Urokinase-mediated posttranscriptional regulation of urokinase-receptor expression in non small cell lung carcinoma

ARTICLE *in* INTERNATIONAL JOURNAL OF CANCER · JUNE 2003

Impact Factor: 5.09 · DOI: 10.1002/ijc.11091 · Source: PubMed

7 AUTHORS, INCLUDING:

Nunzia Montuori
University of Naples Federico II
63 PUBLICATIONS **1,054** CITATIONS
SEE PROFILE

Alessandro Mancini
Second University of Naples
12 PUBLICATIONS **322** CITATIONS
SEE PROFILE

Paola Taglialatela
Dulbecco Telethon Institute
4 PUBLICATIONS **122** CITATIONS
SEE PROFILE

Pia Ragno
Università degli Studi di Salerno
49 PUBLICATIONS **1,146** CITATIONS
SEE PROFILE

Int. J. Cancer: **105,** 353–360 (2003)

Publication of the International Union Against Cancer

UROKINASE-MEDIATED POSTTRANSCRIPTIONAL REGULATION OF UROKINASE-RECEPTOR EXPRESSION IN NON SMALL CELL LUNG CARCINOMA

Nunzia MONTUORI[1], Amalia MATTIELLO[2], Alessandro MANCINI[2], Paola TAGLIALATELA[3], Mario CAPUTI[2], Guido ROSSI[1,3] and Pia RAGNO[1]*

[1]*Istituto di Endocrinologia ed Oncologia Sperimentale (IEOS), Consiglio Nazionale delle Ricerche, Naples, Italy*
[2]*Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Ospedale Monaldi, Naples, Italy*
[3]*Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università "Federico II" di Napoli, Naples, Italy*

The urokinase-type plasminogen activator (uPA) and its cellular receptor (uPAR) are involved in the proteolytic cascade required for tumor cell dissemination and metastasis, and are highly expressed in many human tumors. We have recently reported that uPA, independently of its enzymatic activity, is able to increase the expression of its own receptor in uPAR-transfected kidney cells at a posttranscriptional level. In fact, uPA, upon binding uPAR, modulates the activity and/or the level of a mRNA-stabilizing factor that binds the coding region of uPAR-mRNA. We now investigate the relevance of uPA-mediated posttranscriptional regulation of uPAR expression in non small cell lung carcinoma (NSCLC), in which the up-regulation of uPAR expression is a prognostic marker. We show that uPA is able to increase uPAR expression, both at protein and mRNA levels, in primary cell cultures obtained from tumor and adjacent normal lung tissues of patients affected by NSCLC, thus suggesting that the enzyme can exert its effect in lung cells. We investigated the relationship among the levels of uPA, uPAR and uPAR-mRNA binding protein(s) in NSCLC. Lung tissue analysis of 35 NSCLC patients shows an increase of both uPA and uPAR in tumor tissues, as compared to adjacent normal tissues, in 27 patients (77%); 19 of these 27 patients also show a parallel increase of the level and/or binding activity of a cellular protein capable of binding the coding region of uPAR-mRNA. Therefore, in tumor tissues, a strong correlation is observed among these 3 parameters, uPA, uPAR and the level and/or the activity of a uPAR-mRNA binding protein. We then suggest that uPA regulates uPAR expression in NSCLC at a posttranscriptional level by increasing uPAR-stability through a cellular factor that binds the coding region of uPAR-mRNA.

Key words: *urokinase; urokinase-receptor; posttranscriptional regulation; mRNA binding protein; lung cancer*

The receptor of the urokinase-type plasminogen activator (uPAR) is a heavily glycosylated single-chain protein with a molecular weight of 45–60 kDa. It consists of 3 different domains; the N-terminal domain (D_1) binds the serine-protease uPA, which converts the zymogen plasminogen to plasmin.[1] Plasmin is a broad-spectrum protease able to promote extracellular-matrix (ECM) degradation, both by directly degrading ECM components and by activating latent collagenases and metalloproteases.[2] uPAR focuses uPA activity on the cell membrane, thus regulating cell-surface associated proteolysis.[1,2] The uPA receptor is also involved in the regulation of cell adhesion and migration, independently of the enzymatic activity of its ligand,[3] because it interacts with different types of integrins[4,5] and with vitronectin (VN), a component of the ECM.[6] The linker region, connecting domains 1 and 2 of uPAR, can be cleaved by several proteases, including uPA, which promote the release of D_1,[7] thus unmasking a short region with chemotactic properties.[8]

uPAR is anchored to the cell-surface by a glycosyl-phosphatidylinositol (GPI) tail and lacks a transmembrane domain and a cytosolic tail;[1] however, it is capable of transmitting uPA-mediated extracellular signals inside the cell, probably through the association with some cell-membrane molecules, such as integrins and caveolin[3,9] or other unidentified adaptors.[10] Therefore, the uPA-uPAR system promotes cell migration and tissue invasion through plasmin dependent and independent mechanisms, and plays a preminent role in the regulation of the invasive potential and the migratory capability of tumor cells.

The human uPAR gene consists of 7 exons and 6 introns extending over 23 kb of genomic DNA.[11] Mature uPAR-mRNA is 1,4 kb long and encodes a 313-amino acid polypeptide with a 21-residue signal peptide.[12] uPAR gene expression is influenced by several stimuli, such as tumor promoters, growth factors, cytokines, hormones and hypoxia, both at transcriptional and post-transcriptional levels.[13]

The first evidence for posttranscriptional regulation of uPAR expression was obtained in a human lung carcinoma cell line.[14] The main mechanism of the posttranscriptional regulation of gene expression is the control of the rate of mRNA turnover. Several sequence elements regulating mRNA decay have been identified throughout the mRNA of different proteins. The best characterized determinants are AU-rich elements (ARE) in the 3′-untranslated region (UTR) of several cytokines and oncoproteins; these determinants regulate mRNA degradation by binding stabilizing or destabilizing trans-acting factors.[15–17] The presence of functional AREs in the 3′ UTR of uPAR-mRNA has been reported.[18] ARE-mediated posttranscriptional regulation has been shown also for other components of the plasminogen activation system, such as uPA[19] and its specific inhibitors, PAI-1[20] and PAI-2.[21]

Stability determinants have ben identified within the coding region of very few mRNAs, such as beta-tubulin, c-*fos*, c-*myc* mRNAs[22–24] and, interestingly, in the coding region of 2 components of the plasminogen activation system: PAI-2 and uPAR.[25,26] Increased instability of uPAR-mRNA has been correlated, in human mesothelioma cells, with the presence of a 50 kDa cytoplasmic protein that binds a sequence of 51 nucleotides (nt) located in the coding region of uPAR-mRNA (nt 195–246).[26]

Several types of tumors express high levels of uPAR, but the mechanisms regulating its expression in neoplastic cells have not been clearly elucidated. We have recently reported that uPA upregulates the cell-surface expression of its own receptor in several cell types, independently of its enzymatic activity.[27] uPA increases uPAR expression in uPAR-transfected kidney cells at a posttranscriptional level, by increasing the level and/or the activity of a protein that binds the coding region of uPAR-mRNA, thus stabi-

Grant sponsor: Associazione Italiana per la Ricerca sul Cancro (AIRC).

*Correspondence to: Istituto di Endocrinologia ed Oncologia Sperimentale (IEOS), Consiglio Nazionale delle Ricerche, Via Pansini 5, I-80131, Naples, Italy. Fax: +39-081-7701016. E-mail: ragno@cds.unina.it

Received 8 July 2002; Revised 15 November 2002, 23 January 2003; Accepted 24 January 2003

DOI 10.1002/ijc.11091

lizing the transcript.[28] In the present study, we investigate the relevance of this posttranscriptional mechanism of regulation of uPAR expression and its correlation with uPA in non small cell lung carcinoma (NSCLC), where high levels of uPAR expression are an important prognostic factor.[29]

MATERIAL AND METHODS

Reagents

uPAR cDNA was kindly provided by Dr. M.P. Stoppelli (IIGB, CNR, Naples, Italy). uPA, rabbit polyclonal anti-uPAR and anti-uPA antibodies were from American Diagnostica (Greenwich, CT). Anti-actin antibody and the protease inhibitor cocktail were from SIGMA (St. Louis, MO). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit immunoglobulin G (IgG) were from BIORAD (Richmond, CO). The enhanced chemioluminescence (ECL) detection kit was from Amersham International (Amersham, England) and polyvinylidene fluoride (PVDF) filters were from Millipore (Windsor, MA). The polymerase chain reaction (PCR) kit was from Perkin-Elmer (Branchburg, NJ), T7 and SP6 polymerases were from Promega (Madison, WI), and TRIzol reagent and Superscript II (M-MLV Reverse Transcriptase) were from Life Technologies (Gaithersburg, MD).

Tissue collection

Primary lung cancer tissues and adjacent normal tissues were obtained immediately after surgery from 35 patients affected by NSCLC (19 adenocarcinomas and 16 squamous cell carcinomas), classified as N0 (21), N1 (7) and N2 (7).

Cell culture

Primary cell cultures were prepared as described previously[30] from lung cancer tissue and from adjacent normal lung tissue of patients undergoing surgery for NSCLC. Cells were grown in basal epithelial growth medium (Clonetics-Biowittaker, Walkersville, MD) supplemented with 10% fetal calf serum (FCS) and basal epithelial growth factors (Clonetics-Biowittaker).

Inactivation of uPA

uPA was inactivated by 2 hr incubation with 10 mM diisopropylfluorophosphate (DFP).[31]

Western blot

Lung tissues and cells were lysed in 1% Triton X-100/phosphate buffered saline (PBS) in the presence of protease inhibitors; the protein content was measured by a colorimetric assay (BIORAD). Fifty μg of cell lysate proteins and 100 μg of tissue extract proteins were electrophoresed on a 9% SDS-Polyacrylamide-Gel-Electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane. The membrane was blocked with 5% nonfat dry milk, and probed with anti-uPAR or anti-uPA antibodies at a concentration of 1 μg/ml. Finally, washed filters were incubated with horseradish peroxidase-conjugated anti-rabbit antibody and detected by ECL. Filters were washed and reprobed with an anti-actin antibody (1 μg/ml), as a loading control.

Reverse-PCR

Total cellular RNA was isolated by lysing cells in TRIzol solution according to the supplier's protocol. RNA was precipitated and quantitated by spectroscopy. An amount of 5 μg of total RNA was reversely transcribed with random hexamer primers and 200 U of M-MLV reverse transcriptase. Reverse-transcribed DNA of 1 μl was then amplified, using uPAR-specific 5′ sense (CTG CGG TGC ATG CAG TGT AAG) and 3′ antisense (GGT CCA GAG GAG AGT GCC TCC) 21 mer cDNA primers or GAPDH-specific 5′ sense (TTC ACC ACC ATG GAG AAG GCT) and 3′ antisense (ACA GCC TTG GCA GCA CCA GT) 20 mer cDNA primers, as a control. Semiquantitative PCR was performed for different number of cycles at 62°C in a thermocycler, and the reaction products were analyzed by electrophoresis in 1% agarose gel containing ethidium bromide, followed by photography under ultraviolet illumination.

In vitro transcription

Linearized plasmid of 1 μg containing the coding region of human uPAR was transcribed *in vitro* with T7 RNA polymerase, according to the supplier's protocol, in the presence of 50 μCi (alfa-^{32}P)UTP. Precipitation in 1 M NH_4 acetate was performed to remove unincorporated radioactivity. The specific activity of the product was 10^8 cpm/μg. The size of the labeled mRNA transcript was confirmed by electrophoresis on a 5% urea gel.

Unlabeled RNA competitors were prepared by *in vitro* transcription of 10 μg of the linearized plasmid, according to the supplier's protocol, using T7 RNA polymerase for sense uPAR-mRNA, and SP6 RNA polymerase for antisense uPAR-mRNA.

RNA binding assay

RNA binding assay was performed as previously described.[26] Twenty μg of tissue extract proteins was incubated with 4×10^5 cpm of ^{32}P-labeled transcript in a mixture containing 15 mM KCl, 5 mM $MgCl_2$, 0.25 mM EDTA, 0.25 mM dithiothreitol, 12 mM HEPES (pH 7.9), 10% glycerol and 200 ng/μl of *E. coli* tRNA, in a total volume of 20 μl at 30°C for 30 min. The mixture was then treated with 50 U of RNAase A at 37°C for 30 min and with 5 mg/ml of heparin at room temperature for 10 min, to avoid nonspecific protein binding. Samples were finally analyzed by 5% native PAGE and autoradiography.

RESULTS

uPA up-regulates uPAR expression in primary cell cultures derived from NSCLC

We have previously reported that uPA is able to increase uPAR expression in a kidney cell line transfected with the uPAR-cDNA.[28] To investigate if the up-regulation of uPAR expression mediated by uPA occurs also *in vivo*, we prepared primary cell cultures from lung carcinoma and adjacent normal tissues of 3 patients affected by NSCLC. Cells were then stimulated with or without 5 nM DFP-inactivated-uPA for 16 hr and lysed in Triton X-100 for total proteins analysis. uPA was inactivated in order to observe only the effects mediated directly by its interaction with uPAR, thus excluding those due to its enzymatic activity.

Total proteins were analyzed by 9% SDS-PAGE and western blot with an anti-uPAR antibody. uPA treatment increased uPAR expression in both normal (N) and tumor (T) cells obtained from the 3 patients (Fig. 1). The additional faster (35 kDa) uPAR band, present in the lysates, corresponds to a cleaved form of uPAR (c-uPAR) previously described.[7]

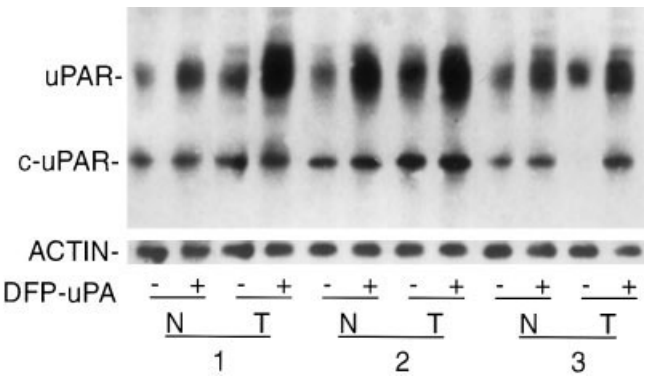

FIGURE 1 – uPA up-regulates uPAR expression in NSCLC primary cell cultures at the protein level. Primary cell cultures derived from normal (N) and tumor (T) tissues of 3 patients affected by NSCLC were stimulated with or without 5 nM DFP-uPA for 16 hr and then lysed. Fifty μg of total proteins was analyzed by 9% SDS-PAGE and western blot with an anti-uPAR antibody. The filter was then hybridized with an anti-actin antibody as a control for protein loading.

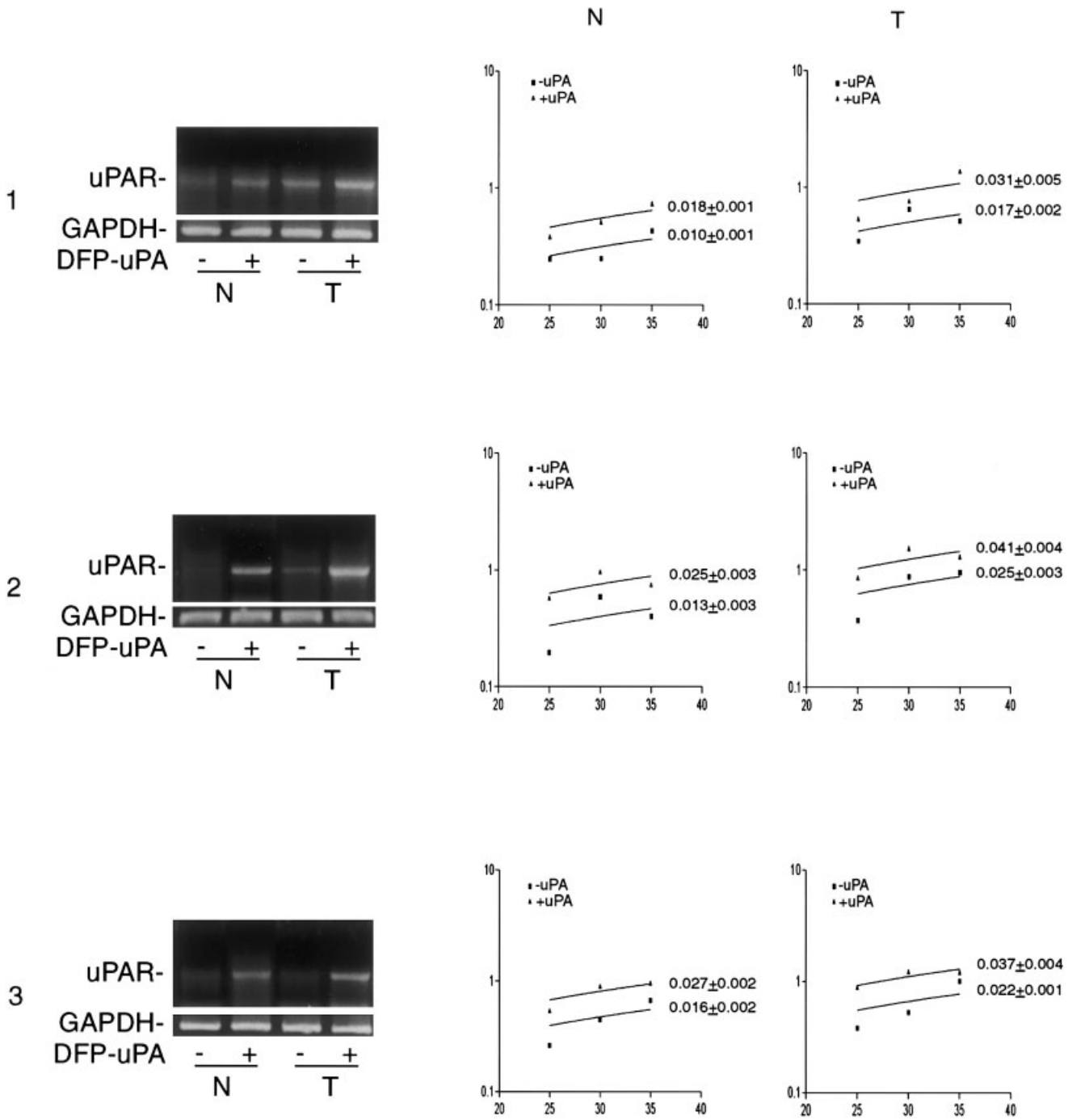

FIGURE 2 – uPA up-regulates uPAR expression in NSCLC primary cell cultures at mRNA level. Primary cell cultures derived from normal (N) and tumor (T) tissues of 3 patients affected by NSCLC were stimulated with or without 5 nM DFP-uPA for 16 hr and then lysed in TRIzol. Total RNA was prepared, reverse-transcribed and amplified by 30 cycles of PCR using primers for uPAR, or for GAPDH as a control for DNA loading. PCR products were analyzed by electrophoresis in 1% agarose gel containing ethidium bromide, followed by photography under ultraviolet illumination (left). The levels of uPAR and GAPDH PCR products after 25, 30 and 35 cycles were estimated by densitometric scanning. uPAR levels, normalized to GAPDH levels, and the number of PCR cycles were plotted on a semilog plot. The different slopes are indicated on the graphs (right).

We then investigated if the observed increase in uPAR expression at a protein level correlated with an increase in uPAR-mRNA. Cells were stimulated with or without 5 nM DFP-uPA for 16 hr and lysed in TRIzol for total RNA preparation. RNA was then reverse-transcribed and amplified by 30 cycles PCR in the presence of primers for uPAR or for GAPDH, as a loading control. The analysis of PCR products by electrophoresis in agarose gel (Fig. 2, left panels) showed the presence of uPAR-mRNA both in N and T lung cells, and its increase after treatment with DFP-uPA, thus demonstrating that the uPA-mediated increase of uPAR expression observed by western blot analysis was due to an increase of the specific uPAR-mRNA. The levels of PCR products after 25, 30 and 35 cycles, estimated by densitometric scanning, and plotted *vs.* the number of cycles, indicated a uPA-dependent increase of uPAR mRNA, ranging between 64% and 92% (Fig. 2, right panels).

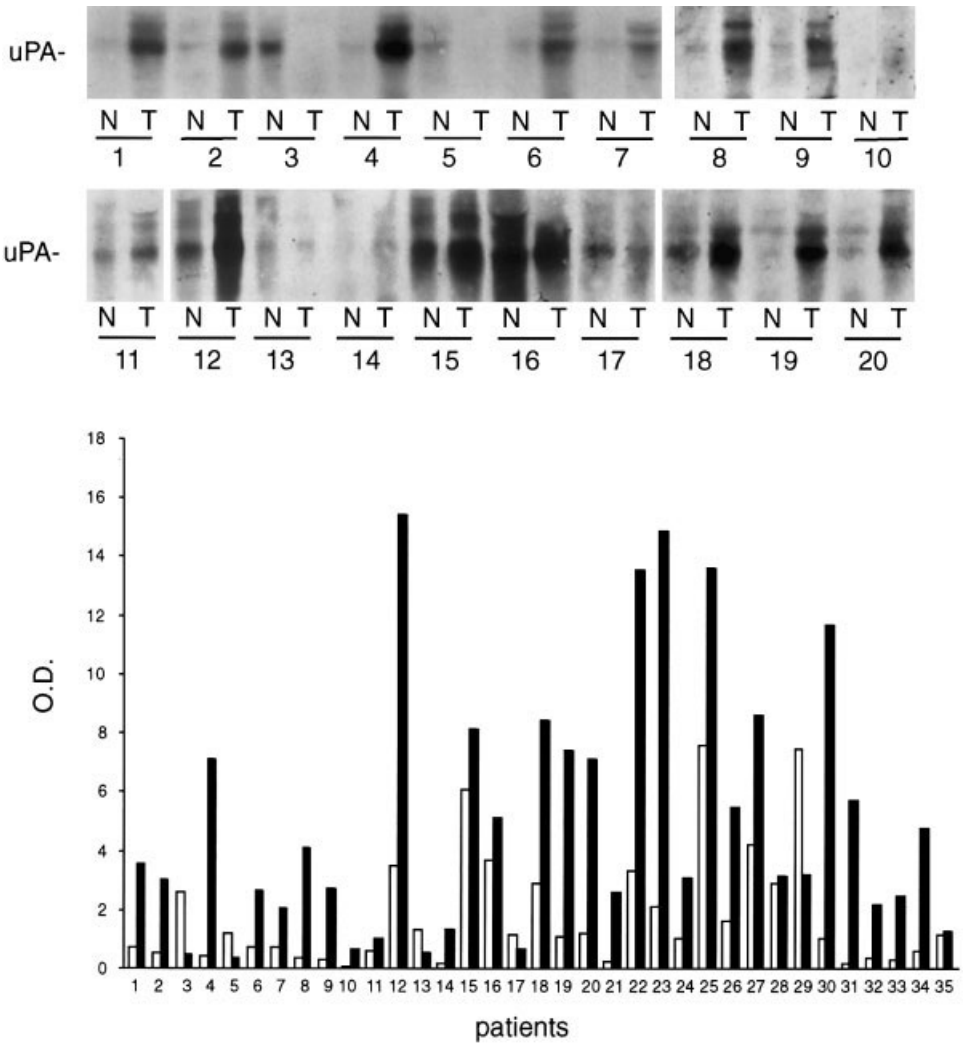

FIGURE 3 – uPA expression in NSCLC. An amount of 100 μg of total proteins of normal (N) and tumor (T) tissue extracts of 35 patients affected by NSCLC was analyzed by 9% SDS-PAGE and western blot with anti-uPA antibodies; the levels of uPA were estimated by densitometric scanning. Upper panel: western blot analysis of 20 out of 35 patients. Lower panel: densitometric scanning of western blot of all patients. The white columns correspond to normal tissues, the black columns to tumor tissues.

Because uPA increases uPAR expression at protein and mRNA levels in primary cell cultures of lung tissues, it is reasonable to assume that it is able to up-regulate uPAR also in lung tissues *in vivo*.

uPA and uPAR expression in NSCLC

We then examined uPA and uPAR levels in NSCLC tissue extracts, in order to investigate a relation between uPA levels and uPAR expression in this type of tumor. Lung cancer and adjacent normal tissues of 35 patients were lysed and analyzed by 9% SDS-PAGE and western blot with anti-uPA and anti-uPAR antibodies. Western blots of N and T tissues of the first 20 patients with anti-uPA and anti-uPAR antibodies are shown in the upper panels of Figures 3 and 4, respectively. uPA and uPAR levels of all 35 patients were estimated by densitometric scanning (Figs. 3 and 4, lower panels).

The results of the western blot analysis and densitometric scanning are summarized in Table I. Only variations higher than 30% were taken into account. uPAR was increased in tumor tissue, as compared to normal tissue, in 33 patients (94%); both uPA and uPAR expression increased in tumor tissue in 27 patients (77%). uPA and uPAR expression in normal and tumor tissues of the other few cases appeared not correlated (Table I).

Western blot analysis and densitometric scanning also showed a high correlation between uPA and uPAR expression in NSCLC tissues (correlation coefficient $r = 0.36$, $p = 0.002$).

uPA and uPAR levels increased significantly in tumor tissues as compared to normal tissues ($p = 0.0001$ and $p < 0.0001$, respectively, as determined by the Student *t*-test; Fig. 5*a*). uPA and uPAR levels were highly variable in the tumor tissues and did not become more homogeneous when the samples were separated according to histologic type or lymph node status of the tumors (Fig. 5*b* and 5*c*, respectively).

uPARmRNA-protein complex in NSCLC

We recently reported that uPA up-regulates uPAR expression in uPAR-transfected kidney cells through a posttranscriptional mechanism, by increasing the level and/or the activity of a cellular factor that binds and stabilizes the coding region of uPAR-mRNA.[28] A RNA binding assay was then performed in order to investigate the presence of uPAR-mRNA binding protein(s) in lung tissues. The labeled coding region of uPAR-mRNA was incubated with the same extracts of normal and tumor tissues from NSCLC patients, previously analyzed by western blot for uPA and uPAR expression. The analysis by a 5% nondenaturing polyacrylamide gel of the first 20 patients and a competition experiment performed on tissue extracts of 2 patients are shown in Figure 6*a* and 6*b*, respectively. The RNA binding assay showed a band corresponding to a single RNase resistant RNA-protein complex in the tissues of most patients. Binding of labeled uPAR-mRNA to cellular protein(s) was competed by a 100-fold excess of cold sense uPAR-mRNA, whereas it was not affected by antisense uPAR-mRNA (Fig. 6*b*). Levels of the uPARmRNA-protein complex were estimated by densitometric scanning in all 35 patients

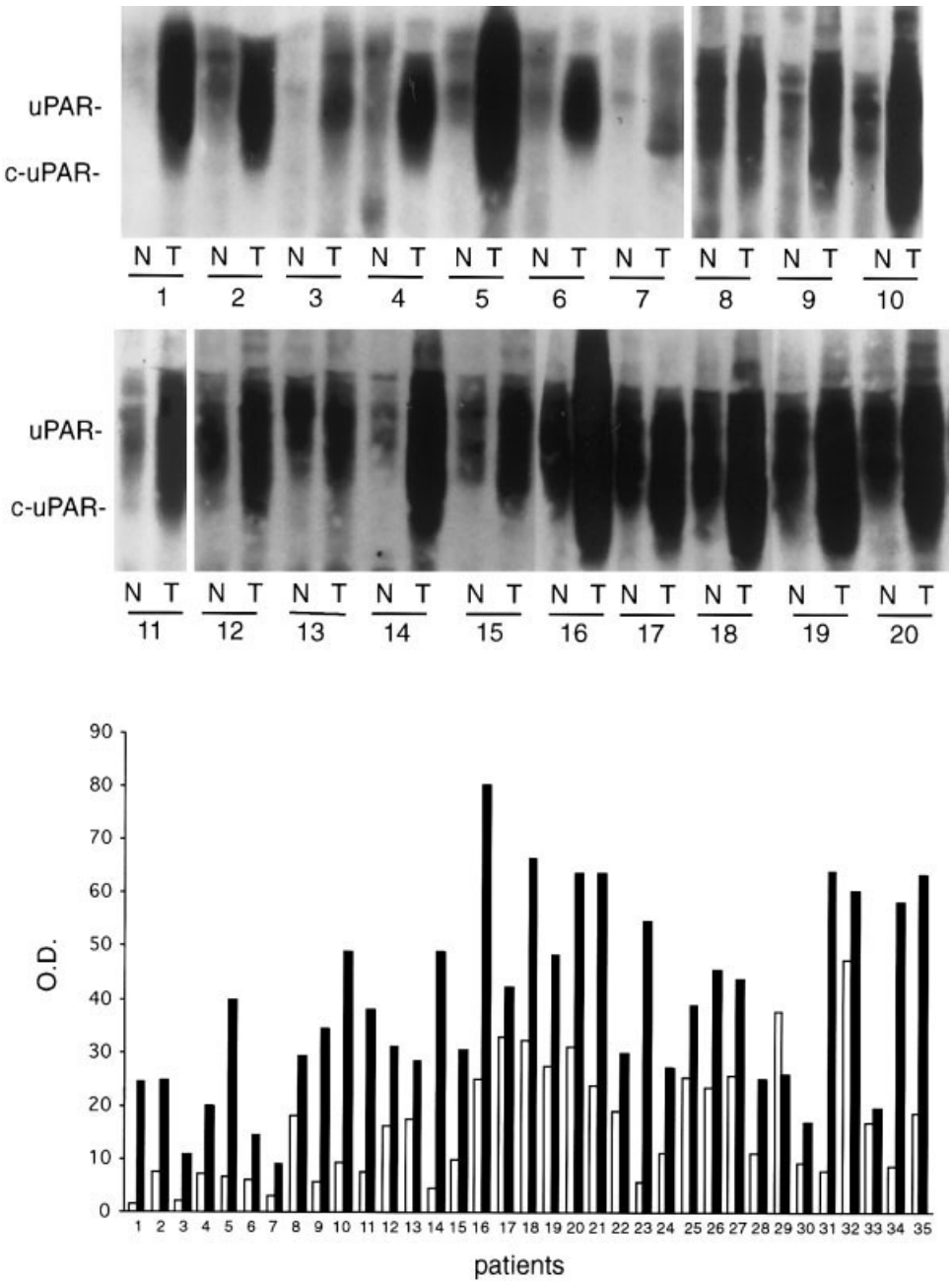

FIGURE 4 – uPAR expression in NSCLC. An amount of 100 μg of total proteins of normal (N) and tumor (T) tissue extracts of 35 patients affected by NSCLC was analyzed by 9% SDS-PAGE and western blot with anti-uPAR antibodies; the levels of uPAR were estimated by densitometric scanning. Upper panel: western blot analysis of 20 out of 35 patients. Lower panel: densitometric scanning of western blot of all patients. The white columns correspond to normal tissues, the black columns to tumor tissues.

TABLE I – UPA, UPAR AND UPARMRNA-PROTEIN COMPLEX IN NSCLC

uPA	uPAR	uPARmRNA-pc	Patients
I	I	I	19
I	I	D	4
I	I	=/ND	4
I	=	I	1
D	I	I	2
D	I	D	1
D	I	=	1
D	D	=	1
=	I	I	2

The expression of uPA and uPAR and the presence of uPARmRNA-protein complex (uPARmRNA-pc) have been analyzed in normal and tumor tissues of 35 patients affected by NSCLC. For each patient, the levels of uPA, uPAR and uPARmRNA-protein complex in the tumor tissue have been compared to the corresponding levels in normal tissue. I, increased level in tumor tissue as compared to normal tissue; D, decreased level in tumor tissue as compared to normal tissue; =, equal levels in tumor and normal tissues; ND, not detected in both tissues.

(Fig. 7). The results of RNA binding assays were summarized in Table I. Only variations higher than 30% in tumor tissue as compared to normal tissue were taken into account. The uPARmRNA-protein complex (uPARmRNA-bp complex) was increased in the tumor tissue of 24/35 patients (68.5%); 19 of these 24 patients showed a parallel increase of both uPA and uPAR (Table I). In these 19 patients, the amount of the uPARmRNA-bp significantly correlated with both uPA ($r = 0.33$; $p = 0.04$) and uPAR ($r = 0.46$; $p = 0.003$) levels.

All together, these results indicate that the coding region of uPAR-mRNA is able to bind specifically a cellular factor present in lung tissue extracts and that the level and/or the binding activity of this factor increases in the majority of tumor tissues as compared to their adjacent normal tissues. The increased level of the complex is strongly related to increased levels of uPAR and uPA, thus supporting the hypothesis that uPA regulates uPAR expression in NSCLC tissues by increasing the level and/or promoting the binding of a stabilizing protein to the coding region of uPAR-mRNA, as previously shown *in vitro*.[28]

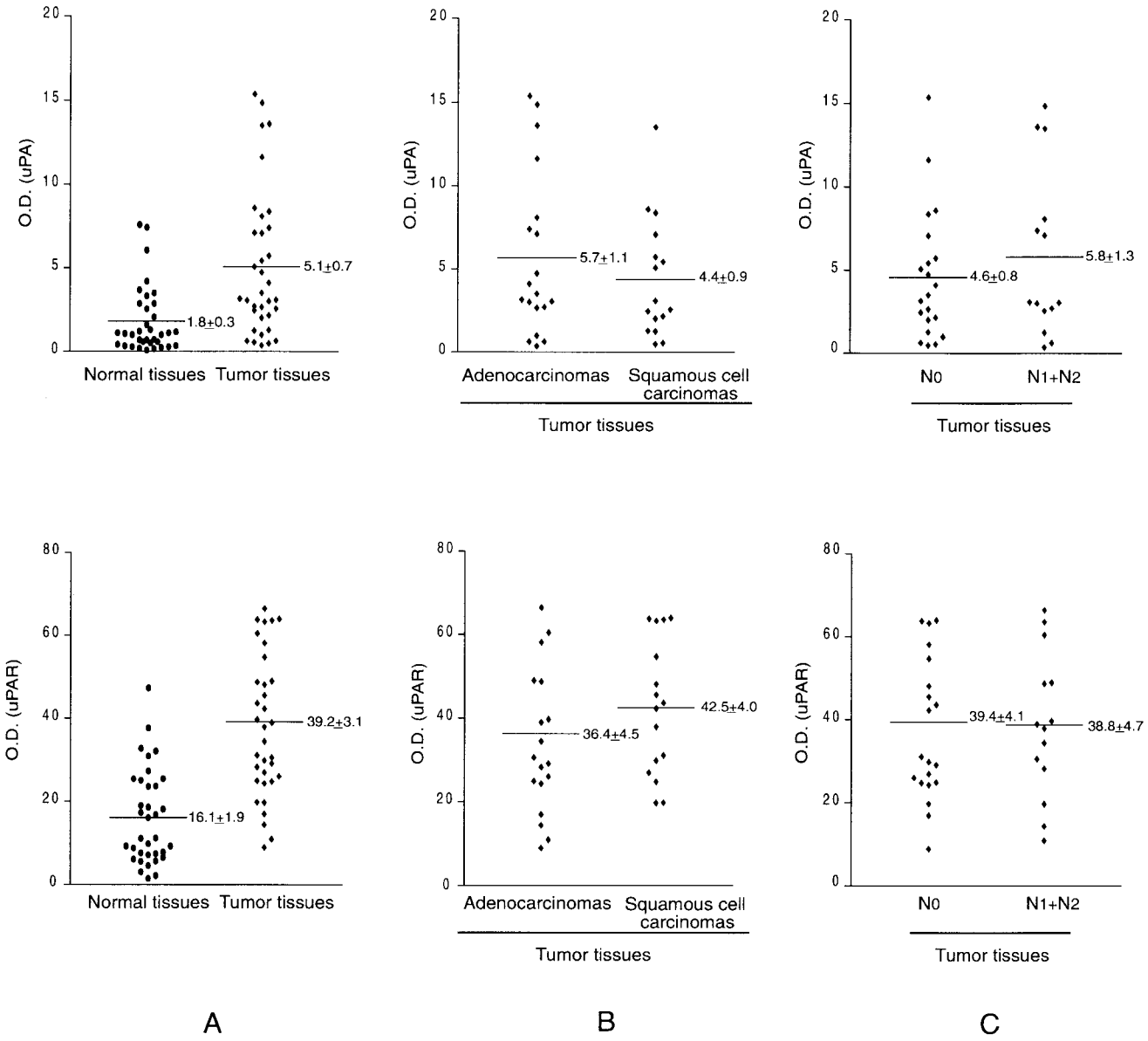

FIGURE 5 – uPA and uPAR variations in NSCLCs. uPA and uPAR levels in normal and tumor tissues (*a*); in adenocarcinomas and squamous cell carcinomas (*b*); in tumors of lymph node status N0 and N1+N2 (*c*). Upper panels: uPA levels; lower panels: uPAR levels. The values on the right of horizontal bars represent the means ± SEM for each group. ($p \leq 0.0001$ tumor tissues *vs.* normal tissues, both panels *a*; $p > 0.05$ squamous cell carcinomas *vs.* adenocarcinomas, both panels *b*; $p > 0.05$ N1+N2 *vs.* N0, both panels *c*).

DISCUSSION

A large body of evidence clearly indicates that the uPA-uPAR system is correlated with the invasive properties of malignant cells, and is overexpressed in several types of tumor, both on the cell surface and in plasma.[32]

In vitro uPAR expression can be regulated by numerous stimuli, such as tumor promoters, growth factors, cytokines and hormones,[13] that act at transcriptional as well as at posttranscriptional levels. We have previously shown that uPA, upon binding uPAR, is able to increase the expression of its receptor on the cell surface of uPAR-transfected kidney cells through a posttranscriptional mechanism. In fact, uPA increases the level and/or the activity of a factor that forms a complex with the coding region of uPAR-mRNA, thus prolonging its half-life.[28]

In order to evaluate the relevance of this posttranscriptional mechanism in NSCLC, we studied the effects of uPA on uPAR expression in lung cells *in vitro* in primary cell cultures obtained from tumor and adjacent normal tissues of NSCLC patients. We thus demonstrated that uPA increased uPAR expression in lung cells, both at protein and mRNA levels.

We then analyzed NSCLC tissues, to determine if, *in vivo*, the levels of uPA could be correlated to the expression of uPAR and to the level and/or activity of uPAR-mRNA binding protein(s). The results, reported in Table I, showed that uPAR expression was increased in the tumor tissue, as compared to the normal tissue, in 33 out of 35 patients affected by NSCLC (94%); this increase was correlated to an enhanced presence of uPA in 27/35 patients (77%). Nineteen of these 27 patients also showed increased levels of the uPARmRNA-protein complex. On the basis of these strong correlations among uPA, uPAR and uPAR-mRNA binding protein(s), we suggest that in most NSCLCs an increased amount of uPA, which, *in vivo*, could be provided by stromal cells as shown for other types of tumor,[1] up-regulates uPAR expression on the tumor cell surface, and that a posttranscriptional mechanism of

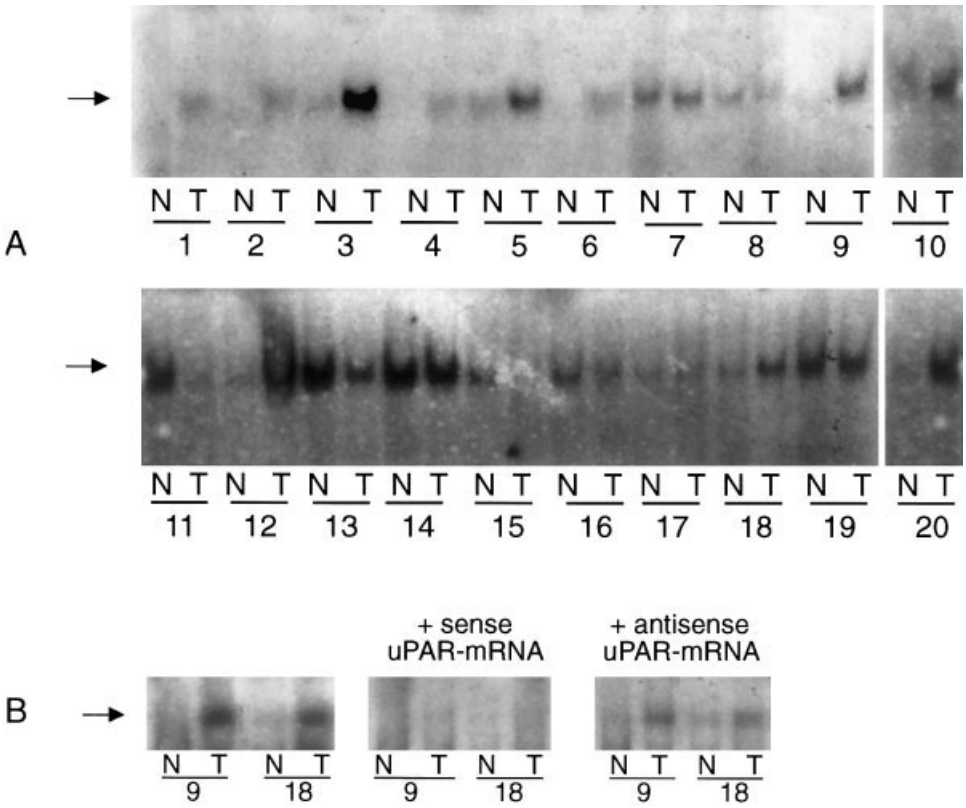

FIGURE 6 – Identification of a uPARmRNA-protein complex in NSCLC by RNA binding assay. Twenty g of total proteins of normal (N) and tumor (T) tissue extracts of 35 patients affected by NSCLC was incubated with ^{32}P-labeled uPAR-mRNA. The mixtures were then treated with RNase A and analyzed by 5% nondenaturing polyacrylamide gel and autoradiography. Panel *a*: RNA binding assay of 20 out of 35 patients. Panel *b*: N and T tissue extracts of patients 9 and 18 incubated with ^{32}P-labeled uPAR-mRNA in the absence of a competitor, in the presence of a 100-fold excess of cold sense uPAR-mRNA or in the presence of a 100-fold excess of cold antisense uPAR-mRNA. The arrows indicate the RNA-protein complex.

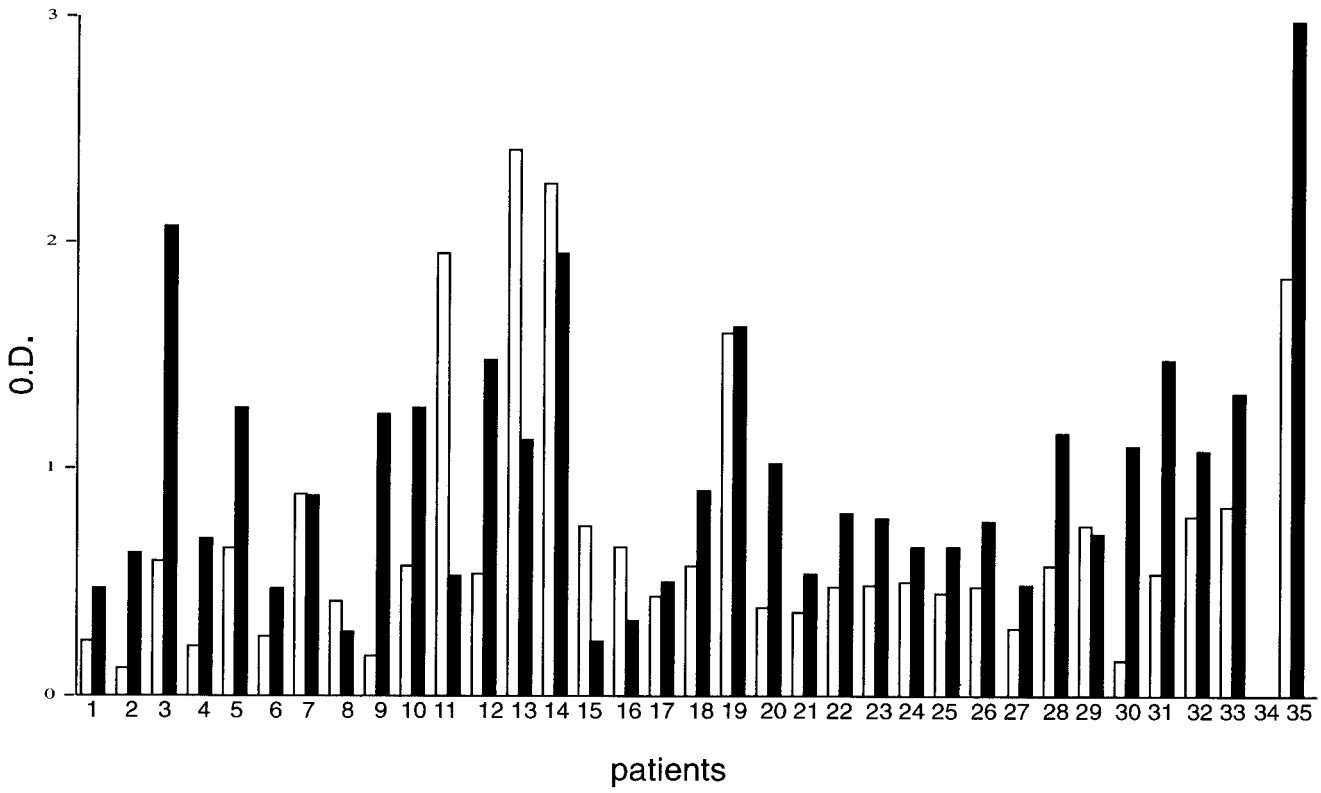

FIGURE 7 – Levels of uPARmRNA-protein complex in NSCLC. The levels of uPARmRNA-protein complex in RNA binding assay of all 35 NSCLC patients were estimated by densitometric scanning. The white columns correspond to normal tissues, the black columns to tumor tissues.

regulation mediated by a uPAR-mRNA stabilizing protein could occur in the majority of these cancers. In the 8 patients not showing an up-regulation of the uPARmRNA-protein complex, uPA could increase uPAR expression only at a transcriptional level, as described in breast carcinoma.[33] Interestingly, 4 of 35 patients showed an increased amount of the uPARmRNA-protein complex in tumor tissue as compared to normal tissue, in spite of the fact that the uPA level was decreased or unchanged, thus suggesting that the complex can be regulated also by factors other than uPA. These results strongly suggest a relevant role *in vivo* of the posttranscriptional regulation of uPAR expression mediated by regulatory motifs located inside the coding region of uPAR-mRNA.

ACKNOWLEDGEMENTS

We thank Dr. M.P. Stoppelli, Istituto Internazionale di Genetica e Biofisica (Naples, Italy) for kindly providing uPAR cDNA. Our study was partly supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC).

REFERENCES

1. Dano K, Behrendt N, Brunner N, Ellis V, Ploug M, Pyke C. The urokinase receptor. Protein structure and role in plasminogen activation and cancer invasion. Fibrinolysis 1994;8:189–203
2. Vassalli JD. The urokinase receptor. Fibrinolysis 1994;8:172–81
3. Chapman HA. Plasminogen activators, integrins and the coordinated regulation of cell adhesion and migration. Curr Opin Cell Biol 1997; 9:714–24
4. Wei Y, Lukashev M, Simon DI, Bodary SC, Rosenberg S, Doyle MV, Chapman HA. Regulation of integrin function by the urokinase receptor. Science 1996;273:1551–5
5. Xue W, Mizukami I, Todd III RF, Petty HR. Urokinase-type plasminogen activator receptors associate with beta-1 and beta-3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. Cancer Res 1997;57:1682–9
6. Wei Y, Waltz DA, Rao N, Drummond RJ, Rosenberg S, Chapman HA. Identification of the urokinase receptor as an adhesion receptor for vitronectin. J Biol Chem 1994;269:32380–8
7. Hoyer-Hansen G, Ronne E, Solberg H, Behrendt N, Ploug M, Lund RL, Ellis V, Dano K. Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. J Biol Chem 1992;267:18224–9
8. Fazioli F, Resnati M, Sidenius N, Higashimoto Y, Appella E, Blasi F. A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. EMBO J 1997;16:7279–86
9. Ossowskj L, Aguirre-Ghiso JA. Urokinase-receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. Curr Opin Cell Biol 2000;12:613–20
10. Blasi F. The urokinase receptor. A cell surface, regulated chemokine. APMIS 1999;107:96–101
11. Casey JR, Petranka JG, Kottra J, Fleenor DE, Rosse WF. The structure of the urokinase-type plasminogen activator receptor gene. Blood 1994;84:1151–6
12. Roldan AL, Cubellis MV, Masucci MT, Behrendt N, Lund LR, Dano K, Appella E, Blasi F. Cloning and expression of the receptor for human urokinase plasminogen activator, a central molecule in cell surface, plasmin dependent proteolysis. EMBO J 1990;9:467–74
13. Irigoyen JP, Munoz-Canoves P, Montero L, Koziczak M, Nagamine Y. The plasminogen activator system: biology and regulation. Cell Mol Life Sci 1999;56:104–32
14. Lund LR, Ellis V, Ronne E, Pyke C, Dano K. Transcriptional and post-transcriptional regulation of receptor for urokinase-type plasminogen activator by cytokines and tumor promoters in the human lung carcinoma cell line A549. Biochem J 1995;310:345–52
15. Gillis P, Malter JS. The adenosine-uridine binding factor recognizes the AU-rich elements of cytokine, limphokine, and oncogene mRNAs. J Biol Chem 1991;266:3172–7
16. Rajagopalan LE, Malter JS. Modulation of granulocyte-macrophage colony-stimulating factor mRNA stability *in vitro* by the adenosine-uridine binding factor. J Biol Chem 1994;269:23882–8
17. Myer VE, Fan XC, Steitz JA. Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. EMBO J 1997;16: 2130–9
18. Wang GJ, Collinge M, Blasi F, Pardi R, Bender JR. Posttranscriptional regulation of urokinase plasminogen activator receptor messenger RNA levels by leukocyte integrin engagement. Proc Natl Acad Sci U S A 1998;95:6296–301
19. Nanbu R, Menoud P-A, Nagamine Y. Multiple instability-regulating sites in the 3′ untranslated region of the urokinase-type plasminogen activator mRNA. Mol Cell Biol 1994;14:4920–8
20. Tillmann-Bogush M, Heaton JH, Gelehrter TD. Cyclic nucleotide regulation of PAI-1 mRNA stability. Identification of cytosolic proteins that interact with an a-rich sequence. J Biol Chem 1999;274: 1172–9
21. Maurer F, Tierney M, Medcalf RL. An AU-rich sequence in the 3′-UTR of plasminogen activator inhibitor type-2 (PAI-2) mRNA promotes PAI-2 mRNA decay and provides a binding site for nuclear Hur. Nucleic Acid Res 1999;27:1664–73
22. Yen TJ, Machlin PS, Cleveland DW. Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin. Nature 1988;334:580–5
23. Wellington CL, Greenberg ME, Belasco JG. The destabilizing elements in the coding region of c-fos mRNA are recognized as RNA. Mol Cell Biol 1993;13:5034–42
24. Yeilding NM, Lee WMF. Coding elements in exons 2 and 3 target c-myc mRNA downregulation during myogenic differentiation. Mol Cell Biol 1997;17:2698–707
25. Tierney MJ, Medcalf RL. Plasminogen activator inhibitor type 2 contains mRNA instability elements within exon 4 of the coding region. Sequence homology to coding region instability determinants in other mRNAs. J Biol Chem 2001;276:13675–84
26. Shetty S, Kumar A, Idell S. Posttranscriptional regulation of urokinase receptor mRNA: identification of a novel urokinase receptor mRNA binding protein in human mesothelioma cells. Mol Cell Biol 1997; 17:1075–83
27. Montuori N, Salzano S, Rossi G, Ragno P. Urokinase-type plasminogen activator up-regulates the expression of its cellular receptor. FEBS Lett 2000;476:166–70
28. Montuori N, Mattiello A, Mancini A, Santoli M, Taglialatela P, Caputi M, Rossi G, Ragno P. Urokinase-type plasminogen activator up-regulates the expression of its cellular receptor through a post-transcriptional mechanism. FEBS Lett 2001;508:379–84
29. Pedersen H, Brunner N, Francis D, Osterlind K, Ronne E, Hansen HH, Dano K, Grondahl-Hansen J. Prognostic impact of urokinase receptor, and plasminogen activator inhibitor in squamous and large cell lung cancer tissue. Cancer Res 1994;54:4671–5
30. Ragno P, Montuori N, Covelli B, Hoyer-Hansen G, Rossi G. Differential expression of a truncated form of the urokinase-type plasminogen-activator receptor in normal and tumor thyroid cells. Cancer Res 1998;58:1315–9
31. Vassalli JD, Dayer JM, Wohlwend A, Belin D. Concomitant secretion of pro-urokinase and of a plasminogen activator-specific inhibitor by cultured human monocytes-macrophages. J Exp Med 1984;159: 1653–68
32. Andreasen PA, Egelund R, Petersen HH. The plasminogen activation system in tumor growth, invasion, and metastasis. Cell Mol Life Sci 2000;57:25–40
33. Zannetti A, Del Vecchio S, Carriero MV, Fonti R, Franco P, Botti G, D'Aiuto G, Stoppelli MP, Salvatore M. Coordinate up-regulation of Sp1 DNA-binding activity and urokinase receptor expression in breast carcinoma. Cancer Res 2000;60:1546–51