The fibrinolytic system and the regulation of lung epithelial cell proteolysis, signaling, and cellular viability

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Shetty S, Padijnayveetil J, Tucker T, Stankowska D, Idell S. The fibrinolytic system and the regulation of lung epithelial cell proteolysis, signaling, and cellular viability. Am J Physiol Lung Cell Mol Physiol 295: L967–L975, 2008. First published October 3, 2008; doi:10.1152/ajplung.90349.2008.—The urokinase-type plasminogen activator (uPA), its receptor (uPAR), and plasminogen activator inhibitor-1 (PAI-1) are key components of the fibrinolytic system and are expressed by lung epithelial cells. uPA, uPAR, and PAI-1 have been strongly implicated in the pathogenesis of acute lung injury (ALI) and pulmonary fibrosis. Recently, it has become clear that regulation of uPA, uPAR, and PAI-1 occurs at the posttranscriptional level of mRNA stability in lung epithelial cells. uPA further mediates its own expression in these cells as well as that of uPAR and PAI-1 through induction of changes in mRNA stability. In addition, uPA-mediated signaling controls the expression of the tumor suppressor protein p53 in lung epithelial cells at the posttranslational level. p53 has recently been shown to be a trans-acting uPA, uPAR, and PAI-1 mRNA-binding protein that regulates the stability of these mRNAs. It is now clear that signaling initiated by uPA mediates dose-dependent regulation of lung epithelial cell apoptosis and likewise involves changes in p53, uPA, uPAR, and PAI-1 expression. These findings demonstrate that the uPA-uPAR-PAI-1 system of lung epithelial cells mediates a broad repertoire of responses that encompass but extend well beyond traditional fibrinolysis, involve newly recognized interactions with p53 that influence the viability of the lung epithelium, and are thereby implicated in the pathogenesis of ALI and its repair.

fibrinolysis; urokinase; plasminogen activator inhibitor-1; fibrin; acute lung injury

Relevance and Goals

Lung epithelial cells express urokinase-type plasminogen activator (uPA), its receptor (uPAR), plasminogen activator inhibitor-1 (PAI-1), and p53. Decreased alveolar fibrinolysis and apoptosis of airway epithelial cells due to altered expression of components of the fibrinolytic system or p53, respectively, are independently and strongly implicated in the pathogenesis of acute lung injury (ALI) and subsequent fibrosis. In this article, we describe the intricate relationship between these processes and newly recognized interactions involving coordinate interactions between the tumor suppressor protein p53 and components of the fibrinolytic pathways that may influence epithelial cell responses to ALI and fibrotic repair. We hope and anticipate that the better understanding of these interactions will ultimately lead to the identification of better treatment strategies and improved patient outcomes.

Lung Epithelial Cell Expression of uPA, uPAR, and PAI-1 and Control of Fibrinolytic Activity in the Normal Lung

Plasminogen in plasma or extravascular fluids can be converted to the active endopeptidase plasmin by uPA or tissue-type plasminogen activator (tPA) (13, 16, 60, 68). Two major endogenous plasminogen activator inhibitors have been identified, PAI-1 and PAI-2, which are produced by platelets and endothelial, mesothelial, and epithelial cells, including those of the lung (47, 60, 90). PAI-1 is the major PAI in plasma and extravascular fluids and has been implicated in the fibrinolytic defect associated with acute respiratory distress syndrome (ARDS) and various forms of lung injury (15, 37). Receptors for uPA and plasminogen allow proximate cell surface activation of plasminogen by lung epithelial and other cell types.

The fibrinolytic system is operational and prevents fibrin deposition in the small airways and alveolar compartment of the normal lung. In bronchoalveolar lavage (BAL) fluids obtained from patients with no overt lung disease, uPA- rather than tPA-related fibrinolytic activity is readily detectable, whereas there is little detectable procoagulant activity (37, 38). Plasma substrates of the procoagulant and fibrinolytic systems are virtually undetectable in these fluids, an observation that in part explains the absence of extravascular fibrin in the normal lung. In the normal murine lung, fibrinolytic activity has been shown to be expressed at the epithelial surface of the airways and alveoli (86), which may likewise limit extravascular fibrin deposition. Alveolar macrophages and lung fibroblasts likewise express uPA, which may supplement the fibrinolytic activity that is constitutively expressed by the epithelium of the normal lung.
In the setting of ALI, homeostatic control of fibrin turnover is disrupted with dramatic impairment of BAL fibrinolytic activity (Fig. 1). A robust procoagulant response characteristically occurs in BAL fluids from patients with ALI or in accelerated forms of pulmonary fibrosis in association with a concurrent decrement in fibrinolytic activity (1, 11, 12, 17, 18, 30, 35, 37, 95, 97-99). These changes reflect initiation of coagulation by increased local expression of tissue factor associated with factor VIIa, and the decrement of fibrinolytic activity is primarily attributable to increased expression of PAI-1 and antiplasmins. The elaboration of PAI-1 and antiplasmins including α2-antiplasmin and α2-macroglobulin suppress BAL fibrinolytic activity in series by inhibition of uPA and plasmin, respectively (29, 30, 59, 86-88, 100). Whereas the fibrinolytic activity of the lung epithelium is preserved after ALI (48, 75), the changes in extravascular lung lining fluids collectively promote alveolar fibrin deposition, which is consistently observed in virtually all forms of clinically encountered ALI. If persistent, the fibrinous transitional neomatrix may rapidly organize with accelerated fibrotic repair (86).

Expression of uPA, uPAR, and PAI-1 are of particular importance in the control of alveolar proteolytic activity and lung remodeling in virtually all forms of ALI, including ARDS (36, 38, 40) and the interstitial lung diseases (2, 4, 6-10, 15, 16, 20-23, 40). These molecules and p53 are all elaborated by lung epithelial cells, including simian virus 40 (SV40)-transformed Beas2B human airway epithelial cells and primary small airway epithelial cells. We reported that these cells regulate expression of uPA, uPAR, and PAI-1 at the posttranscriptional level, extending prior work showing that these molecules are subject to transcriptional control (47, 49, 70-72, 76-79). We therefore infer that these pathways may similarly be operative in small airways or the alveoli in the setting of lung injury and remodeling and that they may precisely regulate the generation of plasmin in these processes (24, 41, 89). Whereas alveolar fibrinolysis can appropriately limit excessive deposition of the fibrinous neomatrix after injury, unrestricted or excessive generation of plasmin could theoretically effect pathophysiological degradation of extracellular matrix proteins such as laminin, fibronectin, collagen, and elastin by direct effects or activation of latent matrix metalloproteinases. It may be that posttranscriptional control provides lung epithelial cells with the capacity to fine-tune and calibrate appropriate expression of the components of the fibrinolytic system during remodeling after ALI.

The Role of Components of the Fibrinolytic System in Outcomes of ALI

Evidence from preclinical and clinical studies. A large body of evidence implicates components of the fibrinolytic system in the pathogenesis of ALI and its repair, as recently reviewed in great detail by Sisson and Simon (86). A number of studies implicate uPA and PAI-1 in the pathogenesis of pulmonary fibrosis induced by bleomycin (25, 46, 54). In brief review, bleomycin- or hyperoxia-induced lung injury in mice with deficiency of PAI-1 is attenuated. Overexpression or exogenous administration of uPA is likewise associated with reduced fibrosis in lung injury induced by bleomycin. Interestingly, deficiency of uPAR does not appear to substantively alter the outcome of bleomycin-induced lung injury in mice (78).

While fibrinogen is not required for the development of bleomycin-induced pulmonary fibrosis (reviewed in Ref. 75), the observations clearly link uPA and PAI-1 to the pathogenesis of ALI and its repair.

The lung epithelium has been shown to be a persistent source of fibrinolytic activity during bleomycin lung injury (53), even though lung lavage fibrinolytic activity is concurrently depressed (reviewed in Ref. 30). These observations support the concept that lung epithelial cells retain the ability

Fig. 1. Inflammatory cell infiltration and fibrin deposition characterize alveolar inflammation in acute lung injury (ALI) and its repair and are associated with a local increase in procoagulant activity with a concurrent decrement in alveolar fibrinolysis compared with the situation in the absence of lung injury. Fibrin is undetectable in the alveolar compartment under normal circumstances.
to remodel airway or alveolar fibrin even when this activity is quenched in BAL in the face of saturating levels of PAI-1 and antiplasmin. In patients with ALI, elevated levels of PAI-1 in pulmonary edema fluid samples are likewise associated with increased mortality, suggesting the probability that the effect may relate to impaired capacity to clear florid transitional fibrin (57).

Induction of uPA, uPAR, and PAI-1 by Lung Epithelial Cells

Epithelial cells synthesize and secrete a 55-kDa proenzyme single chain form of uPA, or prouPA, which is activated by plasmin and other proteases. Expression of uPA by these cells is augmented by proinflammatory stimuli or particulates that may be inhaled (10, 28, 29, 39, 61, 66, 72). Lung epithelial cells also synthesize and express uPAR, which is likewise implicated in a wide range of processes in which the lung epithelium participates: cellular signaling (3, 75), adhesion (14, 20), proteolysis, differentiation (16, 37, 47), regulation of inflammatory cell traffic (32, 33), and cellular proliferation (16, 72, 75–79). Lung epithelial cells further express PAI-1 (68, 74, 85, 91), which is involved in the internalization of trimeric uPA-uPAR-PAI-1 complexes from the cell surface, which results in restoration of uPAR at the surface of lung epithelial cells.

Regulation of uPA, uPAR, and PAI-1 by Lung Epithelial Cells at the Level of mRNA Stability

Although it is well-established that genes coding for uPA, uPAR, and PAI-1 are regulated at the transcriptional level, recent observations indicate that these genes are also subject to regulation at the posttranscriptional level in lung epithelial and pleural mesothelial cells, as we (65) have previously reviewed in detail. Updated information about how these pathways relate to uPA-mediated signaling, control of p53 expression, and viability of lung epithelial cells is considered in the following sections. These pathways have been shown to be operative in cultured bronchial and primary small airway epithelial cells (Fig. 2). It is likely that alveolar epithelial cells respond similarly as these pathways are operative in diverse cell types including pleural mesothelial, endothelial cells, monocytes, and lung carcinoma cells. The stability of each of these mRNAs depends on coordinate interactions between specific mRNA sequences/determinants or "cis elements" that contain information that specifically regulates mRNA turnover and newly identified specific mRNA-binding proteins. These proteins, otherwise called "trans elements," possess the capacity to bind single or multiple coding 3′- or 5′-untranslated mRNA sequences.

Fig. 2. The diagram depicts regulation of the urokinase-type plasminogen activator (uPA), its receptor (uPAR), and plasminogen activator inhibitor-1 (PAI-1) expression in lung epithelial cells at the posttranscriptional level of mRNA stability by mRNA-binding proteins. Phosphorylation of mRNA-binding proteins by stimuli such as proinflammatory cytokines alters uPA, uPAR, and PAI-1 mRNA-mRNA-binding protein interactions and the expression of these proteins through posttranscriptional regulation of mRNA stability.
Regulation of uPAR at the Level of mRNA Stability

It is now clear that uPAR expression is regulated at the posttranscriptional level in lung epithelial cells as well as other resident lung cells, including lung fibroblasts, pleural mesothelial and malignant mesothelioma cells, or lung carcinoma cells (71, 72, 76). uPAR mRNA stability is increased in these cells by treatment with cytokines and translational inhibitors (76). The ability of translational inhibitors to augment uPAR mRNA stabilization suggests that a destabilizing protein is involved in the regulatory process. A 50-kDa protein, phosphoglycerate kinase (PGK; otherwise a member of the Krebs cycle) selectively interacts with a coding region sequence of uPAR mRNA to regulate its stability (76, 80). The binding sequence for PGK on uPAR mRNA has been mapped and encompasses a 51-nt sequence within the coding region (76). A number of proinflammatory agents, PMA, LPS, TNF-α, and TGF-β, are now known to induce cell surface uPAR expression through stabilization of uPAR mRNA. This effect is related to tyrosine phosphorylation of PGK that occurs when cells are stimulated by these agents (80). Tyrosine-phosphorylated PGK fails to interact with the uPAR mRNA coding region, which results in stabilization of uPAR mRNA (Ref. 80; Fig. 2). Treatment of lung epithelial cells with a tyrosine kinase inhibitor reverses stabilization of uPAR mRNA by PMA or TGF-β and enhances the interaction of PGK with uPAR mRNA (80). Inhibition of tyrosine phosphatase activity conversely enhances uPAR mRNA stability and cell surface uPAR expression. The ability of PGK to regulate uPAR mRNA stability is independent of the catalytic activity of PGK.

It is somewhat predictable that the regulation of uPAR mRNA stability would be more complex, and our more recent findings support this inference. Other proteins, including the 40-kDa hnRNPC (67) and 30-kDa HuR (94), likewise regulate uPAR mRNA stability in lung epithelial cells. Unlike the PGK-uPAR mRNA coding region interaction, the hnRNPC-uPAR mRNA 3’-UTR interaction decelerates uPAR mRNA degradation (67). Based on chimeric gene analyses, it appears that the hnRNPC-binding 3’-UTR determinant, like the PGK-binding uPAR coding region determinant, contains uPAR mRNA degradation activity. The relative contribution of the hnRNPC and PGK-binding sequence determinants to the regulation of uPAR mRNA stability was confirmed by creation of deletion mutant mRNAs. Deletion of either of these determinants stabilizes uPAR mRNA, and deletion of both has an additive effect on uPAR mRNA stability (S. Shetty, unpublished observations). Like PGK, hnRNPC undergoes tyrosine phosphorylation when lung epithelial cells are exposed to selected proinflammatory cytokines. However, unlike PGK, tyrosine phosphorylation enhances the ability of hnRNPC to associate with the 110-nt 3’-UTR determinant and stabilize of uPAR mRNA (67). It is possible that uPAR mRNA stability in these cells may also be regulated by adenylylate/uridylylate-rich elements, a possibility to be addressed in future studies.

Regulation of PAI-1 at the Level of mRNA Stability

Two forms of PAI-1 mRNA have been described, a labile 3.2 kb (half-life 0.85 h) and a relatively more stable 2.2 kb (half-life 2.5 h) species. The 3.2-kb species of human PAI-1 mRNA, characterized by a long 3’-UTR, has a relatively short half-life. Stabilities of both mRNA species are regulated at the posttranscriptional level. PAI-1 mRNA binds complexes with proteins of molecular masses ranging from 38 to 76 kDa that interact with cAMP responsive sequences to regulate PAI-1 mRNA in murine hepatocytes (93). These observations indicate that a posttranscriptional component contributes to expression of PAI-1 in a variety of cell types, thereby suggesting the possibility that PAI-1 mRNA-binding protein interactions could likewise influence PAI-1 mRNA stability in lung epithelial cells.

In studies performed to address this possibility, we (74) previously reported that human tracheal epithelial cells express PAI-1 and recently determined that selected lung carcinoma cells express more PAI-1 antigen than primary cultures of normal small airway epithelial cells. The regulation of PAI-1 mRNA stability may be of particular clinical importance in the context of ALI, as previous reports suggest that increased expression of PAI-1 antigen relates to poor outcome in lung cancer patients as well as those with ALI (46, 47, 59, 91).

We recently extended this work and found that cytokines including TNF-α and TGF-β increase the stability of PAI-1 mRNA in Beas2B and primary small airway epithelial cells (S. Shetty, unpublished observations). uPA similarly induces PAI-1 in Beas2B and primary small airway epithelial cells through posttranscriptional stabilization of PAI-1 mRNA in a concentration-dependent manner (68). These effects likely contribute to the increased expression of this inhibitor in the injured lung. The question as to whether this pathway is operative in vivo or if it is altered in ALI or its repair is a priority that is now being addressed by our group.

Although the mechanism of PAI-1 regulation by lung epithelial cells at the mRNA stability level is incompletely understood at present, the interaction between a sequence within the untranslated region of PAI-1 mRNA and a 60-kDa PAI-1 mRNA-binding protein appears to be integral to the process. In small airway epithelial cells, this interaction is readily detectable and correlates with a relatively short PAI-1 mRNA half-life. In TGF-β- or TNF-α-treated Beas2B cells or malignant lung carcinoma-derived cells, the binding interaction is attenuated or undetectable. This inverse relationship between PAI-1 mRNA stability and the PAI-1 mRNA-binding protein-PAI-1 mRNA interaction suggests that the interaction is likewise destabilizing.

The posttranscriptional regulation of PAI-1 mRNA appears to differ in lung epithelial cells vs. that previously described in other cell types. For example, PAI-1 mRNA is also regulated at the posttranscriptional level in hepatocytes by 8-bromo-cAMP, and cAMP-mediated destabilization of PAI-1 mRNA in these cells involves association of a 134-nt PAI-1 3’-UTR mRNA sequence with a 50-kDa PAI-1 mRNA-binding protein (93). Recently, a 81-kDa nuclear protein was shown to bind the 3’-UTR of PAI-1 mRNA in an iron-sensitive manner, and iron deprivation stabilizes PAI-1 mRNA (58). Our recent findings demonstrate that the regulation of PAI-1 expression occurs mainly through alternative means in lung epithelial cells.

uPA-Mediated Regulation of the Fibrinolytic System in Lung Epithelial Cells

Treatment of Beas2B lung epithelial cells or primary cultures of human small airway epithelial cells with two-chain high molecular weight uPA increases uPA protein and mRNA
expression. The process is mediated through the binding interaction between uPA and uPAR and does not require uPA enzymatic activity. uPA-induced uPA autoinduction by lung epithelial cells involves stabilization of uPA mRNA rather than transcriptional activation (81). These observations define a new paradigm for the control of uPA expression through signaling by uPA that is selectively linked to alterations in its message stability (Fig. 3).

In a related vein, uPA enhances uPAR expression as well as uPA binding in Beas2B lung epithelial cells (75). Induction of uPAR by uPA also involves posttranscriptional stabilization of uPAR mRNA. Lastly, we found that uPA enhances Beas2B lung epithelial and small airway epithelial cell PAI-1 protein and mRNA expression in a dose-dependent manner (Ref. 19; Fig. 3). Here again, we found that uPA-mediated induction of PAI-1 involves posttranscriptional modification involving stabilization of PAI-1 mRNA. Inactivation of the catalytic activity of uPA has little effect on PAI-1 induction, and the activity of the isolated NH₂-terminal fragment of the uPA molecule was comparable to that of full-length single- or two-chain uPA. The induction of components of the fibrinolytic system by uPA therefore involves specific, distinct interactions involving uPAR or other receptors at the surface of lung epithelial cells.

Additional uPA-mediated signaling events have recently been identified. Abraham and colleagues found that uPA enhances activation of neutrophils exposed to submaximal stimulatory doses of LPS. Transgenic mice lacking uPA (uPA−/−) were protected from endotoxemia-induced lung injury as regulatory doses of LPS. Transgenic mice lacking uPA (uPA enhances Beas2B lung epithelial and small airway epithelial cell PAI-1 protein and mRNA expression in a dose-dependent manner (Ref. 19; Fig. 3). Here again, we found that uPA-mediated induction of PAI-1 involves posttranscriptional modification involving stabilization of PAI-1 mRNA. Inactivation of the catalytic activity of uPA has little effect on PAI-1 induction, and the activity of the isolated NH₂-terminal fragment of the uPA molecule was comparable to that of full-length single- or two-chain uPA. The induction of components of the fibrinolytic system by uPA therefore involves specific, distinct interactions involving uPAR or other receptors at the surface of lung epithelial cells.

Additional uPA-mediated signaling events have recently been identified. Abraham and colleagues found that uPA enhances activation of neutrophils exposed to submaximal stimulatory doses of LPS. Transgenic mice lacking uPA (uPA−/−) were protected from endotoxemia-induced lung injury as determined by development of lung edema, pulmonary neutrophil accumulation, lung IL-1β, macrophage inflammatory protein-2, and TNF-α levels (Ref. 1; Fig. 3). These results demonstrate that uPA can also potentiate LPS-induced neutrophil-mediated inflammatory responses, including that associated with the development of ALI. Induction of cytokines by alveolar macrophages and neutrophils by uPA is accomplished by the uPA kringle domain (45), providing further evidence that pathophysiologically relevant cellular signaling by uPA involves nonproteolytic as well as proteolytic mechanisms. Whether uPA modulates the release of inflammatory mediators by lung epithelial cells is at present unknown but represents an intriguing avenue for future investigation.

**The Role of uPA-uPAR-Mediated Control of Epithelial Cell Apoptosis/Viability**

In addition to activation of plasminogen, uPA stimulates proliferation of epithelial cells (26, 48, 89). uPA induces proliferation in several cell lines including lung epithelial cells, and the process appears to be uPAR-dependent (43, 50, 76–79, 82). In addition, the uPA-uPAR system exhibits cooperativity with selected integrins to induce phenotypic changes in epithelial cells exposed to hypoxia that are similar to epithelial-mesenchymal transition. Newly recognized pathways by which the lung epithelium can regulate the uPA-uPAR-PAI-1 fibrinolytic system include regulation through uPA signaling, uPA-induced mediator expression, and cross talk between uPA and p53 by which uPA can regulate epithelial cell viability.

A wide range of lung diseases including ARDS, interstitial lung diseases, bronchiolitis obliterans, and drug-induced lung diseases are characterized by lung epithelial cell apoptosis. The interstitial lung diseases are characterized by the development of progressive fibrosis, and accelerated pulmonary fibrosis is a potentially grave consequence of ARDS (21). In asthma and chronic obstructive pulmonary disease (COPD), fibrotic changes alternately occur in the subepithelium of the conducting airways (42, 92), and epithelial cell apoptosis may likewise play a role in the airway remodeling that occurs in association with these diseases. The evidence linking epithelial cell apoptosis and remodeling in the injured lung has been reviewed by Chapman (21). Although the Fas/Fas ligand pathway has been implicated in the pathogenesis of epithelial cell apoptosis in ARDS or the interstitial lung diseases (5, 44, 96), the role of the fibrinolytic system in control of viability of the lung epithelium has until recently not been appreciated.

We (69) recently found that uPA regulates the tumor suppressor protein p53 in lung epithelial cells in a biphasic, concentration-dependent manner, and the changes in p53 expression influence epithelial cell apoptosis or survival. Although the uPA concentration in the alveolar compartment is probably <1 nM, uPA expression is induced by proinflammatory cytokines during injury despite the fact that fibrinolytic activity is considerably depressed due to a disproportionate increase in PAI-1. It is therefore conceivable that the concentrations of uPA in alveolar lining fluids may be considerably elevated during the course of ALI and its repair. The effects are observed in primary airway epithelial cells and in Beas2B human airway epithelial cells. This pathway represents a newly appreciated, direct link between uPA, uPAR, and p53-mediated epithelial cell survival and alveolar fibrinolysis.

The induction of p53 by uPA occurs at the translational level, without increased p53 mRNA expression. At relatively low concentrations of uPA, <5 nM, p53 is induced, and lung...
epithelial cells are stimulated to undergo apoptosis. The response differs at higher, 10–20 nM concentrations of uPA stimulation, as p53 is suppressed, lung epithelial cell viability is maintained, and cellular proliferation is stimulated (Fig. 4). This pathway could regulate pathophysiological alterations of p53 expression and thereby influence epithelial cell apoptosis or viability in the setting of ALI and its repair. At this time, the contribution of uPA-mediated alteration of p53 to lung epithelial cell viability and fibrosis in vivo, in various forms of alveolitis, remains to be defined. Studies to determine how the system operates in bleomycin-induced lung injury in mice have recently been proposed by our group to address the issue. Further studies to elucidate the receptor interactions and signaling pathways by which uPA switches p53 “on” or “off” in lung epithelial cells are also being pursued at this time. Along these lines, we (69) found that uPA induces the oncoprotein MDM2 in a concentration-dependent manner, which may serve to limit p53 expression in lung epithelial cells at relatively high concentrations (10–20 nM) of uPA exposure and therefore promote cellular proliferation.

Evidence supporting an independent role for p53 in the pathogenesis of ALI derives from prior studies, especially those in which bleomycin was used to induce lung injury. Bleomycin, a potent chemotherapeutic agent, causes ALI and subsequent fibrosis in humans, rats, and mice (7, 41, 46, 51, 56, 85, 90). The cytotoxic effect of bleomycin is generally believed to involve its ability to bind and cleave DNA (7, 53, 55, 90), effects that lead to increased intrapulmonary expression of p53 (51). Asbestos and ionizing radiation, agents known to cause clinical pulmonary disease, have likewise been shown to induce p53 and PCNA in injured lungs in situ and in cultured lung fibroblasts (52, 101). Deletion of p53 augments alveolar inflammation suggesting that residual apoptotic cells serve as a proinflammatory trigger (27).

Protection after bleomycin-induced lung injury by PAI-1 deficiency and lung-specific uPA induction (34, 53, 90, 91) and increased lung injury with deletion of p53 (27) strongly suggests the possibility that interactions between uPA and p53 could cooperatively contribute to the pathogenesis of ALI. We (64, 83, 84) recently provided evidence supporting this possibility by confirming a direct link between p53 and regulation of uPA-uPAR-PAI-1 fibrinolytic system. We characterized p53 as a specific mRNA-binding protein that controls uPA, uPAR, and PAI-1 expression by modulating stabilities of these mRNAs in primary lung epithelial cells and Beas2B cells. This observation provides another level at which p53 may influence the viability of lung epithelial cells as well as alveolar fibrinolysis. Further elucidation of the regulatory mechanisms may offer new molecular targets that could be exploited to protect the epithelium against injury and perhaps mitigate the severity or fibrotic sequelae of ALI.

Regulation of uPA Expression in Lung Epithelial Cells by p53

Building on the observations described in the previous section, we next sought to determine whether p53 could reciprocally induce components of the fibrinolytic system. p53-deficient lung carcinoma cell express large amounts of uPA protein and mRNA and exhibit slower degradation of uPA mRNA than that of p53-bearing nonmalignant Beas2B human airway epithelial cells. Reintroduction of p53 protein in p53-deficient cells suppresses basal and uPA-induced uPA expression and destabilizes the level of uPA mRNA primarily due to augmented uPA mRNA degradation. Conversely, suppression of p53 in Beas2B cells increases basal uPA protein and mRNA expression and potentiates uPA autoinduction through uPA mRNA stabilization. We recently found that p53 protein directly binds to a 35-nt uPA mRNA 3’-UTR in a sequence-specific manner and that endogenous uPA mRNA similarly associates with p53 protein isolated from Beas2B cytosolic extracts. Insertion of this sequence into β-globin mRNA ac-

Fig. 4. Model illustration of uPA-mediated control of lung epithelial cell viability. uPA serves as a concentration-dependent molecular switch in the regulation of apoptosis or proliferation through control of uPA, uPAR, and PAI-1 as well as p53 expression by lung epithelial cells. At relatively low concentrations of uPA stimulation, ≤5 nM, uPA primarily binds its high-affinity receptor, uPAR. This interaction signals for induction of p53, which binds uPAR, uPA, and PAI-1 mRNAs and results in destabilization of uPAR and uPA mRNA with concurrent stabilization of PAI-1 mRNA. This results in a reduction of uPAR and uPA in the cells with a concurrent induction of PAI-1 expression and subsequent apoptosis. Conversely, at relatively higher uPA concentrations, ≥10 nM, uPA binds to alternate receptors as well as uPAR. These interactions trigger signaling that suppresses p53 and reduces its interaction with and stabilizes uPAR or uPA mRNA with destabilization of PAI-1 mRNA, leading to increased uPAR at the cell surface, increased cellular uPA, and decreased PAI-1, with preservation of the viability of lung epithelial cells.
celerates decay of otherwise stable β-globin mRNA. These observations indicate a direct role for p53 as a uPA mRNA-binding protein that destabilizes uPA mRNA to decrease cellular uPA expression.

Regulation of uPAR Expression in Lung Epithelial Cells by p53

We next evaluated whether p53 likewise regulates uPAR expression. Along these lines, we found that inhibition of p53 in Beas2B cells increased basal as well as uPA-mediated uPAR expression and stabilized uPAR mRNA (Fig. 4). p53 protein also specifically binds to a 37-nt uPAR 3′-UTR sequence. These observations demonstrate a novel regulatory role for p53 as a uPAR mRNA-binding protein that downregulates uPAR expression, destabilizes uPAR mRNA, and thereby contributes to the viability of human airway epithelial cells (84). This represents yet another function of p53 that could influence lung epithelial cell fibrinolytic capacity or cell survival. How the pathway operates in the context of ALI or fibrosing alveolitis likewise remains to be determined.

Regulation of PAI-1 Expression by p53

Because uPA regulates its own expression as well as that of its receptor through elaboration of lung epithelial cell p53, we next tested whether p53 further controls the expression of PAI-1 by lung epithelial cells. We found that p53-deficient lung carcinoma cells express minimal PAI-1 protein and mRNA. PAI-1 mRNA is highly unstable in cells where p53 expression is minimal. Introduction of p53 in p53-deficient cells enhanced PAI-1 expression and stabilized PAI-1 mRNA. On the contrary, blocking p53 expression in nonmalignant human lung epithelial (Beas2B) cells decreased basal PAI-1 expression, and uPA failed to increase PAI-1 expression due to accelerated degradation of PAI-1 mRNA. Interestingly, p53 specifically binds to a 70-nt PAI-1 mRNA 3′-UTR sequence, and endogenous PAI-1 mRNA copurifies with p53 protein in Beas2B cells. Surprisingly, insertion of the p53-binding 70-nt sequence into β-globin mRNA destabilized the chimeric transcript. These observations demonstrate a novel role for p53 as an mRNA-binding protein that regulates increased PAI-1 expression and stabilization of PAI-1 mRNA in human lung epithelial and carcinoma cells. Coordinated interaction of p53 with uPA, uPAR, and PAI-1 mRNA results in reciprocal suppression of uPA and uPAR with an increment in PAI-1 expression leading to suppression of survival signals and contributing to epithelial cell apoptosis. Our group is in the process of determining whether protection of the lung epithelium can prevent the ALI and/or pulmonary fibrosis associated with bleomycin challenge in mice. This strategy is supported by the recent observation that transplantation of alveolar type II cells protects against pulmonary fibrosis induced by bleomycin (63).

Summary

The lung epithelium regulates expression of uPA, uPAR, and PAI-1 through specific, newly recognized posttranscriptional mechanisms that control expression of these proteins by lung epithelial cells at the level of mRNA stability. The posttranscriptional regulation involves interaction of newly recognized mRNA-binding proteins with specific sequences of uPA, uPAR, or PAI-1 mRNA, which affect the decay of the respective mRNAs. uPA signals for autoinduction as well as induction of uPAR and PAI-1 by lung epithelial cells and the mechanisms all involve altered mRNA stability rather than transcriptional control. uPA also regulates expression of p53 by lung epithelial cells through translational control rather than regulation of p53 mRNA stability. uPA-mediated induction of p53 represents another newly recognized pathway by which lung epithelial cell apoptosis or viability may be determined in the context of ALI. Lastly, p53 regulates uPA, uPAR, and PAI-1 expression by lung epithelial cells, indicating a feedback mechanism by which cellular proteolytic capacity and viability can be influenced by the interaction of p53 with uPA, uPAR, or PAI-1 mRNA. The complex interplay between these pathways underscores the versatility of interactions by which lung epithelial cells may regulate local fibrinolysis, cellular viability, inflammation, and aberrant fibrin turnover in ALI or its repair. As better understanding of the underlying mechanisms and their relationship to ALI emerges, new opportunities to successfully limit alveolar injury or pulmonary fibrosis in the clinical setting may derive.

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