times (0–24 h) in the same media. Total RNA was isolated using TriReagent, and RNA (20 μg) was hybridized with [32P]dCTP using a Rediprime labeling kit from Promega (Madison, WI) followed by hybridizations and washes for 15 min at 65 °C in 1× SSC, 1% SDS, and 1× SSC, 1% SDS and 0.1% SSC, 1% SDS. The RNA was transferred to Hybond N+ according to the instructions of the manufacturer. Prehybridization and hybridization were done at 65 °C in NaCl (1 mM), SDS (1%) and 100 μg/ml salmon sperm DNA. Hybridization was performed with p53 cDNA probes (1 ng/ml) labeled to ~6–7 × 10⁶ cpm/μg of DNA overnight. After hybridization, the filters were washed twice for 15 min at 65 °C with 2× SSC, 1% SDS, 1× SSC, 1% SDS, and 0.1% SSC, 1% SDS. The RNA was size-fractionated by gel electrophoresis and visualized with ethidium bromide. Alternately, we also determined the programmed cell death by measuring the annexin V-phosphatidylserine interaction using BD ApoAlert kit (BD Biosciences) followed by flow cytometry analysis as described by the manufacturer.

DNA Synthesis—Beas2B cells treated with or without tcuPA for 48 h were pulse-labeled with 1 μCi/ml [3H]thymidine for 8 h. After labeling, cells were washed once with cold 15% (w/v) trichloroacetic acid followed by four subsequent washes with cold 10% trichloroacetic acid. The cells were lysed, and cell associated radioactivity was measured in a liquid scintillation counter.
higher uPA concentrations beyond 5 nM, uPA inhibited p53 expression in Beas2B cells.

To rule out that contaminating lipopolysaccharides (LPS) in the high molecular weight uPA (HMW uPA) preparation we used was responsible for the induction of p53, we measured the LPS content of this preparation by the Limulus amebocyte lysate enzyme-linked immunosorbent assay method. We found that this HMW uPA preparation contains very negligible amounts (about 1 pg/ml) of LPS, making it unlikely that LPS could be responsible for the induction of p53 in these experiments.

**Induction of p53 by Endogenous uPA**—To confirm independently that the LPS content of the HMW uPA preparation did not account for the induction of p53, we next prepared uPA-overproducing Beas2B cells and vector-containing controls by transfecting these cells with the eukaryotic expression vector pRc/CMV2 containing uPA cDNA or pRc/CMV2 cDNA using lipofection. We analyzed the p53 expression of the stable cell lines by Western blotting. As shown in Fig. 2, Beas2B cells transfected with uPA cDNA expressed 3.5-fold more p53 in comparison to vector-transfected or nontransfected control cells. We also found a comparable increase in p53 protein expression by uPA cDNA-transfected cells compared with vector cDNA or nontransfected control cells by metabolic labeling.
with [35S]methionine, followed by immunoprecipitation using an anti-p53 monoclonal antibody (data not shown).

Induction of p53 mRNA Expression by uPA in Lung Epithelial Cells—By having determined that uPA mediates time-dependent p53 protein expression in Beas2B cell, we next sought to evaluate whether the increased expression of p53 was attributable to an increased level of p53 mRNA. We measured the steady state levels of p53 mRNA in uPA-treated Beas2B cells by Northern blotting using a p53 cDNA probe. As shown in Fig. 3, uPA failed to induce p53 mRNA in Beas2B cells. These data demonstrate that uPA increases p53 protein expression by Beas2B cells, as determined by Western blotting without altering expression of p53 mRNA.

Effect of uPA on Phosphorylation of p53 and MDM2 Expression—Because uPA did not induce p53 mRNA expression and p53 undergoes phosphorylation at multiple sites both in vitro and in vivo (32, 33), we asked whether uPA regulates p53 expression through phosphorylation at serine 15 and 20 on p53, which leads to reduced interaction with its negative regulator oncoprotein MDM2 (34). To test this hypothesis, we treated cells with varying amounts of uPA (0–1000 ng/ml) for 24 h at 37 °C, and we analyzed the cell lysates for phosphorylation of p53 at Ser-15 and Ser-20 by Western blotting using phosphospecific antibodies. As shown in Fig. 4a, uPA (50 ng/ml; 1 nm) induced Ser-15 phosphorylation. However, at higher concentrations beyond 250 ng/ml (5 nm), uPA failed to induce Ser-15 phosphorylation. uPA did not induce Ser-20 phosphorylation to any significant extent in Beas2B cells. We next treated Bas2B cells with uPA (50 ng/ml) for 0–24 h and analyzed for the phosphorylation of p53 at Ser-15, and we found that uPA induced Ser-15 phosphorylation as early as 10 min after the treatment, and this elevated level was maintained up to 24 h (Fig. 4b).

We next wanted to test whether uPA regulates p53 expression by altering MDM2 expression, an oncoprotein that prevents the accumulation of p53 by targeting it for ubiquitination and proteosomal degradation (35, 36). We initially treated Beas2B cells with two concentrations of uPA (50 and 1000 ng/ml) for 0–24 h and analyzed for the expression of MDM2 protein by Western blot using anti-MDM2 antibody. At concentrations of 50 ng/ml, uPA induced MDM2 expression around 3 h followed by a sharp decline to basal level around 24 h (Fig. 4c), whereas at higher uPA concentrations, MDM2 expression was induced, and this elevated level was maintained up to 24 h (Fig. 4d). We next treated Beas2B cells with varying amounts of uPA and found that uPA induces MDM2 expression in a concentration-dependent manner (Fig. 4e).

Effects of Phosphatase and Phosphotyrosine Kinase Inhibitors on uPA-mediated p53 Induction—To address our strong inference that uPA-mediated p53 expression involves cellular signaling, we pretreated Beas2B cells with herbinycin A and genistein separately or in combination with uPA. As shown in Fig. 5a, herbinycin A and genistein alone did not induce p53 expression nor did either inhibitor reverse uPA-mediated p53 expression by Beas2B cells. However, pretreatment of cells with sodium orthovanadate (a tyrosine phosphatase inhibitor) reversed uPA-mediated p53 expression. In order to determine the protein-tyrosine phosphatase involved in uPA-mediated p53 expression, we infected adenovirus overexpressing catalytically inactive mutants of SHP-1, SHP-2, as well as vector cDNA. As shown in Fig. 5b, both basal and uPA-induced p53 expression was inhibited in the Beas2B cells infected with dominant negative SHP-2 mutants. In order to confirm further the involvement of SHP-2 in p53 expression, we generated stable Beas2B cells overexpressing SHP-2 and determined the p53 expression by Western blot. As shown in Fig. 5c, SHP-2 overexpression induced p53 expression, indicating that protein-tyrosine phosphatase SHP-2 regulates p53 expression.

Effect of ATF and LMW uPA on p53 Expression—We next sought to determine the molecular domains of uPA that participate in the induction of p53. We therefore treated Beas2B cells with either the ATF or LMW fragment of uPA to determine whether the induction of p53 in Beas2B cells by uPA is mediated by the occupancy of its receptor uPAR. As shown in Fig. 6a, HMW uPA and the ATF alone or in combination induced p53 expression. Similarly single chain inactive HMW uPA also induced p53 expression. LMW uPA, a form of uPA that does not bind to uPAR, failed to induce p53 expression, indicating that the process requires the interaction of uPA with uPAR.

Effect of uPA Enzymatic Activity on uPA-induced Expression of p53—We next used alternative independent techniques to
ascertain the role of uPA enzymatic activity in the induction of p53 by uPA treatment of Beas2B cells. We first tested the ability of an anti-uPA monoclonal antibody to inhibit p53 expression. As shown in Fig. 6b, an antibody that blocks uPA enzymatic activity alone did not induce p53 expression and failed to alter uPA-mediated p53 expression. Similarly, pretreatment of uPA with B428 (an inhibitor of uPA) or the plasminogen activator inhibitor PAI-1 had no effect on uPA-mediated induction of p53. These data confirm that catalytic activity is not required for uPA-mediated induction of p53 in Beas2B cells.

Fig. 4. a, Effect of uPA on phosphorylation of p53 and MDM2 expression. Beas2B cells grown to confluence were treated with varying amounts of uPA (0–1000 ng/ml) for 24 h at 37 °C in basal medium containing 0.5% BSA. The total proteins were separated on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was immunoblotted with anti-phospho-Ser-15 and anti-p53 antibody. The same membrane was stripped and developed with anti-β-actin antibody. b, Beas2B cells grown to confluence were treated with 50 ng/ml uPA for 0–24 h, and cell lysates were subjected to Western blotting for the phosphorylation of the Ser-15 residue on p53 as described above (a). For the effect of uPA on MDM2 expression, Beas2B cells were treated with 50 ng/ml (c) or 1000 ng/ml (d) of uPA for varying (0–24 h) times as described above (a). Total proteins were separated on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was developed with anti-MDM2 antibody. The same membrane was stripped and developed with anti-β-actin antibody for equal loading. c, Beas2B cells grown to confluence were treated with varying amounts (0–1000 ng/ml) of uPA for 4 h, and lysate was subjected to Western blotting using anti-MDM2 antibody as described above (c and d). The figure shown is representative of three separate experiments.

Role of uPAR in uPA-mediated p53 Expression—To confirm further that the interaction between uPA and uPAR is required to induce p53 in Beas2B cells, we pre-treated the cells with selected agents that block uPAR for 2 h and then treated with uPA for 24 h. As shown in Fig. 6c, a uPAR antibody that blocks uPA-uPAR interaction alone or pretreatment with uPAR antibody followed by stimulation with uPA inhibited p53 expression. To confirm that uPA-mediated p53 expression involves uPAR, we next treated Beas2B cells with A5 compound (a uPAR antagonist) alone or in combination with uPA. As shown in Fig. 6c, A5 alone did not induce p53. However, A5 in combi-
nation with uPA inhibited uPA-mediated p53 expression. In a third approach, we removed uPAR from the cell surface by treating with phosphatidylinositol-phospholipase C (PI-PLC) and then tested to see if uPA would stimulate p53 expression. It is known that uPAR is a GPI-linked protein and that PI-PLC removes GPI-linked proteins, including uPAR, from the cell surface (19). PI-PLC treatment inhibited uPA-mediated p53 expression. These data confirm the involvement of uPAR in uPA-mediated p53 expression. In a separate experiment we found that PI-PLC completely cleaved uPAR from the cell surface (data not shown). These experiments provide further evidence that the uPA-mediated induction of p53 is mediated by the association of uPA with uPAR at the cell surface.

**Fig. 6. Effect of different fragments of uPA on p53 expression.**

**a,** Beas2B cells grown to confluence were treated with or without tcuPA (50 ng/ml), ATF (50 ng/ml), single chain uPA (scuPA) (50 ng/ml), and LMW (50 ng/ml) of uPA for 24 h at 37 °C in basal medium containing 0.5% BSA. Beas2B cells were treated with ATF for 2 h followed by tcuPA for 24 h (ATF+tcuPA). The total proteins were separated on an 8% SDS-polyacrylamide gel and then transferred to nitrocellulose membrane. The membrane was immunoblotted with an anti-p53 antibody. The data are representative of the findings of three separate experiments, and the mean density is presented as bar graph. PBS, phosphate-buffered saline. **b,** effect of uPA inhibitors on uPA-mediated p53 expression. Beas2B cells grown to confluence were treated with or without B-428 (0.02 mM), anti-uPA monoclonal antibody (mAb) (2 μg/ml), or plasminogen activator inhibitor (PAI-1) (4 μg/ml) for 2 h followed by uPA for 24 h at 37 °C in basal medium containing 0.5% BSA. The total proteins were separated on an 8% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was immunoblotted with anti-p53 antibody. Data representative of three independent experiments are illustrated. **c,** role of uPAR in uPA-mediated p53 induction. Beas2B cells were grown to confluence and then treated with or without anti-uPAR antibody (2 μg/ml), A5 (1 μg/ml) compound, and PI-PLC (10 units/ml) for 2 h followed by uPA for 24 h at 37 °C in basal medium containing 0.5% BSA, and proteins were isolated. The total proteins were separated on an 8% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was immunoblotted with an anti-p53 antibody. Data representative of four independent experiments are shown, and mean densities of individual bands are presented as bar graphs.
Effect of Integrins on p53 Expression—Because uPA-mediated p53 expression involves uPA interaction with uPAR, and uPAR cannot transduce signals directly to the interior because of its GPI anchor, we sought to determine the accessory molecule involved in uPA-mediated p53 expression. We therefore treated Beas2B cells with anti-αvβ3, β1, CD18/CD11b, anti-uPAR antibodies, or IgG for 2 h followed by stimulation with uPA for 24 h. The cell lysates were prepared, and p53 expression was determined by Western blot. As shown in Fig. 7, only the anti-β1 and the anti-uPAR antibody-treated cells inhibited uPA-induced p53 expression.

Effect of Plasmin on uPA-mediated p53 Expression—We next sought to determine whether plasmin, a product of uPA-mediated plasminogen activation known to be involved in remodeling of the extracellular matrix, might influence uPA-induced p53 expression. Treatment with plasmin alone or in combination with uPA failed to alter p53 expression (data not shown).

Effect of uPA Concentration on Beas2B Cell Apoptosis—To confirm that uPA-mediated expression of p53 induces apoptosis, we treated Beas2B cells with varying concentrations of uPA (0–3000 ng/ml) and quantified apoptosis by flow cytometry. Flow cytometric analysis of annexin V-treated cells indicated that uPA at 10–100 ng/ml concentration induced apoptosis at a moderate (35%) level. A similar result was also observed when apoptosis was determined by DNA fragmentation analysis using agarose gel electrophoresis and ethidium bromide staining of genomic DNA (data not shown).

Induction of DNA Synthesis by uPA—uPA-induced DNA synthesis in Beas2B cells in a concentration-dependent manner with maximal induction at 500 ng/ml (Fig. 8b).

**DISCUSSION**

uPA-dependent proteolysis is critical for cellular migration and tissue remodeling in inflammation, tumor growth, and metastasis (25–28). The uPA-uPAR interaction can promote cellular movement and decrease cellular attachment to the surrounding ground substance, processes that may contribute to remodeling of the lung in the acute respiratory distress syndrome or the interstitial lung diseases (37, 38). The interaction between uPA and uPAR at the cancer cell surface also appears to be a critical event in the pathogenesis of neoplastic growth and metastasis, mediating tissue remodeling and desmplasia, tumor cell invasion, adhesion, and proliferation of neoplastic cells (1, 5, 10). Binding of uPA to uPAR mediates cell proliferation in several cell types, including nonmalignant lung epithelial cells, lung carcinoma-derived cells, and malignant mesothelioma (12, 39). Tumor cell invasion is often facilitated by a dramatic increase in the production of the plasminogen activators, particularly uPA (39–43). The tumor suppressor p53 gene is mutated or suppressed in the majority of human tumors (22), and introduction of the wild-type p53 gene into p53-deficient tumor cells suppresses transformation and tumorigenesis and inhibits proliferation of many malignant cell types (24). Pathways that regulate the uPA-uPAR system and p53 expression are therefore germane to the pathogenesis of lung injury and neoplasia.

A shift toward proteolysis is more prevalent in malignant tissues, which probably facilitates tumor invasion of adjacent normal tissues. In this context, uPA appears to play a cooperative role in the propagation and spread of neoplasms (5). A change in proto-oncogene and tumor-suppressor gene expression (41) may facilitate epithelial cell transformation and alter the capacity of lung epithelial cells to effect proteolytic degradation of the extracellular matrix. The activity of the p53 tumor suppressor protein in particular has a key role in this process. We therefore sought to elucidate interactions by which uPA and p53 can interact and to determine specifically how p53 is expressed and regulated by uPA in lung epithelial cells.

In this study, we present evidence that uPA induces expression of p53 in cultured Beas2B and that the effect is also observed in primary SAE cells. This pathway provides a versatile regulatory system through which the uPA concentration of the ambient microenvironment and pericellular proteolysis could regulate cellular transformation by up-regulating expression of p53. This molecular mechanism may be relevant for understanding the role of uPA in lung injury and neoplasia.
a crucial determinant of epithelial cell transformation or the invasiveness of lung carcinomas, in which excessive uPA-dependent pericellular proteolysis increases cellular proliferation and invasiveness (5).

uPA-mediated p53 expression does not induce p53 mRNA induction, indicating the involvement of post-transcriptional mechanisms such as enhanced translation of p53 mRNA and/or decreased proteolytic degradation of the protein (42). Activation of p53 following genotoxic damage is achieved by induction of p53 levels and by modifications of the p53 protein such as phosphorylation and acetylation (43–47). DNA damage caused by ionizing radiation, chemotherapeutic drugs, UV radiation, alkylation agents, and environmental carcinogens induces p53 through alteration of p53 protein stability (48–54). However, uPA is a serine protease involved in extracellular matrix degradation to which no DNA damage or repair function has been attributed. Altered p53 protein expression because of translation rather than transcription or mutation in the p53 gene has been observed in blast cells obtained from patients with acute myelogenous leukemia (55). In the present study we have observed that uPA induces phosphorylation of the Ser-15 residue of p53 in a concentration-dependent manner consistent with the uPA-mediated induction of p53. Activation of p53 can lead either to cell arrest, DNA repair, or apoptosis. At low concentrations of uPA, Beas2B cells undergo apoptosis as a consequence of increased p53 accumulation. This is accomplished by two independent mechanisms. First, uPA induces Ser-15 phosphorylation at a concentration of 50 ng/ml, which impairs p53 interaction with MDM2 and its degradation. Second, at a low concentration uPA also fails to maintain elevated levels of MDM2 protein required for p53 turnover. However, at higher uPA concentrations induction of MDM2 protein as well as inhibition of Ser-15 phosphorylation leads to increased degradation of p53, which results in reduced apoptosis and increased DNA synthesis.

In previous studies, we and others have found that uPA influences uPA, uPAR, and PAI-1 expression through post-transcriptional pathways (19–21). Similar influences on uPA, uPAR, and PAI-1 mRNA stabilities are also discovered in lung cancer-derived cell lines (25, 56). It has been reported previously that p53 regulates fibrinolysis by inhibiting uPA expression through suppression of promoter and enhancer activity of the uPA gene and the activation of the PAI-1 promoter (58, 59). The present study suggests that uPA may overcome the transcriptional control of p53 over the expression of uPA and PAI-1 by inhibiting the p53 expression itself at higher a concentration. Activation of c-Myc stabilizes p53 protein in quiescent mouse fibroblasts (57), indicating the involvement of p53-induced apoptosis and cell cycle reentry to prevent cell proliferation induced by oncogene activation. Because uPA is known to induce cell proliferation on a wide variety of cell types, including fibroblasts (8–15), uPA-mediated p53 induction probably mediates apoptosis as a safeguard to prevent unrestrained cell proliferation induced by uPA. It is highly likely that uPA at a higher concentration probably induces cell proliferation by disrupting this safeguard mechanism through MDM2-mediated ubiquitination.

The identification of a newly defined mechanism by which the uPA regulates expression of p53 suggests the possibility that other novel pathways could likewise influence cellular transformation. Based upon our previous observations, the induction of uPA, uPAR, and PAI-1 by uPA in lung epithelial cells could likewise contribute to altered p53 expression, possibly by influencing the kinetics of internalization of trimeric complexes of uPA, PAI-1, and uPAR. The precise regulatory levels by which uPA induces p53 expression by lung epithelial cells remain to be elucidated in studies that extend this report.

We found that the induction of p53 by uPA in Beas2B cells requires receptor interaction and that the LMW fragment could not reproduce the effect. Engagement of β1 integrin with specific antibody inhibited uPA-induced p53 expression, indicating the intricate connections between expression of p53 and uPA–uPAR integrin-mediated adhesion. There are precedents for this mode of uPA-mediated induction of cellular responses. uPA is mitogenic for several different cell types. The mitogenic response likewise requires receptor interaction with uPA in pleural mesothelioma as well as epidermal tumor cell lines (8–15). The inhibitory effect of a tyrosine phosphatase inhibitor on p53 expression indicates that the process involves cellular signaling mainly through activation of tyrosine phosphatases. Alternatively, uPA stimulation of Beas2B cells may also induce synthesis of growth factors or cytokines that in turn may induce p53 protein. The elucidation of the mechanisms responsible for the prolonged effect of uPA on the p53 protein level remains to be determined. Phosphorylation of serines 15 and 20 following genotoxic stress (25, 43–56) has been demonstrated to impair interaction between p53 and MDM2, resulting in enhanced p53 accumulation (45). In contrast to DNA damage, differential expression of p53 predicated on uPA concentration can act as a selective pressure during tumor growth for the elimination of cells with wild-type p53 and clonal expansion of cells with diminished p53 expression. Induction of p53 by uPA in Beas2B cells clearly is not mediated via generation of plasmin because exposure of cells to treatment with plasmin did not affect p53 expression.

In summary, we now report that uPA stimulates expression of p53 by lung epithelial cells in culture. If operative in vivo, this pathway could contribute to the relative local overexpression of uPA and the neoplastic transformation associated with lung cancer. To our knowledge, this newly identified pathway is the first description of the ability of uPA to regulate the expression of p53 in any cell type.

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The identification of a newly defined mechanism by which the uPA regulates expression of p53 suggests the possibility that other novel pathways could likewise influence cellular transformation. Based upon our previous observations, the induction of uPA, uPAR, and PAI-1 by uPA in lung epithelial cells could likewise contribute to altered p53 expression, possibly by influencing the kinetics of internalization of trimeric complexes of uPA, PAI-1, and uPAR. The precise regulatory levels by which uPA induces p53 expression by lung epithelial cells remain to be elucidated in studies that extend this report. We found that the induction of p53 by uPA in Beas2B cells requires receptor interaction and that the LMW fragment could not reproduce the effect. Engagement of β1 integrin with specific antibody inhibited uPA-induced p53 expression, indicating the intricate connections between expression of p53 and uPA–uPAR integrin-mediated adhesion. There are precedents for this mode of uPA-mediated induction of cellular responses. uPA is mitogenic for several different cell types. The mitogenic response likewise requires receptor interaction with uPA in pleural mesothelioma as well as epidermal tumor cell lines (8–15). The inhibitory effect of a tyrosine phosphatase inhibitor on p53 expression indicates that the process involves cellular signaling mainly through activation of tyrosine phosphatases. Alternatively, uPA stimulation of Beas2B cells may also induce synthesis of growth factors or cytokines that in turn may induce p53 protein. The elucidation of the mechanisms responsible for the prolonged effect of uPA on the p53 protein level remains to be determined. Phosphorylation of serines 15 and 20 following genotoxic stress (25, 43–56) has been demonstrated to impair interaction between p53 and MDM2, resulting in enhanced p53 accumulation (45). In contrast to DNA damage, differential expression of p53 predicated on uPA concentration can act as a selective pressure during tumor growth for the elimination of cells with wild-type p53 and clonal expansion of cells with diminished p53 expression. Induction of p53 by uPA in Beas2B cells clearly is not mediated via generation of plasmin because exposure of cells to treatment with plasmin did not affect p53 expression. In summary, we now report that uPA stimulates expression of p53 by lung epithelial cells in culture. If operative in vivo, this pathway could contribute to the relative local overexpression of uPA and the neoplastic transformation associated with lung cancer. To our knowledge, this newly identified pathway is the first description of the ability of uPA to regulate the expression of p53 in any cell type.

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