Regulation of urokinase receptor expression by protein tyrosine phosphatases

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Submitted 1 April 2006; accepted in final form 2 October 2006

Shetty S, Velusamy T, Idell S, Tang H, Shetty PK. Regulation of urokinase receptor expression by protein tyrosine phosphatases. Am J Physiol Lung Cell Mol Physiol 292: L414–L421, 2007. First published October 6, 2006; doi:10.1152/ajplung.00121.2006.—Urokinase-type plasminogen activator (uPA) and its receptor (uPAR) play a major role in several physiological processes such as cell migration, proliferation, morphogenesis, and regulation of gene expression. Many of the biological activities of uPA depend on its association with uPAR. uPAR expression and its induction by uPA are regulated at the posttranscriptional level. Inhibition of protein tyrosine phosphatase-mediated dephosphorylation by sodium orthovanadate induces uPAR expression and, with uPA, additively induces cell surface uPAR expression. Sodium orthovanadate induces uPAR by increasing uPAR mRNA in a time- and concentration-dependent manner. Both sodium orthovanadate and uPA induce uPAR mRNA stability, indicating that dephosphorylation could contribute to uPA-induced posttranscriptional regulation of uPAR expression. Induction of the tyrosine phosphatase SHP2 in Beas2B and H157 cells inhibits basal cell surface uPAR expression and uPA-induced uPAR expression. Sodium orthovanadate also increases uPAR expression by decreasing the interaction of a uPAR mRNA coding region sequence with phosphoglycerate kinase (PGK) as well as by enhancing the interaction between a uPAR mRNA 3′ untranslated sequence with heterogeneous nuclear ribonucleoprotein C (hnRNPC). On the contrary, overexpression of SHP2 in Beas2B cells increased interaction of PGK with the uPAR mRNA coding region and inhibited hnRNPC binding to the 3′ untranslated sequence. These findings confirm a novel mechanism by which uPAR expression of lung airway epithelial cells is regulated at the level of mRNA stability by inhibition of protein tyrosine phosphatase-mediated dephosphorylation of uPAR mRNA binding proteins and demonstrate that the process involves SHP2.

phosphatase; messenger ribonucleic acid stability; messenger ribonucleic acid binding proteins

UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA) and its receptor (uPAR) control a promitogenic signaling system in eukaryotic cells, and elevated levels of uPA and uPAR expression are associated with invasive potential of tumors (2, 17). Information supporting the involvement of uPA, uPAR, and plasminogen activator inhibitor-1 in lung injury and repair or lung neoplasia has been steadily increasing (1, 13, 23, 24, 26, 36, 37). Because many biological activities of uPA depend on association with uPAR, this receptor plays a central role in localized uPA-mediated plasminogen activation at the cell surface. Interaction of uPA with its cellular receptor (uPAR) also promotes cellular signaling, which can thereby influence the course of lung inflammation or cancer. Better understanding of the specific pathways that regulate uPAR expression is therefore germane to the pathogenesis of lung injury or the spread of lung epithelial neoplasms.

uPA regulates diverse functions, such as cell adhesion, signaling, and mitogenesis, and most of the biological activities of uPA are dependent on its association with uPAR (4, 7, 14–16, 18, 25, 34, 39, 41, 42). It is noteworthy that lung squamous cell carcinoma is characterized by elevated uPAR expression and that inhibition of uPAR reduces proliferation as well as migration of H157 cells (33). Synthesis of uPAR is regulated by a variety of hormones, growth factors, and cytokines at either the transcriptional or posttranscriptional level (4, 7, 14–16, 18, 20, 21, 25, 33, 34, 39, 41, 42). The posttranscriptional mechanisms that regulate uPAR expression involve interaction of phosphoglycerate kinase (PGK) with a 51-nt coding region (CDR) determinant (28–29, 34) and heterogeneous nuclear ribonucleoprotein C (hnRNPC) interaction with a 110-nt 3′-untranslated region (UTR) sequence (31), respectively. Interaction of PGK with the 51-nt uPAR mRNA CDR determinant destabilizes uPAR mRNA (29), whereas hnRNPC-110-nt 3′-UTR interaction stabilizes uPAR mRNA (31). uPA also induces cell surface uPAR expression in diverse cell types (7, 20–22, 28, 29, 31, 33).

Earlier work from several laboratories, including ours, indicates a role for activation of cellular signaling in the expression of cell surface uPAR (6, 8, 9, 11, 15, 16, 20–22, 25, 27–31, 33–35, 38, 41, 42). However, the mechanism by which uPA induces uPAR expression at the posttranscriptional level in these cells remains unclear. In this study, we address this gap in current knowledge. We now demonstrate that protein tyrosine dephosphorylation induces uPAR expression via posttranscriptional regulation at the level of mRNA stability and we further elucidate the responsible mechanism.

MATERIALS AND METHODS

Materials. Culture media, penicillin, streptomycin, and fetal calf serum (FCS) were purchased from Gibco BRL (Grand Island, NY). Tissue culture plastics were obtained from Becton Dickinson Labware (Lincoln Park, NJ). Bovine serum albumin (BSA), ovalbumin, Tris base, aprotinin, dithiothreitol, phenylmethylsulfonyl fluoride (PMSF), and ammonium persulfate were obtained from Sigma Chemical (St. Louis, MO). Acrylamide, bisacrylamide, and nitrocellulose were obtained from Bio-Rad Laboratories (Richmond, CA). Anti-uPAR antibody was obtained from American Diagnostics (Greenwich, CT). Anti-SHP2, phospho-SHP2, and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). XAR X-ray film was purchased from Eastman Kodak (Rochester, NY).

Cell culture. Human bronchial epithelial cells (Beas2B) were obtained from the ATCC. These cells were maintained in LHC-9 medium containing 1% antibiotics as previously described (33). Hu-
man lung squamous cell carcinoma (H157) cells were maintained in RPMI 1640 medium containing 10% heat-inactivated FCS, 1% glutamine, and 1% antibiotics as previously described (29, 31). Primary cultures of human small airway epithelial cells (SAEC) were obtained from Clonetics (San Diego, CA) and maintained in SAGM medium (Clonetics).

**Total cellular membrane extraction and Western blotting.** Beas2B cells grown to confluence in LHC-9 medium were treated with sodium orthovanadate in serum-free medium containing 0.5% BSA. The cells were washed with PBS, and receptor-bound uPA was removed with glycine-HCl treatment as described earlier (33). We used Western blotting to measure uPAR at the cell surface. Membrane proteins were isolated as previously described (32, 33), separated using 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 uPAR monoclonal antibody in the same buffer at 4°C; membranes were washed and uPAR proteins were detected using enhanced chemiluminescence (ECL).

**Total protein extraction and Western blotting.** Beas2B cells were grown to confluence and serum-starved overnight with RPMI 1640 medium. The cells were then incubated in serum-free medium or in the same medium supplemented with sodium orthovanadate or recombinant human two-chain uPA for selected times. After these treatments, the cells were suspended in lysis buffer (10 mM Tris·HCl, pH 7.4, containing 150 mM NaCl, 1% Triton X-100, 15% glycerol, 1 mM NaF, 1 mM EDTA, 1 mM PMSF, and 3–10 μg of aprotinin per 100 ml). Cell lysates were prepared using three cycles of freezing and thawing. Proteins from Beas2B cell lysates (50 μg) were separated using 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-phospho-SHP2 monoclonal antibodies in the same buffer at 4°C. The membrane was then washed, and tyrosine-phosphorylated SHP2-immunoreactive proteins were detected using ECL. Membranes were stripped with β-mercaptoethanol and subjected to Western blotting using a monoclonal antibody to total SHP2 or β-actin.

**Plasmid construction.** Plasmid uPAR or SHP2 cDNA was subcloned to HindIII and XhoI sites of pcDNA3.1 (Invitrogen), and the sequences of the clones were confirmed by sequencing.

**Random priming of uPAR or SHP2 cDNA.** The full-length template of uPAR or SHP2 cDNA was released with HindIII or XhoI, purified on 1% agarose gels, and labeled with [32P]dCTP using a rediPrime labeling kit (Amersham, Arlington Heights, IL). Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activity of the product was 6 × 106 cpm/μg.

**Northern blotting of uPAR or SHP2 mRNA.** A Northern blotting assay was used to assess the level of uPAR or SHP2 mRNA. Beas2B cells were treated with or without uPA for various time periods in RPMI medium. Total RNA was isolated using TRI reagent. RNA (20 μg) was isolated on agarose-formaldehyde gels. After electrophoresis, 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 M)/ SDS (1%) and 100 μg/ml salmon sperm DNA. Hybridization was performed with uPAR or SHP2 cDNA probe (1 ng/ml) labeled to ~6 × 107 cpm/μg 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, respectively. The membranes were next exposed to X-ray film at −70°C overnight. The intensity of the bands was measured using densitometry and normalized against that of β-actin.

**Transfection of Beas2B and H157 cells.** To further confirm that SHP2 regulates uPAR expression, in a separate experiment we cloned SHP2 cDNA into a eukaryotic expression vector, pcDNA3.1. The Beas2B cells were transfected with vector cDNA or vector DNA containing SHP2 cDNA by using Lipofectamine, and stable cell lines were created by treating Beas2B cells with neomycin over 3 mo as described above. The effect of SHP2 overexpression on uPA-mediated uPAR induction was confirmed by treating the cells with PBS or uPA, and the plasma membrane proteins were analyzed by Western blot. To confirm that SHP2 regulates uPAR expression, we transfected uPAR-overexpressing squamous cell carcinoma H157 cells with or without SHP2 cDNAs or empty vector by lipofection, as described above. Stable cell lines were generated by antibiotic selection, after which the cells were cultured in large amounts and uPAR expression was confirmed by Western blotting using an anti-uPAR antibody.

**In vitro transcription.** Linearized plasmids containing the human uPAR mRNA transcriptional templates of uPAR cDNA were transcribed in vitro with T7 or Sp6 polymerase (Ambion). The uPAR mRNA transcripts were synthesized according to the supplier’s protocol except that 50 μCi of [32P]UTP were substituted for unlabeled UTP in the reaction mixture. Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activity of the product was 4.9 × 108 cpm/μg.

**Northernwestern assay.** To confirm the role of protein tyrosine phosphatases on posttranscriptional regulation of uPAR expression, we initially transfected Beas2B cell lysates treated with sodium orthovanadate to uPAR mRNA-uPAR mRNA binding protein interaction by Northernwestern assay (28). PGK and hnRNPC proteins isolated from Beas2B cell extracts with the use of specific antibodies were separated on 8% SDS-PAGE and then blotted to a nitrocellulose membrane. The membrane was blocked with gel shift buffer containing 1% BSA and 20 μg of ribosomal RNA for 1 h. The membrane was replaced with fresh buffer containing 32P-labeled uPAR mRNA (2 × 108 cpm/ml) and incubated for an additional hour at room temperature. The membrane was later washed three times with 50 ml of gel shift buffer for 10 min each, air-dried, and exposed to X-ray film. Since both coding and 3'-UTR determinants regulate uPAR expression at the posttranscriptional level by interacting with PGK (29) and hnRNPC (31), we used uPAR CDR and 3'-UTR mRNAs for Northernwestern studies. We also determined the effect of sodium orthovanadate on PGK and hnRNPC expression and tyrosine phosphorylation by using Western blotting, as previously described by Shetty and Idell (28).

To directly confirm the role played by the protein tyrosine phosphatase SHP2 on uPAR expression through posttranscriptional regulation, we induced SHP2 overexpression by cDNA transfection, and the cell lysates of cells treated with or without uPA were subjected to Northernwestern assay for PGK and hnRNPC interaction with uPAR CDR or 3'-UTR, respectively, or expression and tyrosine phosphorylation of PGK or hnRNPC as described above. We also measured the stability of uPAR mRNA in these cell lines by performing transcription chase experiments as described earlier by our group (34).

**RESULTS**

**Induction of uPAR expression by sodium orthovanadate.** Beas2B cells were treated with sodium orthovanadate for 0–24 h, and the effect of this treatment on uPAR expression was analyzed by Western blotting using an anti-uPAR antibody. We found that inhibition of protein tyrosine phosphatase activity induced uPAR expression in a time-dependent manner with maximum expression detected ~6–12 h after the treatment (Fig. 1A). We also found increased induction when Beas2B cells were treated with sodium orthovanadate and uPA together (Fig. 1B). To confirm the effect of sodium orthovanadate on uPAR expression, we treated Beas2B cells with varying amounts of sodium orthovanadate and measured cell surface uPAR expression. As shown in Fig. 1C, sodium orthovanadate induced uPAR expression in a concentration-dependent manner with maximum induction occurring at a 25 μM concentration and inhibition of uPAR expression beyond 25 μM. This effect may be attributable to cellular toxicity.
of the relatively high concentration of sodium orthovanadate. We later treated primary SAEC with uPA and sodium orthovanadate and found that sodium orthovanadate likewise induced uPAR expression. Sodium orthovanadate has an additive effect when added with uPA (Fig. 1D).

Effect of sodium orthovanadate on uPAR mRNA expression.

We next tested the effect of sodium orthovanadate on uPAR mRNA expression. As shown in Fig. 2A, sodium orthovanadate induced uPAR mRNA expression in a concentration-dependent manner with a maximal effect around 5–25 μM concentration. However, at concentrations beyond 25 μM sodium orthovanadate, uPAR expression was reduced to basal levels. Sodium orthovanadate also induced uPAR mRNA expression in a time-dependent fashion with maximal induction noted ~3 h after the treatment (Fig. 2B). uPA induced uPAR mRNA in a time-dependent manner with maximal induction observed between 6 and 24 h (Fig. 2C).

We next sought to confirm that the induction of cell surface uPAR expression in Beas2B cells occurs via regulation of cellular uPAR mRNA stability. We determined the effect of uPA and sodium orthovanadate on uPAR mRNA stability in these cells. As shown in Fig. 3, Beas2B cells treated with uPA or sodium orthovanadate stabilized uPAR mRNA severalfold compared with PBS-treated cells. These data show that both uPA treatment and inhibition of protein tyrosine phosphatase activity by sodium orthovanadate induce cell surface uPAR expression through increased uPAR mRNA stability.

Effect of sodium orthovanadate and uPA on SHP2 expression.

Since uPA and sodium orthovanadate, a protein tyrosine phosphatase inhibitor, induced uPAR protein (Fig. 1, A–C) and mRNA expression (Fig. 2) and the protein tyrosine phosphatase SHP2 associates with uPAR protein (6), we speculated that uPA induces uPAR expression by regulating SHP2 at the posttranscriptional level. We next wanted to determine whether sodium orthovanadate- or uPA-mediated induction of uPAR mRNA expression in Beas2B cells was due to protein tyrosine phosphatase SHP2. To address this possibility, we cultured Beas2B cells in 150-mm culture dishes and treated the cells with either sodium orthovanadate or uPA for 0–24 h. The cell lysates were subjected to Western blot analysis. The results indicate that both uPA and sodium orthovanadate induced tyrosine phosphorylation of SHP2 in a time-dependent fashion.

Fig. 1. Expression of cell surface urokinase-type plasminogen activator receptor (uPAR) by inhibition of protein tyrosine phosphatase. A and B: bronchial epithelial (Beas2B) cells grown to confluence were treated with 25 μM sodium orthovanadate alone (Naor; A) or with 1 μg/ml urokinase-type plasminogen activator (Naor + uPA; B) for 0–24 h in serum-free medium. Cell membrane proteins were isolated, and membrane proteins were separated on 8% SDS-PAGE. The proteins were later transferred to a nitrocellulose membrane and subjected to Western blotting using anti-uPAR monoclonal antibodies. uPAR expression was detected by enhanced chemiluminescence (ECL). The data are representative of 3 independent experiments.

C: Beas2B cells were treated with varying amounts (0–250 μM) of sodium orthovanadate for 12 h, and uPAR expression was detected by Western blotting as described in A. D: small airway epithelial cells were treated with PBS, uPA, Naor, and Naor + uPA for 24 h. The membrane proteins were analyzed for uPAR expression by Western blotting.

Fig. 2. Regulation of uPAR mRNA expression by protein tyrosine phosphatase inhibitors in Beas2B cells. A: Beas2B cells grown to confluence were treated with varying amounts of Naor for 3 h, and total RNA was isolated using TRI reagent. uPAR mRNA was analyzed by Northern blotting using 32P-labeled uPAR cDNA. As shown in Fig. 2A, sodium orthovanadate induced uPAR mRNA expression in a concentration-dependent manner with a maximal effect around 5–25 μM concentration. However, at concentrations beyond 25 μM sodium orthovanadate, uPAR expression was reduced to basal levels. Sodium orthovanadate also induced uPAR mRNA expression in a time-dependent fashion with maximal induction noted ~3 h after the treatment (Fig. 2B). uPA induced uPAR mRNA in a time-dependent manner with maximal induction observed between 6 and 24 h (Fig. 2C).

C: Beas2B cells were treated with varying amounts (0–250 μM) of sodium orthovanadate for 12 h, and uPAR expression was detected by Western blotting as described in A. D: small airway epithelial cells were treated with PBS, uPA, Naor, and Naor + uPA for 24 h. The membrane proteins were analyzed for uPAR expression by Western blotting.

Fig. 3. Regulation of uPAR mRNA expression by protein tyrosine phosphatase inhibitors in Beas2B cells. A: Beas2B cells grown to confluence were treated with varying amounts of Naor for 3 h, and total RNA was isolated using TRI reagent. uPAR mRNA was analyzed by Northern blotting using 32P-labeled uPAR cDNA. As shown in Fig. 3, Beas2B cells treated with uPA or sodium orthovanadate stabilized uPAR mRNA severalfold compared with PBS-treated cells. These data show that both uPA treatment and inhibition of protein tyrosine phosphatase activity by sodium orthovanadate induce cell surface uPAR expression through increased uPAR mRNA stability.
Fig. 3. Stabilization of uPAR mRNA by uPA and protein tyrosine phosphatase inhibition in Beas2B cells. Beas2B cells were treated with PBS, uPA, or Naor to induce maximal uPAR mRNA. The cells were later treated with 5,6-dichloro-1β-D-ribofuranosylbenzamidazole (DRB; 10 μg/ml) for 0, 3, 6, 12, and 24 h in the same medium to inhibit ongoing transcription, and the decay of uPAR mRNA was then determined by Northern blotting using 32P-labeled uPAR cDNA. The same membrane was stripped and developed with β-actin cDNA to control for equal loading. The data shown are representative of 3 independent experiments.

Without altering the total SHP2 expression (Fig. 4, A and C). We next analyzed the SHP2 mRNA expression by Beas2B cells and found that sodium orthovanadate or uPA had no effect on SHP2 mRNA expression (Fig. 4, B and D).

Since both sodium orthovanadate and uPA induce tyrosine phosphorylation of SHP2 and uPAR mRNA stability, we inferred that uPA regulates uPAR expression at the posttranscriptional level through SHP2. To determine whether SHP2 contributes to the regulation of uPAR expression, we generated Beas2B cells stably overexpressing SHP2 and determined uPAR expression by Western blot. As shown in Fig. 5, A and B, SHP2 overexpression inhibited basal and uPA-induced uPAR protein and mRNA expression, indicating that SHP2 regulates uPAR expression and that the process involves tyrosine dephosphorylation. We next overexpressed SHP2 in uPAR-overproducing H157 cells (33) by cDNA transfection and analyzed cell surface uPAR protein and mRNA expression by using Western and Northern blotting, respectively. As shown in Fig. 5, C and D, both uPAR protein and mRNA are reduced in SHP2 cDNA-transfected H157 cells compared with vector cDNA-transfected cells. Since sodium orthovanadate inhibits SHP2 activity and induces uPAR mRNA stability, and H157 cells express stable uPAR mRNA (32), we next tested the effect of SHP2 overexpression on uPAR mRNA stability in H157 cells. As shown in Fig. 6, H157 cells express stable uPAR mRNA and overexpression of SHP2 destabilizes uPAR mRNA, confirming that SHP2 regulates uPAR mRNA stability.

Inhibition of dephosphorylation by sodium orthovanadate induced uPAR mRNA stability (Fig. 3), and we previously showed that posttranscriptional regulation uPAR expression is mediated by the interaction of uPAR mRNA binding proteins (PGK and hnRNPC) with coding and 3′-UTR, respectively (29, 31, 34). Therefore, we next sought to determine whether protein tyrosine phosphatase-mediated stabilization of uPAR mRNA is due to altered interaction of PGK or hnRNPC with uPAR mRNA. To examine this possibility, we treated PGK isolated from Beas2B cell lysates with sodium orthovanadate and subjected them to Northwestern assay. The data (Fig. 7A) show that inhibition of dephosphorylation leads to decreased binding of PGK proteins isolated from sodium orthovanadate-treated Beas2B cells to the uPAR mRNA CDR. When the same sample was analyzed to determine the tyrosine phosphorylation status of PGK, we found increased tyrosine phosphorylation of PGK with increasing time after sodium orthovanadate treatment. However, we did not observe any significant change in PGK protein expression. The same material was next analyzed to interrogate the interaction of 3′-UTR with the uPAR 3′-UTR mRNA. We also found that sodium orthovanadate increased the interaction of hnRNPC with the uPAR 3′-UTR mRNA. Inhibition of protein tyrosine phosphatases also increased tyrosine phosphorylation of hnRNPC without altering the level of hnRNPC protein expression.

To confirm that the protein tyrosine phosphatase SHP2 directly regulates uPAR mRNA stability through PGK- and hnRNPC-mediated posttranscriptional stabilization of uPAR, we treated stable Beas2B cells overexpressing SHP2 cDNA or...
control vector cDNA with or without uPA. The lysates were analyzed for uPAR mRNA and PGK or hnRNPC interaction. The data (Fig. 8A) show that overexpression of SHP2 leads to increased binding of PGK proteins to the uPAR mRNA CDR compared with overexpression of control vector cDNA. However, we did not observe any significant change in PGK protein expression. When the same material was analyzed for hnRNPC interaction with uPAR 3′-UTR mRNA, we found that SHP2 decreased hnRNPC binding with the uPAR 3′-UTR (Fig. 8B) without altering the level of hnRNPC protein expression. These data suggest that the protein tyrosine phosphatase SHP2 regulates uPAR mRNA turnover by altering both PGK-mediated degradation and hnRNPC-mediated stabilization of uPAR mRNA due to changes in the tyrosine phosphorylation of PGK and hnRNPC and their subsequent binding with uPAR mRNA coding and 3′-UTR, respectively. Inhibition of dephosphorylation of SHP2-overexpressing cells with sodium orthovanadate also reversed the inhibitory effect of SHP2 on uPAR expression (Fig. 9).

DISCUSSION

The uPA and uPAR interaction is involved in the proteolytic cascade that mediates tissue remodeling, tumor cell invasion,
and metastasis (13, 17). Both uPA and uPAR are highly expressed in many human tumors. Many biological activities of uPA depend on association with its receptor, and uPAR plays a principal role in localizing uPA-mediated plasminogen activation and cellular signaling (2, 3, 43). uPAR is also involved in the regulation of cell proliferation, adhesion, and migration independent of the enzymatic activity of its ligand (6, 30, 35, 40). In addition, outcome studies in cancer patients have shown that high levels of either uPA or uPAR in tumors correlate with poor prognosis (2, 17, 43). Therefore, regulation of uPAR by lung epithelial cells is germane to the pathogenesis of a broad range of lung diseases. Current understanding of mechanisms by which these cells regulate this receptor remain poorly understood, leading us to pursue this study.

We (3, 30, 35) and others (6, 43) previously reported that uPA interacts with uPAR to induce proliferation of several cell types, including lung epithelial cells. We also previously reported that overexpression of uPAR appears to increase the invasiveness of lung carcinomas (33, 35). Recently, we reported that uPA induces uPAR expression in lung epithelial cells (33). Other groups have reported that the amino-terminal fragment of uPA interacts with uPAR to induce cell surface uPAR (20–22). The potential importance of tyrosyl phosphorylation in regulating uPAR expression is firmly established, since inhibition of tyrosine kinase activity inhibits both basal and uPA-induced uPAR expression (33). Therefore, phosphorylation of proteins on tyrosine residues appears to regulate uPAR expression and uPA-uPAR-mediated cellular processes such as proliferation, adhesion/migration, and responses to mitogens. Because the deregulation of tyrosine phosphorylation regulates uPAR expression (33), this process could substantially contribute to epithelial cell responses to injury and to their ability to regulate remodeling of proteolysis in the airways and alveolar compartment.

Fig. 8. Effect of SHP2 overexpression on uPAR mRNA-uPAR mRNA binding protein interactions in Beas2B cells. Stable Beas2B cells transfected with vector cDNA or SHP2 cDNA were treated with or without uPA for 24 h. Total proteins were obtained by lysing the cells in a Western lysis buffer, and PGK (A) and hnRNPC proteins (B) were isolated from the total lysates using specific antibodies. The proteins were then separated on 8% SDS-PAGE. The proteins were later transferred to a nitrocellulose membrane and subjected to Western assay using anti-PGK and anti-hnRNPC antibodies to determine the levels of total PGK or hnRNPC protein expression by ECL. The data shown are representative of the findings of 3 independent experiments.

Fig. 9. Effect of Naor on SHP2-mediated inhibition of uPAR expression in Beas2B cells. Stable Beas2B cells transfected with vector cDNA or SHP2 cDNA were treated with or without 25 μM Naor for 24 h. The total membrane proteins were then separated on 8% SDS-PAGE, transferred to a nitrocellulose membrane, and subjected to Western blotting using anti-uPAR monoclonal antibody to determine the uPAR expression by ECL. The data shown are representative of the findings of 2 independent experiments.

uPAR induces tyrosine phosphorylation of several cellular proteins, including uPAR-associated 38-kDa protein in U937 cells (10), 78-kDa cytoplasmic proteins in H157 cells (3), and Src kinases (5). uPA also induces tyrosine phosphorylation of STAT proteins (8, 9). We therefore reasoned that the control of uPAR and induction of this receptor by uPA could involve alterations of the phosphorylation status of regulatory proteins. We inferred that this process could extend to the control of uPAR expression at the level of mRNA stability and confirmed that impression in the studies we report in this article. Inhibition of protein tyrosine phosphatase activity by sodium orthovanadate failed to inhibit uPA-mediated DNA synthesis in H157 cells, whereas inhibition of tyrosine kinase activity inhibits uPA-induced DNA synthesis (3). We now report that inhibition of protein tyrosine phosphatase-mediated dephosphorylation by sodium orthovanadate induces uPAR expression. We also found that inhibition of protein tyrosine phosphatase activity by treatment with inhibitor sodium orthovanadate potentiates uPA-induced uPAR expression.

uPAR expression is regulated by both transcriptional and posttranscriptional mechanisms (15, 16, 20–22, 25, 33, 34, 41, 42). Induction of uPAR protein by uPA was reversed by inhibition of tyrosine kinases (33). uPA induces uPAR expression in lung epithelial cells by posttranscriptional stabilization of uPAR mRNA. Inhibition of protein tyrosine dephosphorylation by sodium orthovanadate induces basal and uPA-induced cell surface uPAR expression (33). The present study demonstrates that the mechanism involves stabilization of uPAR mRNA, providing evidence for a newly recognized mechanism by which tyrosine phosphorylation of cellular proteins is involved in uPAR gene expression at the posttranscriptional level.

Our results provide the first demonstration that uPA induces cell surface uPAR expression by stabilization of uPAR mRNA that is mediated by the protein tyrosine phosphatase SHP2. To confirm the role of SHP2 on cell surface uPAR expression, we overexpressed SHP2 in uPAR-overproducing H157 cells by using SHP2 cDNA transfection. Overexpression of SHP2 protein inhibited uPAR expression in these cells, as well as in Beas2B cells in which uPA was likewise overexpressed. A recent report indicated the association of the tyrosine phosphatase SHP2 with uPAR expression and its role in uPA- and PDGF-mediated cell migration (6, 43), supporting the central role of SHP2 in the expression of uPAR by lung carcinoma and nonmalignant lung epithelial cells.
Recently, we found that posttranscriptional regulation of uPAR expression is mediated by determinants present in uPAR mRNA coding and 3′-UTR. Posttranscriptional regulation of uPAR expression involves interaction of PGK with a 51-nt determinant present in the uPAR mRNA coding region (29, 34). We found that inhibition of protein tyrosine phosphatase by sodium orthovanadate induced tyrosine phosphorylation of PGK; upon tyrosine phosphorylation, we found that PGK induced cell surface uPAR expression by stabilization of uPAR mRNA (28). Inhibition of protein tyrosine phosphates also induced tyrosine phosphorylation of hnRNPC. Unlike PGK, tyrosine-phosphorylated hnRNPC increased interaction with the uPAR 3′-UTR, stabilized uPAR mRNA, and increased cell surface uPAR. Two recent studies showed that p38 MAPK, JNK1, and ERK regulate uPAR mRNA stabilities (12, 19). Our findings extend this information and define a new mechanism by which uPAR-dependent responses of the lung epithelium may be controlled in lung injury and repair or in neoplasia.

**ACKNOWLEDGMENTS**

We are grateful to Katy Windham and Brad Low for technical assistance.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grants R01 HL-71147, R01 HL-62453, and PO1 HL076406-01.

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