Posttranscriptional Regulation of Urokinase Receptor Expression by Heterogeneous Nuclear Ribonuclear Protein C[†]

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Received November 27, 2007; Revised Manuscript Received March 12, 2008

ABSTRACT: Interaction of urokinase-type plasminogen activator (uPA) with its receptor, uPAR, is a key regulatory step in uPA-mediated cell proliferation and migration. Our previous studies demonstrated that posttranscriptional stabilization of uPAR mRNA by uPA contributes to the induction of cell surface uPAR expression, and heterogeneous nuclear ribonuclear protein C1 (hnRNPC) binds to a 110 nt sequence of uPAR mRNA 3′-UTR, thereby preventing its degradation. These observations indicate that hnRNPC could be involved in the induction of uPAR expression by uPA. In the present study, we investigated this possibility and confirmed that uPA increased the binding of hnRNPC to the 3′-UTR of uPAR mRNA. Furthermore, uPA induced tyrosine phosphorylation of hnRNPC and uPAR expression through mRNA stabilization. Inhibition of hnRNPC tyrosine phosphorylation abolished its interaction with uPAR mRNA and suppressed mRNA stabilization and cell surface uPAR expression. Deletion experiments revealed that hnRNPC binds to uPAR mRNA through its RNA binding domain (RBD). Site-directed mutagenesis studies further indicated that phosphorylation of tyrosine residue 57 (Y57) present in RBD of hnRNPC by uPA is essential for uPAR 3′-UTR mRNA binding and uPAR expression. Increased hnRNPC interaction with the uPAR mRNA 3′-UTR through phosphorylation of Y57 represents a novel mechanism by which uPA regulates posttranscriptional uPAR mRNA turnover and cell surface uPAR expression.

Plasminogen activation (PA) by uPA[1] plays an important role in stromal remodeling through the breakdown of basement membranes and extracellular matrix proteins under normal physiological conditions as well as in pathological settings such as lung inflammation and tumor growth (*1, 2*). Impaired fibrinolysis during lung inflammation and the ensuing repair is evidenced by the abnormal accumulation of fibrin within the interstitial and alveolar spaces (*3*). Interestingly, uPA-mediated fibrin degradation also plays a major role during neoplasia (*4–6*).

Most of the cellular effects of uPA depend on its interaction with its receptor, uPAR. PA has been shown to be potentiated at least 50−60-fold following cell surface interaction of uPA with the uPAR. uPAR localizes uPA at the leading edge of migrating cells and is expressed by a wide variety of cell types including nonmalignant lung epithelial cells, macrophages, and fibroblasts (*7, 8*). Carcinomas of the epithelial origin from the lung and other tissues such as breast, ovary, prostate, and kidney express increased amounts of both uPA and uPAR at the tumor−stromal interface of the invasive foci. uPA and uPAR are implicated in the tumor cell proliferation, migration, and invasion of local and distant tissues (*9–11*). A higher level of uPAR is presumed to be the predictor of tumor metastasis and recurrence and has a role in prognosis (*12*). Steady states of uPAR mRNA and protein have been shown to increase due to increased mRNA stability in several cancer cell types (*13, 14*). Therefore, studies targeting the expression and/or interaction of uPAR have a physiological importance.

uPA induces the expression of its own cell surface receptor, uPAR, and the process involves posttranscriptional stabilization of uPAR mRNA (*9*). Our previous studies have revealed that uPAR mRNA 3′-UTR contains a 110 nt destabilization determinant, and heterogeneous nuclear ribonuclear protein C1 (hnRNPC) specifically interacts with the 110 nt sequence and induces cell surface uPAR expression (*15*).

We recently reported that inhibition of protein tyrosine phosphatase SHP2-mediated dephosphorylation augments hnRNPC binding to 3′-UTR of uPAR mRNA and increases uPAR mRNA stability (*16*). Conversely, SHP2 overexpression abolishes hnRNPC and uPAR mRNA 3′-UTR interaction. uPA fails to stabilize uPAR mRNA or induce cell surface uPAR expression in Beas2B cells transfected with SHP2 cDNA or treated with tyrosine kinase inhibitors (*14, 16*). These observations indicate that tyrosine phosphorylation of

[†] This work was supported by National Heart, Lung, and Blood Institute Grants Project 2 of P01HL-62453 and R01-HL071147.

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[1] Abbreviations: hnRNPC, heterogeneous nuclear ribonuclear protein C; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; 3′-UTR, 3′-untranslated region; RBD, RNA binding domain; CID, C1−C1 interaction domain; CTF, carboxy-terminal tail fragment.

10.1021/bi702338y CCC: $40.75
Published on Web 05/22/2008

hnRNPC could be involved in the regulation of uPAR expression at the posttranscriptional level. The current study was designed to determine how hnRNPC supports the expression of uPAR by uPA. Our findings indicate that uPA induces the binding of hnRNPC protein to 3′-UTR of uPAR through phosphorylation of tyrosine residue 57 of hnRNPC protein. Inhibition of Y57 phosphorylation abolished hnRNPC-mediated stabilization of uPAR mRNA and cell surface uPAR expression. Our deletion studies further demonstrate that hnRNPC interacts with the uPAR mRNA through its RNA binding domain (RBD), thereby mediating its effect on uPAR expression.

MATERIALS AND METHODS

Materials. Culture media (RPMI), penicillin, streptomycin, and fetal calf serum (FCS) were purchased from Gibco BRL Laboratories (Grand Island, NY). Tissue culture plastics were from Becton Dickinson Labware (Franklin Lakes, NJ). Herbimycin A, genestein, bovine serum albumin (BSA), ovalbumin, Tris-base, aprotinin, dithiothreitol (DTT), phenylmethanesulfonyl fluoride (PMSF), and ammonium persulfate (APS) were from Sigma Chemical Co. (St. Louis, MO). Acrylamide, bisacrylamide, and nitrocellulose membranes were products of Bio-Rad Laboratories (Richmond, CA). Beas2B human bronchial epithelial cells and an hnRNPC1 cDNA clone were obtained from ATCC. LHC-9 media were obtained from Clonetics and Biofluids (Rockville, MD). Anti-uPA and anti-uPAR antibodies were obtained from American Diagnostica (Greenwich, CT). Anti-phosphotyrosine and anti-β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Lipofectamine transfection reagents were obtained from Stratagene (Cedar Creek, TX). *In vitro* transcription kits and 5,6-dichloro-1-β-D-ribofuranosylbenzamidazole (DRB) were purchased from Ambion (Austin, TX) and Calbiochem (La Jolla, CA), respectively. HEPES and other reagents were from Fisher Scientific (Pittsburgh, PA). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, OH). Oligonucleotide primers used in PCR reactions were synthesized by MWG Biotech (Mendenhall, NC). Restriction enzymes were from New England Biolabs (Beverly, MA), and ^{32}P-UTP and ^{32}P-dCTP were from Perkin-Elmer Life and Analytical Sciences (Boston, MA). XAR X-ray films were purchased from Eastman Kodak (Rochester, NY).

Cell Cultures. Human bronchial epithelial cells (Beas2B) were routinely maintained in LHC-9 medium containing 1% antibiotics. 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 (*14*).

In Vitro Transcription. Linearized plasmid containing the human uPAR mRNA 3′-UTR transcriptional template of uPAR cDNA was transcribed *in vitro* using T_7 polymerase (Ambion). The uPAR mRNA 3′-UTR transcript was synthesized according to the supplier's protocol except that 50 μCi of [^{32}P]UTP substituted unlabeled UTP in the reaction mixture. Passage through a Sephadex G-25 column removed unincorporated radioactivity. The specific activities of the product were 4.9×10^8 cpm/μg.

Preparation of Total and Cellular Membrane Extracts and Western Blotting. Beas2B cells grown to confluence were serum-starved overnight with RPMI-glutamine media. On the following day, the cells were treated with PBS or uPA (1 μg/mL) for 12 h and washed with phosphate-buffered saline. The membrane proteins were isolated using the procedures described previously (*17, 18*). Isolated membrane proteins were separated by SDS−PAGE and transferred onto a nitrocellulose membrane. The membrane was then blocked with 1% BSA in wash buffer for 1 h at room temperature followed by overnight incubation with anti-uPAR monoclonal antibody in the same buffer at 4 °C, and washed, and the proteins bound with antibody were detected by enhanced chemiluminescence (ECL) as described earlier (*14*). In a separate experiment, Beas2B cells treated with PBS or uPA were lysed in a buffer containing Triton X-100, and the lysates were similarly analyzed for hnRNPC and β-actin proteins by Western blotting using anti-hnRNPC or anti-β-actin antibody.

Molecular Cloning and Expression of hnRNPC. The sequences coding for different domains of the hnRNPC were PCR-amplified using a previously cloned full-length cDNA packaged in pcDNA3.1 vector as the template (*15*), in conjunction with sense and antisense oligonucleotide primers designed on the basis of 5′- and 3′-regions of the open reading frame of the fragments. Amplification conditions were 2 min at 94 °C and 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min 45 s. The final reaction mixture was applied onto a 1.2% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining. The PCR products corresponding to sequences coding for different domains were excised from the gel, and the cDNAs therein were isolated by spin filtration. Purified PCR products were individually subcloned into the *Hin*dIII/*Xba* I site of pcDNA3.1D/V5-HIS-TOPO (Invitrogen, Carlsbad, CA). Plasmids containing the sequences that code for different hnRNPC domains were isolated and used for transfection of Beas2B cells.

Northwestern Assay. To confirm the effect of uPA on hnRNPC binding to uPAR mRNA 3′-UTR, we initially isolated hnRNPC proteins from the extracts of Beas2B cells treated with PBS or uPA and subjected to Northwestern assay as previously described (*16*). Briefly, hnRNPC proteins isolated from Beas2B cell lysates using specific antibody were separated on 8% SDS−PAGE and blotted to nitrocellulose membrane. The membrane was blocked with gel shift buffer containing 1% BSA and rRNA (20 μg) for 1 h. The membrane was then placed in fresh buffer containing ^{32}P-labeled uPAR mRNA 3′-UTR (200000 cpm/mL) and incubated for an additional 1 h at room temperature. Afterward, the membrane was washed three times with 50 mL of gel shift buffer for 10 min each, air-dried, and exposed to an X-ray film. The membrane was later stripped and subjected to Western blot analysis, using anti-phosphotyrosine or anti-hnRNPC antibody, for verifying tyrosine phosphorylation and expression of hnRNPC proteins.

Gel Mobility Shift Assay. Different domains of hnRNPC protein isolated from the cytoplasmic extracts of stable Beas2B cell lines were incubated with 2×10^4 cpm of a ^{32}P-labeled uPAR 3′-UTR RNA transcript in a mixture containing 15 mM KCl, 5 mM $MgCl_2$, 0.25 mM DTT, 12 mM HEPES (pH 7.9), 10% glycerol, and *Escherichia coli* tRNA (200 ng/μL) along with 150 mM NaCl. The mRNA−protein complexes were subjected to gel mobility

shift assay and visualized by autoradiography as previously described (*15*).

Random Priming of uPAR cDNA. The full-length template of uPAR was released with *Hin*dIII or *Xba* I, purified on 1% agarose gel, and labeled with ^{32}P-dCTP using a rediPrime labeling kit (Amersham, Arlington Heights, IL). Passage through a Sephadex G-25 column removed unincorporated ^{32}P-dCTP. The specific activity of the product was determined to be 6×10^8 cpm/μg.

Northern Blotting of uPAR mRNA. A Northern blotting assay was used to assess the levels of uPAR and hnRNPC mRNA. Total RNA was isolated from Beas2B cells treated with PBS or uPA using TRI reagent. Northern blot analysis was carried out with isolated RNA based on the procedure described previously (*19*).

Assessment of uPAR mRNA Stability by Northern Blotting. uPAR mRNA stability was measured by employing the transcription chase technique. Beas2B cells stimulated with selected agonists were treated with DRB (20 μg/mL) for different lengths of time (0−24 h) to inhibit ongoing transcription. Afterward, total RNA was isolated at selected time points using TRI reagent. RNA (20 μg) was separated by agarose/formaldehyde gel electrophoresis and then subjected to Northern blotting.

Site-Directed Mutagenesis of Tyrosyl Residues of hnRNPC. Mutation of specific tyrosyl residues of hnRNPC was carried out using the QuikChange site-directed mutagenesis kit (Stratagene) based on the manufacturer's instructions. Specific mutagenic primers were designed with mutations at desired sites by replacing codons encoding tyrosine (Y) with that of phenylalanine (F). PCR reactions were carried out using specific mutagenic primers with wild-type hnRNPC cDNA as the template. The reaction conditions were 95 °C for 1 min, followed by 12 cycles of 95 °C for 30 s, 55 °C for 1 min, and 68 °C for 15 min. At the end of the reaction, the PCR product was treated with *Dpn*I to digest the parental DNA template and transformed into XL1-blue supercompetent cells. The positive clones were identified by performing colony PCR. Plasmids were isolated from the positive clones and subjected to nucleotide sequencing to verify the mutation.

Transfection of Beas2B and H157 Cells. pcDNA3.1D/V5-HIS-TOPO harboring individual hnRNPC cDNAs with either mutations at phosphorylation sites or containing sequences that code for each of the three domains were transfected into Beas2B cells by lipofection as described previously (*14*). Vector with or without wild-type hnRNPC cDNA was also transfected in parallel as controls. The stable cell lines were generated by antibiotic selection. Afterward, the cells were cultured in large quantities, and the expression of recombinant protein was confirmed by Western blotting. Squamous carcinoma cells (H157) were similarly transfected with pcDNA3.1 with or without antisense hnRNPC cDNA, and stable cell lines were generated and used in stability studies.

RESULTS

Involvement of hnRNPC in Squamous Carcinoma Cell uPAR mRNA Stabilization. We have earlier reported that lung squamous carcinoma (H157) cells exhibit stable uPAR mRNA and increased cell surface uPAR expression, as well as elevated hnRNPC-uPAR 3′-UTR mRNA binding activity (*15*). To further assess the functional importance of hnRNPC in uPAR mRNA stabilization, hnRNPC protein expression in H157 cells was inhibited by hnRNPC antisense cDNA transfection (Figure 1a). Western blot analysis indicated that the suppression of hnRNPC protein in hnRNPC antisense treated cells resulted in a significant reduction of cell surface uPAR protein when compared to the vector cDNA treated control (Figure 1b). Northern blotting showed that down-regulation of hnRNPC similarly affected uPAR mRNA expression (Figure 1c). Transcription chase experiments further demonstrated that inhibition of hnRNPC expression accelerated uPAR mRNA decay in H157 cells (Figure 1d). Collectively, these observations revealed hnRNPC protein as a major *trans*-acting factor involved in the regulation of uPAR mRNA stabilization.

Expression of hnRNPC in Lung Epithelial Cells. We have recently reported that hnRNPC binds to uPAR mRNA 3′-UTR, and increased expression of hnRNPC stabilizes uPAR mRNA and induces cell surface uPAR protein in lung epithelial cells (*15*). uPA induces uPAR expression through posttranscriptional stabilization of uPAR mRNA (*19, 20*). We therefore sought to find out if hnRNPC-uPAR mRNA 3′-UTR interaction is involved in uPA-induced uPAR expression in Beas2B cells. We initially tested the effect of uPA on hnRNPC expression by treating Beas2B cells with uPA (1 μg/mL) for different lengths of time (0−24 h) and analyzed the cell lysates for hnRNPC protein expression. Results showed that uPA treatment failed to alter the expression of hnRNPC protein (data not shown).

We next examined the effect of uPA on hnRNPC mRNA expression by Northern blotting, and the results showed that uPA likewise had no effect on hnRNPC mRNA expression (data not shown). These results therefore ruled out the possibility that increased uPAR mRNA stabilization by uPA is mediated through increased expression of hnRNPC protein or mRNA.

uPA Upregulates uPAR mRNA Binding of hnRNPC through Tyrosine Phosphorylation. Since uPA failed to induce hnRNPC protein or mRNA expression, we speculated that uPA could influence the binding of the hnRNPC protein without altering its basal expression. To test this possibility, we isolated hnRNPC from uPA-treated Beas2B cell lysates and tested its interaction with ^{32}P-labeled uPAR mRNA 3′-UTR (*16*). The Northwestern analysis indicated that uPA increased hnRNPC binding to uPAR mRNA 3′-UTR in a time-dependent manner with maximal effect observed between 3 and 12 h after uPA treatment (Figure 2a). The hnRNPC-uPAR mRNA binding profile was consistent with that of uPAR mRNA expression by uPA. In view of our earlier observation that uPAR induction by uPA is responsive to both tyrosine kinase and protein tyrosine phosphatase inhibitors, we speculated that increased hnRNPC-uPAR mRNA interaction could be caused by posttranslational tyrosine phosphorylation of hnRNPC. To investigate this possibility, the same membrane was probed with anti-phosphotyrosine antibody. As shown in Figure 2a, uPA indeed induced tyrosine phosphorylation of hnRNPC in a time-dependent manner, and the phosphorylation profile paralleled that of uPAR mRNA binding.

Previous studies have demonstrated that uPA causes tyrosine phosphorylation of hnRNPC, and inhibition of tyrosine kinase activation inhibits uPAR mRNA stability

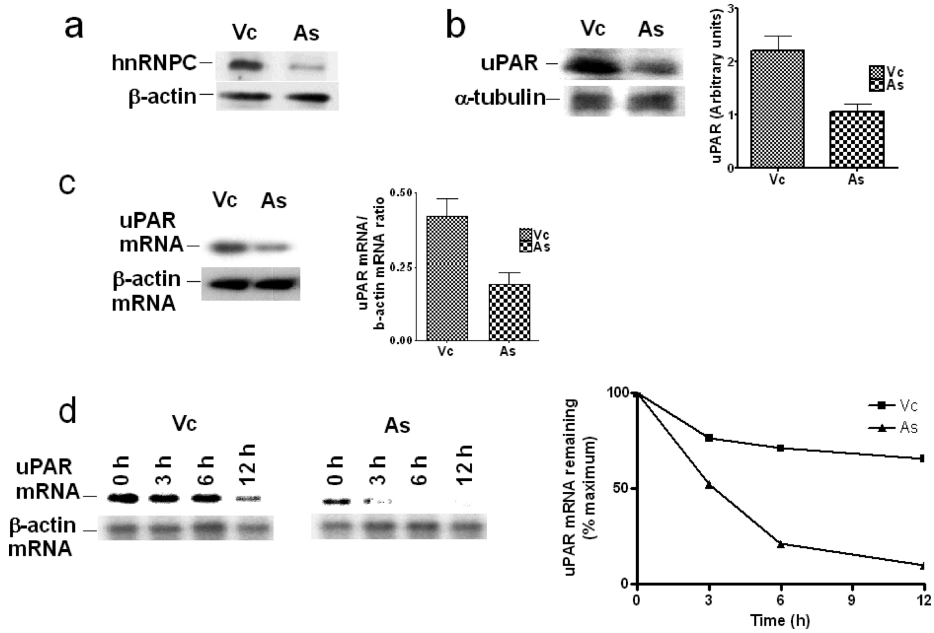

FIGURE 1: Inhibition of hnRNPC downregulates lung squamous carcinoma cell surface uPAR expression. (a) The lysates from stable H157 cells expressing antisense hnRNPC (As) or vector pcDNA3.1 (Vc) were tested for hnRNPC and β-actin expression by Western blotting. (b) Role of hnRNPC expression on cell surface uPAR protein. The membrane proteins isolated from stably transfected H157 cells expressing antisense hnRNPC or vector pcDNA3.1 were separated on SDS−PAGE, and the uPAR expression was determined by Western blotting using anti-uPAR monoclonal antibody. The same membrane was stripped and probed for α-tubulin as a loading control. The mean density of individual uPAR bands is presented as a bar graph. (c) Effect of hnRNPC inhibition on uPAR mRNA expression. Total RNA isolated from H157 cells transfected with antisense hnRNPC cDNA or vector cDNA as described in panel a was subjected to uPAR mRNA analysis by Northern blotting using ^{32}P-labeled uPAR cDNA as a probe. The same membrane was stripped and probed for β-actin mRNA. The mean density of individual bands after normalization with the corresponding β-actin mRNA is presented as a ratio in the bar graph. (d). Inhibition of hnRNPC expression destabilizes uPAR mRNA. Stable H157 cells overexpressing hnRNPC antisense cDNA or vector cDNA were treated with uPA (1 μg/mL) for 12 h to induce maximum uPAR mRNA expression. The ongoing transcription was blocked by treating the cells with DRB (20 μg/mL) for varying time periods (0−12 h). RNA was isolated, and the level of uPAR mRNA at different time points was determined by Northern blot analysis. The same membrane was stripped and probed for β-actin mRNA. The line graph represents percentage mRNA decay calculated from the mean values obtained by integrating the densities at 0 h after normalization to the corresponding β-actin mRNA of the individual bands. The above experiments were repeated three times.

(*16*). In order to further confirm that uPA-mediated tyrosine phosphorylation of hnRNPC is required for its binding to uPAR mRNA 3′-UTR, Beas2B cells were treated individually with herbimycin A (2 μM), a common phosphorylase inhibitor, and genestein (6 μg/mL), a specific tyrosine kinase inhibitor, for 3 h prior to uPA treatment. hnRNPC proteins were isolated 12 h after treatment with uPA and subjected to Northwestern assay using a ^{32}P-labeled uPAR mRNA 3′-UTR probe. Results showed that treatment with herbimycin A or genestein abolished the ability of uPA to induce hnRNPC-uPAR mRNA 3′-UTR interaction (Figure 2b). Moreover, both herbimycin and genestein blocked basal as well as uPA-induced tyrosine phosphorylation of hnRNPC, indicating that uPA-induced tyrosine phosphorylation is crucial for its uPAR mRNA binding activity.

Since it has been demonstrated that uPA increases uPAR expression in a dose-dependent manner with maximal effect at uPA concentration beyond 500 ng/mL (*14*), we treated Beas2B cells with varying amounts (0−2 μg/mL) of uPA and analyzed for hnRNPC binding to uPAR mRNA 3′-UTR using Northwestern assay. uPA increased hnRNPC interaction with uPAR mRNA 3′-UTR in a concentration-dependent manner, beginning at 100 ng/mL, which parallels with the tyrosine phosphorylation of hnRNPC protein (Figure 2c).

To exclude the possibility that contaminants present in the uPA preparation may cause hnRNPC phosphorylation or binding, we transfected Beas2B cells with pcDNA3.1 harboring full-length uPA cDNA and generated stable cell lines endogenously overproducing uPA as well as a control cell line bearing vector cDNA alone. hnRNPC proteins isolated from these cells were tested for uPAR 3′-UTR binding and tyrosine phosphorylation. The hnRNPC proteins isolated from the cells overexpressing uPA showed increased uPAR mRNA 3′-UTR binding affinity and tyrosine phosphorylation compared with vector cDNA transfected control Beas2B cells (Figure 2d). These results confirmed that the effect is uPA-specific and is not due to contaminants present in the preparation.

Identification of the uPAR mRNA 3′-UTR Binding Domain of hnRNPC. In order to identify the specific region of the hnRNPC molecule involved in uPAR mRNA 3′-UTR binding, we divided hnRNPC cDNA into three portions: an amino-terminal RNA binding domain (RBD; amino acid residues 1−110), a middle C1−C1 interaction domain (CID; amino acid residues 111−193), and a carboxy-terminal tail fragment (CTF; amino acid residues 194−310) (Figure 3a). The cDNAs encoding these three fragments were generated and cloned into an eukaryotic vector, pcDNA 3.1. The plasmids were individually transfected into Beas2B cells, and stable cell lines expressing wild-type (Wt) hnRNPC protein and different truncated forms of hnRNPC were generated by antibiotic selection. hnRNPC deletion proteins isolated from these cells were individually tested for uPAR mRNA 3′-UTR binding. Northwestern assay showed that RBD had a strong binding affinity for uPAR mRNA 3′-UTR, while CID exhibited weaker binding. In contrast, CTF did not show

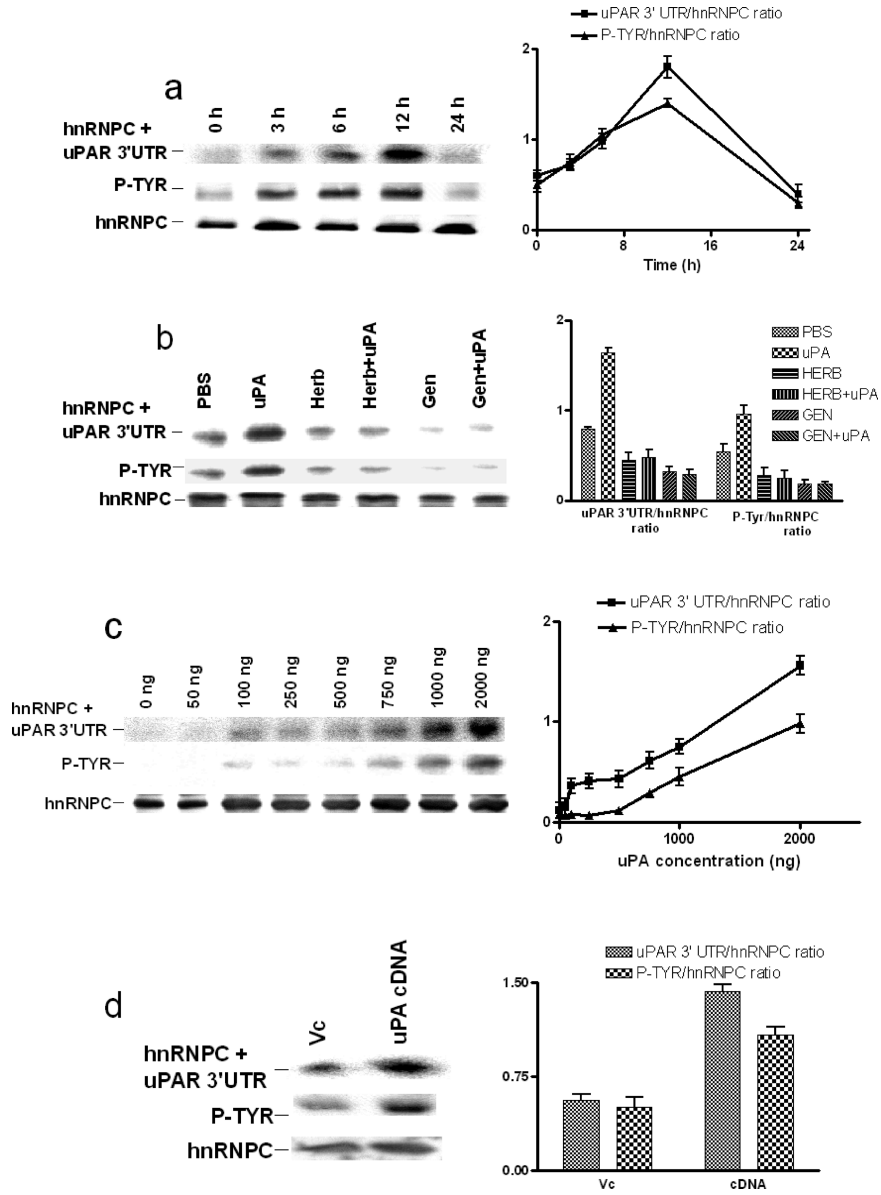

FIGURE 2: Induction of hnRNPC binding to uPAR mRNA 3′-UTR and hnRNPC tyrosine phosphorylation by uPA. (a) Effect of uPA on hnRNPC-uPAR 3′-UTR mRNA binding and tyrosine phosphorylation. Beas2B cells treated with uPA (1 μg/mL) for 0−24 h in serum-free medium were lysed in Western lysis buffer, after which hnRNPC proteins isolated from the total lysates using specific antibody were separated on 8% SDS−PAGE. The hnRNPC proteins were subsequently transferred onto a nitrocellulose membrane and subjected to Northwestern assay using the ^{32}P-labeled uPAR mRNA 3′-UTR transcript to determine their binding efficiency. The same membrane was stripped and analyzed for tyrosine phosphorylation and total hnRNPC by Western blotting using anti-phosphotyrosine and anti-hnRNPC antibodies, respectively. The ratio (mean density of the individual bands) which represents either uPAR 3′-UTR mRNA binding or tyrosine phosphorylation of hnRNPC normalized with total hnRNPC is presented in the line graph. (b) Effect of tyrosine kinase inhibitors on uPA-mediated hnRNPC-uPAR 3′-UTR mRNA interaction. Beas2B cells grown to confluence were treated with herbimycin A (2 μM) or genestein (6 μg/mL) for 3 h followed by uPA treatment. After 12 h the cells were lysed, and hnRNPC proteins were isolated and subjected to ^{32}P-labeled uPAR 3′-UTR binding by Northwestern analysis as described above. This was followed by Western blot assay with anti-phosphotyrosine and hnRNPC antibodies. The ratio (mean density) of individual bands is presented as a bar graph. (c) Effect of uPA concentration on uPAR mRNA 3′-UTR binding and tyrosine phosphorylation of hnRNPC. Beas2B cells were treated with various amounts of uPA ranging from 0 to 2 μg/mL for 12 h. hnRNPC proteins isolated were analyzed by Northwestern assay using ^{32}P-labeled uPAR 3′-UTR. The same membrane was later stripped and developed using anti-phosphotyrosine and anti-hnRNPC antibody to assess the changes in tyrosine phosphorylation status and level of hnRNPC. The ratio of individual bands of the experiments is presented in the line graph. (d) Effect of overexpression of endogenous uPA on hnRNPC binding to uPAR mRNA 3′-UTR. hnRNPC proteins isolated from Beas2B cells overexpressing uPA or vector alone were subjected to Northwestern assay using ^{32}P-labeled uPAR mRNA 3′-UTR. The same membrane was stripped and assessed for tyrosine phosphorylation status and total hnRNPC by Western blotting using anti-phosphotyrosine and anti-hnRNPC antibodies, respectively. The data (mean density) shown as a bar graph are representative of three independent experiments.

any such effect (data not shown). The weak binding exhibited by CID, nevertheless, was questionable due to the low NaCl (15 mM) concentration in the binding buffer and denaturation of proteins during electroblotting to solid surface (nitrocellulose membrane) in the Northwestern procedure. We therefore decided to test the binding specificity of both RBD and CID by gel mobility shift assay using a liquid hybridization buffer containing 150 mM NaCl. Results showed that RBD, but not CID, of hnRNPC protein formed a specific complex with uPAR mRNA 3′-UTR (Figure 3b). To confirm the expression of different fragments, hnRNPC proteins isolated from Beas2B cells were subjected to Western

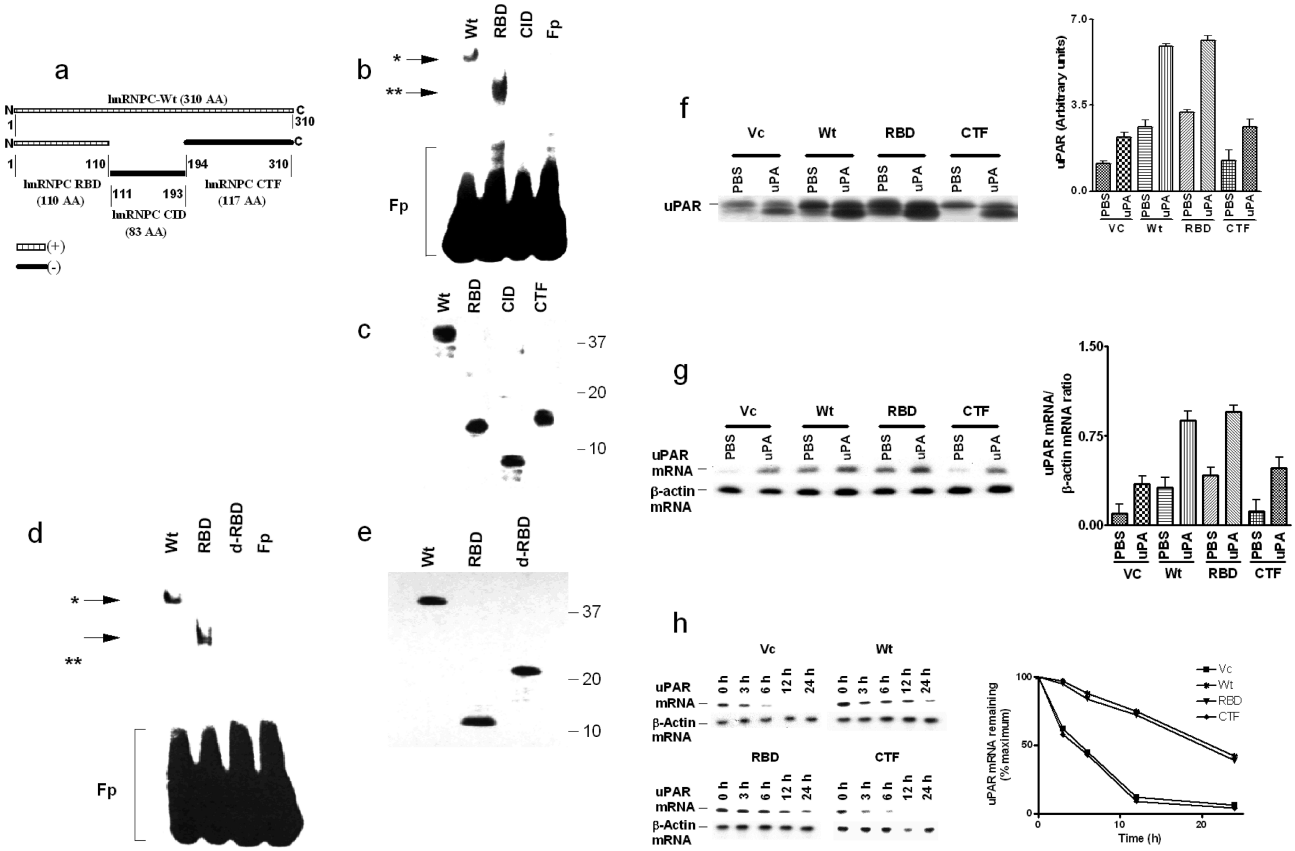

FIGURE 3: Identification of uPAR mRNA binding sites of hnRNPC. (a) Deletion map of hnRNPC. (b) Identification of uPAR 3′-UTR mRNA binding sites of hnRNPC. Recombinant full-length hnRNPC protein (Wt) or its truncated fragments harboring either the RNA binding domain (RBD) or the C1−C1 interaction domain (CID) isolated from the lysates of Beas2B cells overexpressing these recombinant proteins as described in Figure 2 were incubated with ^{32}P-labeled uPAR 3′-UTR mRNA at 30 °C for 30 min. After treatment with RNase T1 and heparin, the samples were separated on native PAGE, dried, and exposed to X-ray film at −70 °C overnight. Free probe (Fp). Asterisks * and ** represent Wt hnRNPC/uPAR 3′-UTR or hnRNPC RBD/uPAR 3′-UTR complexes, respectively. (c) Expression of hnRNPC fragments in lung epithelial cells. The different hnRNPC deletion fragments overexpressed in Beas2B cells were isolated as described in Figure 2b. These proteins were developed by Western blot using hnRNPC antibody to assess their expression levels. Wt full-length hnRNPC, 40 kDa; RBD, 13.1 kDa; CID 9.8 kDa; CTF, 13.8 kDa. (d) Role of RBD on hnRNPC binding to uPAR 3′-UTR. Full-length hnRNPC protein (Wt) or truncated hnRNPC protein lacking both CID and CTF (RBD) or lacking RBD alone (d-RBD) isolated from stable Beas2B cell lines was subjected to gel shift assay as described in panel b. Free probe (Fp). Asterisks * and ** represent Wt hnRNPC/uPAR 3′-UTR or hnRNPC RBD/uPAR 3′-UTR complexes, respectively. (e) The expression of full-length hnRNPC (Wt), RBD, and d-RBD in Beas2B cells was assessed by Western blot using hnRNPC antibody. Full-length hnRNPC, 40 kDa; RBD, 13.1 kDa; d-RBD, 23.6 kDa. (f) Effect of hnRNPC RBD expression on Beas2B cell surface uPAR expression. Stable Beas2B cells transfected with cDNA sequences that code for RBD or CTF were treated with PBS or uPA for 12 h along with Wt hnRNPC (Wt) and vector DNA (Vc) overexpressing cells. Membrane proteins extracted from these cells were immunoblotted with anti-uPAR antibody. (g) Effect of overexpression of RBD on uPAR mRNA expression. Stable cell lines overexpressing the various fragments of hnRNPC as described in panel f were treated with PBS or uPA for 12 h, and total RNA was analyzed for uPAR mRNA expression by Northern blotting using the uPAR cDNA probe. The experiments were repeated three times, and the mean density of the individual bands is presented as a bar graph. (h) Effect of expression of RBD on uPAR mRNA stability. Stable Beas2B cell lines overexpressing RBD or CTF or full-length hnRNPC (Wt) or empty vector (Vc) were treated with uPA for 12 h to induce maximum uPAR mRNA. The next day transcription was blocked by adding DRB (20 μg/mL) to the same media. RNA was isolated, and uPAR mRNA was measured at different time points by Northern blot using the ^{32}P-labeled uPAR cDNA probe followed by hybridization with the β-actin probe for loading equality. The line graph represents percentage mRNA decay calculated from the mean values obtained by integrating the density of the individual bands from two independent experiments as described in Figure 1d.

blotting using anti-hnRNPC antibody. As shown in Figure 3c, hnRNPC fragments with the expected sizes were expressed at a similar level in Beas2B cells. These latter results further confirmed that increased binding activity exhibited by RBD is due to its binding affinity and not because of differences in the level of expression.

Although lone expression of CID fragment failed to bind uPAR mRNA, we speculated that CID may still be able to bind to uPAR mRNA when it is present in the holoprotein. To further confirm that only RBD, but not CID or CTF, binds to uPAR mRNA in the original conformation, we generated a cDNA construct encoding the hnRNPC lacking RBD (d-RBD) but with both CID and CTF in pcDNA 3.1 and transfected into Beas2B cells. Truncated hnRNPC (d-RBD) proteins isolated from these cell lysates were subjected to gel mobility shift assay. RBD was used as a positive control. The results showed that hnRNPC protein lacking RBD failed to bind the mRNA transcript (Figure 3d), even though their expression was comparable (Figure 3e). These results therefore clearly confirmed the role of RBD in mRNA regulation. RNA binding affinities of individual fragments of hnRNPC protein, however, are far from clear; AU-rich element binding activity of hnRNPC has been restricted to the RBD comprising the amino-terminal 94 amino acids

(*21, 22*). More recent studies, nevertheless, demonstrated that hnRNPC lacking the RBD retained considerable U1, U2, and U6 snRNA binding activity *in vitro* (*23, 24*). This effect was attributed to CID, a leucine zipper motif. Furthermore, Wan and co-workers (*25*) found RBD as the main structural motif that contributes to the binding activity and CID only enhances the affinity of hnRNPC to RNA.

To confirm that the interaction of RBD with uPAR mRNA increases uPAR expression at the cell surface, we treated Beas2B cells transfected with vector DNA alone or vector harboring full-length hnRNPC or RBD or CTF of hnRNPC with PBS or uPA and analyzed for cell surface uPAR expression by Western blotting. Results showed that cells overexpressing RBD of hnRNPC increased uPAR protein at the cell surface compared to Beas2B cells expressing vector alone or CTF. As shown in Figure 3f, overexpression of both RBD and Wt hnRNPC enhanced uPAR expression. The diffused migration of uPAR on SDS−PAGE is likely due to its differential glycosylation (*13, 14*). This observation was confirmed at the uPAR mRNA level by Northern blotting (Figure 3g). Transcription chase experiments showed that hnRNPC RBD likewise stabilized uPAR mRNA in Beas2B cells when compared to control cells or CTF-expressing Beas2B cells (Figure 3h). These results further demonstrated that increased uPAR protein or mRNA expression by RBD is due to enhanced uPAR mRNA stabilization.

Inhibition of hnRNPC Tyrosine Phosphorylation Abolishes the uPA Effect. Collectively, the above experiments showed that RBD of hnRNPC upregulates uPAR expression. Since uPA is known to modulate binding of hnRNPC to uPAR mRNA via tyrosine phosphorylation (*16*), we decided to test if the potentially phosphorylated tyrosine residue (Y57) that resides in the RBD is involved in the induction of uPAR expression by uPA. We therefore mutated tyrosine residue 57 to phenylalanine (Y57F) by site-directed mutagenesis using full-length hnRNPC cDNA packaged in a eukaryotic expression vector, pcDNA 3.1, as a template. We also generated a control hnRNPC mutant cDNA by substituting potentially phosphorylated Y126 residue with phenylalanine (Y126F) for comparison. Plasmids carrying wild-type or mutant hnRNPC cDNAs were individually transfected into Beas2B cells, and stable cell lines were generated by antibiotic selection. The cells expressing wild-type or mutant hnRNPC proteins were treated with PBS or uPA for 12 h, and hnRNPC proteins were isolated from the cell lysates and analyzed for uPAR mRNA 3′-UTR binding by Northwestern assay. hnRNPC (Y57F) mutant protein showed very little binding to uPAR mRNA compared with Y126F mutant or wild-type protein. hnRNPC (Y57F) mutant likewise exhibited minimal tyrosine phosphorylation (Figure 4a). Western blot analyses showed that overexpression of wild-type hnRNPC or Y126F increased Beas2B cell surface uPAR, whereas Y57F mutation failed to enhance basal uPAR expression. Further, uPA induced more uPAR expression in cells transfected with hnRNPC wild-type or Y126F mutant cDNAs compared to vector alone or hnRNPC Y57F mutant cDNA treated cells (Figure 4b). Induction of uPAR expression by uPA in Beas2B cells expressing hnRNPC Y57F mutant was comparable to the cells transfected with vector cDNA and is therefore attributed to basal hnRNPC expression. Inhibition of uPA-induced hnRNPC tyrosine phosphorylation by Y57F mutation likewise tapered uPAR mRNA expression (Figure 4c). These experiments confirmed that uPA influences uPAR expression through phosphorylation of Y57 residue and inhibition of Y57 phosphorylation by overexpressing mutant Y57F hnRNPC suppresses expression of cell surface uPAR and uPAR mRNA.

We then analyzed the effect of Y57F mutation on uPAR mRNA stability by transcription chase experiments. Beas2B cells expressing hnRNPC Y57F mutation exhibited increased degradation of uPAR transcript when compared with cells that expressed hnRNPC Y126F mutation or Wt hnRNPC (Figure 4d), demonstrating the importance of Y57 tyrosine phosphorylation on the posttranscriptional stabilization of uPAR mRNA.

DISCUSSION

uPA induces cell surface uPAR expression and proliferation of nonmalignant lung epithelial cells, malignant lung carcinoma-derived cells, and mesothelioma cells (*26*). Studies have shown that binding of uPA to uPAR activates local proteolysis and intracellular signal transduction processes which play a critical role in tissue remodeling (*27–29*). Increased uPAR expression by atherosclerotic coronary arterial smooth muscle cells and migrating keratinocytes in the wounds further indicate its broader pathophysiological involvement. Increased expression of uPAR has also been reported in hepatocellular, endometrial, gastric, pancreatic, and colorectal carcinomas (*12*). Inhibition of uPAR expression suppresses tumor cell growth *in vivo* (*30*) and *in vitro* (*31*). An elevated level of the soluble form of uPAR (suPAR) has been found in the plasma of patients with advanced breast cancer and colon cancer (*32*). Clinical studies have correlated the poor prognosis in a variety of malignancies with increased uPAR expression (*4, 33*). These reports collectively indicate the prognostic significance of uPAR in human malignancies. Therefore, elucidation of regulatory mechanisms that control uPAR expression and its responses to uPA is pivotal in clinical interventions against pathophysiological conditions.

uPAR expression is known to be regulated at both transcriptional and posttranscriptional level by cytokines and tumor promoters (*13, 17, 19, 34, 35*). However, uPA induces uPAR expression only through posttranscriptional mRNA stabilization in diverse cell types including lung epithelial cells ((*14, 16, 20, 36*). hnRNPC, a ribonucleoprotein that belongs to a family of pre-mRNA-binding proteins is involved in the regulation of uPAR expression. The process involves specific binding of hnRNPC with a 110 nt *cis* regulatory element present in the uPAR mRNA 3′-UTR and preventing its degradation (*15*). Induction of amyloid precursor protein in Alzheimer's diseases has been shown to be mediated through posttranscriptional mRNA stabilization by hnRNPC (*37*). In asthma, eosinophil survival through increased GM-CSF expression is likewise caused by increased hnRNPC expression (*38*). Additional involvement of hnRNPC in the translation of c-*myc* mRNA (*39*) and antiapoptotic protein, X-chromosome-linked inhibitor of apoptosis (XIAP) (*40*) demonstrates its critical role in both malignant and nonmalignant human diseases.

The increased rate of proliferation exhibited by highly malignant squamous cell lung carcinoma H157 cells *in vitro* has been attributed to elevated uPAR expression at the cell surface (*15*). These cells also express high levels of hnRNPC

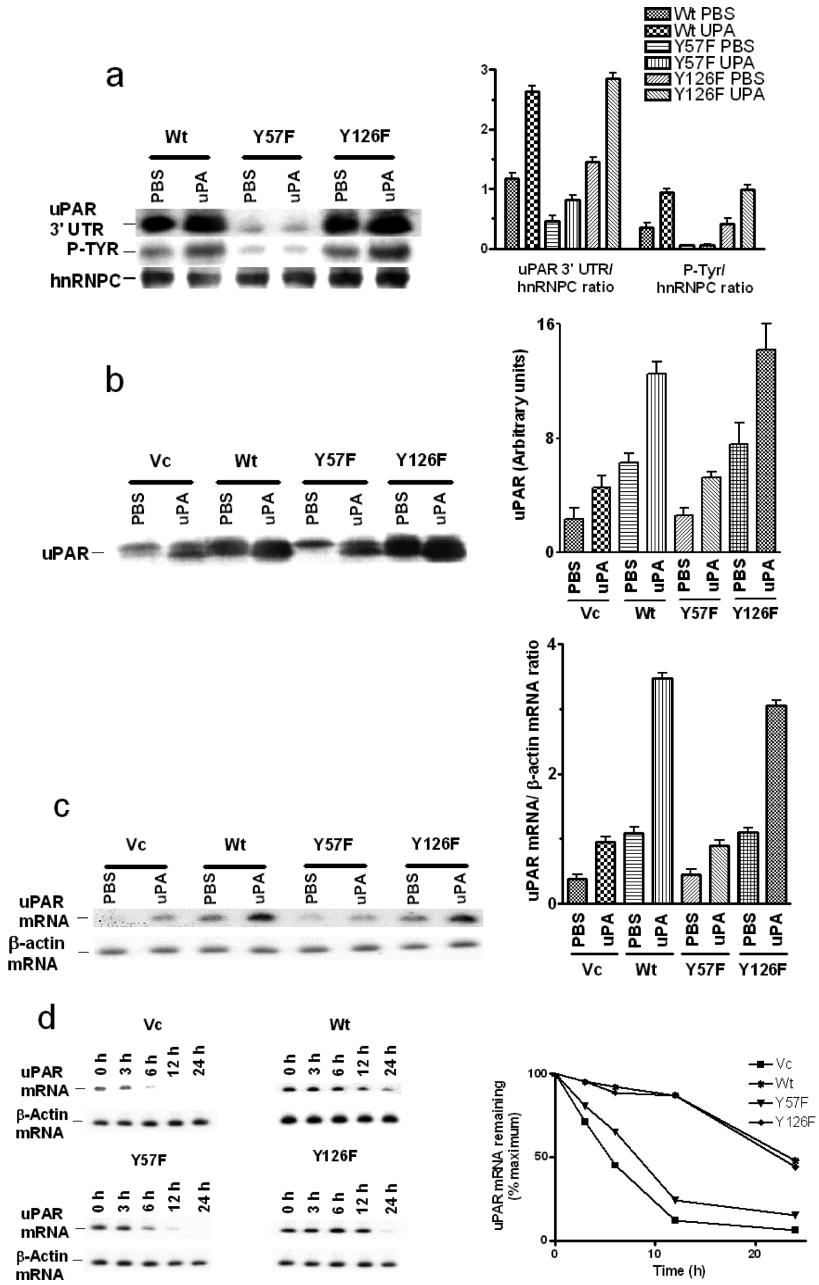

FIGURE 4: Inhibition of Y57 phosphorylation suppresses hnRNPC binding to uPAR mRNA 3′UTR and cell surface uPAR expression. (a) Mutation of hnRNPC tyrosine residues and its ability to interact with uPAR mRNA 3′-UTR. Beas2B stable cell lines expressing either wild-type (Wt) hnRNPC or mutant hnRNPC proteins with tyrosine residues 57 or 126 replaced by phenylalanine (Y57F or Y126F) were treated with PBS or uPA for 12 h. The recombinant hnRNPC proteins isolated from the cell lysates as described in Figure 2 were subjected to uPAR 3′-UTR mRNA binding by Northwestern assay. The same membrane was later stripped and developed by Western blot using anti-hnRNPC or anti-phosphotyrosine antibodies. (b) Effects of mutation of tyrosyl residues on uPAR protein expression. Stable Beas2B cells transfected with pcDNA 3.1 (Vc) or vector containing either wild-type hnRNPC cDNA (Wt) or hnRNPC cDNA with Y57F or Y126F mutations were treated with PBS or uPA for 12 h. The membrane proteins were immunoblotted with anti-uPAR antibody. (c) Role of hnRNPC mutation on uPAR mRNA expression. Stable Beas2B cell lines transfected with vector alone or overexpressing hnRNPC protein with or without Y57F or Y126F mutations were treated with PBS or uPA for 12 h. The total RNA was analyzed for expression of uPAR and β-actin mRNA by Northern blotting. The data shown are representative of three independent experiments. The mean density of the individual bands is presented as a bar graph. (d) Effect of hnRNPC Y57F mutation on uPAR mRNA stability. Stable cell lines overexpressing vector cDNA or Wt hnRNPC or hnRNPC with Y57F or Y126F mutations were treated with uPA for 12 h. The uPAR mRNA was analyzed at different time points after inhibiting on-going transcription by Northern blotting and normalized against the corresponding β-actin mRNA loading control. The line graph represents percentage mRNA decay calculated from the mean values obtained by integrating the density of the individual bands from two separate experiments as described in Figure 1d.

protein and mRNA. In our present study, inhibition of hnRNPC expression in H157 carcinoma cells downregulated the expression of both uPAR protein and mRNA by accelerating mRNA decay. These results support the hnRNPC-mediated uPAR mRNA stabilization as the principal event involved in uPAR expression at the H157 cell surface.

Recently, we reported that TGF-β or PMA induced uPAR expression through posttranscriptional stabilization of uPAR mRNA which can be reversed by treatment with tyrosine kinase inhibitors (*19*). Inhibition of hnRNPC-uPAR mRNA interaction and cell surface uPAR expression by protein tyrosine phosphatase, SHP2 overexpression, and increased

Beas2B and H157 cell hnRNPC-uPAR mRNA binding in the presence of tyrosine phosphatase inhibitor indicate the importance of tyrosine phosphorylation (*16*). Further, uPA failed to increase the expression of either hnRNPC protein or RNA. These observations prompted us to investigate if uPA stabilizes uPAR mRNA through phosphorylation of hnRNPC tyrosyl residues. Results showed that uPA treatment induced tyrosine phosphorylation of hnRNPC and the effect was consistent with the binding efficiency of the protein. Inhibition of hnRNPC binding to uPAR mRNA 3′-UTR and suppression of uPA-mediated uPAR expression in the presence of tyrosine kinase inhibitor herbimycin A or genestein further demonstrate that tyrosine phosphorylation of hnRNPC is critical for uPAR expression.

In the current study, we found that only RBD of the hnRNPC molecule binds to uPAR 3′-UTR mRNA. Although the involvement of regions other than amino-terminal RBD has also been reported in RNA binding (*23, 24*), results from our study showed clearly that only RBD, but not CID and CTF, of hnRNPC is involved in uPAR 3′UTR mRNA binding. Involvement of RBD in the binding activity was further confirmed by the inability of hnRNPC lacking RBD (d-RBD) to bind uPAR mRNA 3′UTR. We found that RBD alone could enhance the expression of uPAR protein and mRNA through mRNA stabilization which further demonstrated the ability of RBD to alter uPAR expression.

The ability of truncated protein (RBD) which contains potentially phosphorylating tyrosine residue Y57 to stabilize uPAR mRNA and respond to uPA stimulation justifies the involvement of this residue in the posttranscriptional regulation of uPAR expression. We subsequently extended our study to determine if phosphorylation of Y57 indeed alters the mRNA binding affinity of hnRNPC protein to regulate uPAR expression. Results showed that mutation of Y57 with phenylalanine (Y57F) on the hnRNPC protein blocked its interaction with uPAR mRNA. This particular point mutation further decreased both basal and uPA-induced cell surface uPAR expression due to impaired stabilization of uPAR mRNA, while Y126F mutation showed no such effect. These observations further confirm the role of tyrosine phosphorylation of hnRNPC in posttranscriptional regulation of uPAR expression, which is mainly mediated through phosphorylation of Y57 residue. Increased hnRNPC phosphorylation by H_2O_2 has been implicated in endothelial cell proliferation and cell survival (*41*). Tyrosine phosphorylation as the primary event involved in uPAR expression could therefore be conceived as the underlying mechanism in uPA/uPAR-mediated mammalian cell proliferation.

In summary, our current study demonstrated for the first time that RBD of hnRNPC binds and stabilizes uPAR mRNA attributing the key role of this domain in hnRNPC-mediated uPAR regulation. Induction of hnRNPC binding to uPAR mRNA by uPA is mediated through phosphorylation of Y57 residue present in the RBD. Furthermore, mutation of Y57 residue abrogates uPA-mediated uPAR induction in lung epithelial cells. The present study provides a clear understanding of the molecular mechanism involved in downstream events that regulate uPAR expression through hnRNPC in lung epithelial cells.

ACKNOWLEDGMENT

The authors are grateful to Janet Harris and Brad Low for technical assistance.

REFERENCES

1. Pollanen, J., Stephens, R. W., and Vaheri, A. (1991) Directed plasminogen activation at the surface of normal and malignant cells. *Adv. Cancer Res. 57*, 273–328.
2. Dano, K., Behrendt, N., Brunner, N., Ellis, V., Ploug, M., and Pyke, C. (1994) The urokinase receptor. Protein structure and role in plasminogen activation and cancer invasion. *Fibrinolysis 8*, 189–203.
3. Kuhn, C. (1993) The pathogenesis of pulmonary fibrosis. *Monogr. Pathol. 36*, 78–92.
4. Mignatti, P., and Rifkin, D. B. (1993) Biology and biochemistry of proteinases in tumor invasion. *Physiol. Rev. 73*, 161–195.
5. Nishiuma, T., Sisson, T. H., Subbotina, N., and Simon, R. H. (2004) Localization of plasminogen activator activity within normal and injured lungs by in situ zymography. *Am. J. Respir. Cell Mol. Biol. 31*, 552–558.
6. Plataki, M., Koutsopoulos, A. V., Darivianaki, K., Delides, G., Siafakas, N. M., and Bouros, D. (2005) Expression of apoptotic and antiapoptotic markers in epithelial cells in idiopathic pulmonary fibrosis. *Chest 127*, 266–274.
7. Blasi, F., Vassalli, J. D., and Dano, K. (1987) Urokinase-type plasminogen activator: proenzyme, receptor, and inhibitors. *J. Cell Biol. 104*, 801–804.
8. Chapman, H. A. (1994) *Fibrinolysis in Disease* (Glas-Green-walt, P., Ed.) pp 253−259, CRC Press, New York.
9. Mustjoki, S., Alitalo, R., Stephens, R. W., and Vaheri, A. (1999) Blast cell-surface and plasma soluble urokinase receptor in acute leukemia patients: relationship to classification and response to therapy. *Thromb. Haemostasis 81*, 705–710.
10. Stephens, R. W., Nielsen, H. J., Christensen, I. J., Thorlacius-Ussing, O., Sorensen, S., Dano, K., and Brunner, N. (1999) Plasma urokinase receptor levels in patients with colorectal cancer: relationship to prognosis. *J. Natl. Cancer Inst. 91*, 869–874.
11. Pappot, H. (1999) The plasminogen activation system in lung cancer with special reference to the prognostic role in "non-small cell lung cancer". *APMIS 107* (Suppl.), 92.
12. Bock, C. E., and Wang, Y. (2004) Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. *Med. Res. Rev. 24*, 13–39.
13. Shetty, S., and Idell, S. (1999) Posttranscriptional regulation of urokinase receptor gene expression in lung carcinoma and malignant mesothelioma cells *in vitro*. *Mol. Cell. Biochem. 199*, 189–200.
14. Shetty, S., and Idell, S. (2001) Urokinase induces expression of its own receptor in Beas2B lung epithelial cells. *J. Biol. Chem. 276*, 24549–24556.
15. Shetty, S. (2005) Regulation of urokinase receptor mRNA stability by hnRNP C in lung epithelial cells. *Mol. Cell. Biochem. 272*, 107–118.
16. Shetty, S., Velusamy, T., Idell, S., Tang, H., and Shetty, P. K. (2007) Regulation of urokinase receptor expression by protein tyrosine phosphatases. *Am. J. Physiol. Lung Cell Mol. Physiol. 292*, L414−L421.
17. Shetty, S., Kumar, A., Johnson, A., Pueblitz, S., and Idell, S. (1995) Urokinase receptor in human malignant mesothelioma cells: Role in tumor cell mitogenesis and proteolysis. *Am. J. Physiol. 268*, L972−L982.
18. Shetty, S., Muniyappa, H., Halady, P. K. S., and Idell, S. (2004) Regulation of urokinase receptor expression by phosphoglycerate kinase. *Am. J. Respir. Cell Mol. Biol. 31*, 100–106.
19. Shetty, S., and Idell, S. (2004) Urokinase receptor mRNA stability involves tyrosine phosphorylation in lung epithelial cells. *Am. J. Respir. Cell Mol. Biol. 30*, 69–75.
20. Montuori, N., Mattiello, A., Mancini, A., Taglialatela, P., Caputi, M., Rossi, G., and Ragno, P. (2001) Urokinase-type plasminogen activator up-regulates the expression of its cellular receptor through a post-transcriptional mechanism. *FEBS Lett. 508*, 379–384.
21. Gorlach, M., Wittekind, M., Beckman, R. A., Mueller, L., and Dreyfuss, G. (1992) Interaction of the RNA-binding domain of the hnRNP C proteins with RNA. *EMBO J. 11*, 3289–3295.

22. Gorlach, M., Burd, C. G., and Dreyfuss, G. (1994) The determinants of RNA-binding specificity of the heterogeneous nuclear ribonucleoprotein C proteins. *J. Biol. Chem. 269*, 23074–23078.
23. Shahied Milam, L., Soltaninassab, S. R., Iyer, G. V., and LeStourgeon, W. M. (1998) The heterogeneous nuclear ribonucleoprotein C protein tetramer binds U1, U2, and U6 snRNAs through its high affinity RNA binding domain (the bZIP-like motif). *J. Biol. Chem. 273*, 21359–21367.
24. McAfee, J. G., Shahied-Milam, L., Soltaninassab, S. R., and LeStourgeon, W. M. (1996) A major determinant of hnrnpC protein binding to RNA is a novel bZIP-like RNA binding domain. *RNA 2*, 1139–1152.
25. Wan, L., Kim, J. K., Pollard, V. W., and Dreyfuss, G. (2001) Mutational definition of RNA-binding and protein-protein interaction domains of heterogeneous nuclear RNP C1. *J. Biol. Chem. 276*, 7681–7688.
26. Bohuslav, J., Horejsi, V., Hansmann, C., Stockl, J., Weidle, U. H., Majdic, O., Bartke, I., Knapp, W., and Stockinger, H. (1995) Urokinase plasminogen activator receptor, beta 2-integrins, and Src-kinases within a single receptor complex of human monocytes. *J. Exp. Med. 181*, 1381–1390.
27. Chapman, H. A., Wei, Y., Simon, D. I., and Waltz, D. A. (1999) Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb. Haemostasis 82*, 291–297.
28. Blasi, F. (1999) Proteolysis, cell adhesion, chemotaxis, and invasiveness are regulated by the uPA-uPAR-PAI-1 system. *Thromb. Haemostasis 82*, 298–304.
29. Koshelnick, Y., Ehart, M., Stockinger, H., and Binder, B. R. (1999) Mechanisms of signaling through urokinase receptor and the cellular response. *Thromb. Haemostasis 82*, 305–311.
30. Mohan, P. M., Chintala, S. K., Mohanam, S., Gladson, C. L., Kim, E. S., Gokaslan, Z. L., Lakka, S. S., Roth, J. A., Fang, B., Sawaya, R., Kyritsis, A. P., and Rao, J. S. (1999) Adenovirus-mediated delivery of antisense gene to urokinase-type plasminogen activator receptor suppresses glioma invasion and tumor growth. *Cancer Res. 59*, 3369–3373.
31. Kook, Y. H., Adamski, J., Zelent, A., and Ossowski, L. (1994) The effect of antisense inhibition of urokinase receptor in human squamous cell carcinoma on malignancy. *EMBO J. 13*, 3983–3991.
32. Stephens, R. W., Pedersen, A. N., Nielsen, H. J., Hammers, M. J. A. G., Hoyer-Hansen, G., Rone, E., Dybkjaer, D. E., and Brunner, N. (1997) ELISA determination of soluble urokinase receptor in blood from healthy donors and cancer patients. *Clin. Chem. 43*, 1868–1876.
33. Andreasen, P. A., Kjoller, L., Christensen, L., and Duffy, M. J. (1997) The urokinase-type plasminogen activator system in cancer metastasis: a review. *Int. J. Cancer 72*, 1–22.
34. Lund, L. R., Ellis, V., Ronne, E., Pyke, C., and Dano, K. (1995) Transcrptional and post-transcriptional regulation of the receptor for urokinase-type plasminogen activator by cytokines and tumour promoters in the human lung carcinoma cell line A549. *Biochem. J. 310*, 435–352.
35. Pedersen, H., Brunner, N., Francis, D., Osterlind, K., Ronne, E., Hansen, H. H., Dano, K., and Grondahl-Hansen, J. (1994) Prognostic impact of urokinase, urokinase receptor, and type 1 plasminogen activator inhibitor in squamous and large cell lung cancer tissue. *Cancer Res. 54*, 4671–4675.
36. Montuori, N., Salzano, S., Rossi, G., and Ragno, P. (2000) Urokinase-type plasminogen activator up-regulates the expression of its cellular receptor. *FEBS Lett. 476*, 166–170.
37. Rajagopalan, L. E., Westmark, C., Jarzembowski, J. A., and Malter, J. S. (1998) hnRNPC increases amyloid precursor protein (APP) production by stabilizing APP mRNA. *Nucleic Acids Res. 26*, 3418–3423.
38. Esnault, S., and Malter, J. S. (2003) Hyaluronic acid or TNF-alpha plus fibronectin triggers granulocyte macrophage-colony-stimulating factor mRNA stabilization in eosinophils yet engages differential intracellular pathways and mRNA binding proteins. *J. Immunol. 171*, 6780–6787.
39. Kim, J. H., Paek, K. Y., Choi, K., Kim, T. D., Hahm, B., Kim, K. T., and Jang, S. K. (2003) Heterogeneous nuclear ribonucleoprotein C modulates translation of c-myc mRNA in a cell cycle phase-dependent manner. *Mol. Cell. Biol. 23*, 708–720.
40. Holcik, M., Gordon, B. W., and Korneluk, R. G. (2003) The internal ribosome entry site-mediated translation of anti apoptotic protein XIAP is mediated by heterogeneous ribonuclear protein C1 and C2. *Mol. Cell. Biol. 23*, 280–288.
41. Kattapuram, T., Yang, S., Maki, J. L., and Stone, J. R. (2005) Protein kinase CK1 alpha regulates mRNA binding by heterogeneous ribonucleoprotein C in response to physiologic levels of hydrogen peroxide. *J. Biol. Chem. 280*, 15340–15347.

BI702338Y