Volume 326, number 1,2,3, 69–74 FEBS 12660 July 1993

An alternatively spliced variant of mRNA for the human receptor for urokinase plasminogen activator

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Received 28 April 1993

Using 3′ RACE (rapid amplification of cDNA ends), we have isolated a cDNA variant for the receptor for human urokinase plasminogen activator (uPAR). The deduced protein includes the amino-terminal ligand binding domain in uPAR, but lacks the carboxy-terminal membrane attachment by a glycolipid anchor. Genomic DNA analysis showed that the uPAR mRNA variant is generated by alternative splicing. The new variant mRNA is expressed in various human cell lines and tissues and both variants are up-regulated by phorbol ester in A549 cells. We propose that the alternatively spliced uPAR mRNA encodes a soluble uPA binding protein, the possible function of which is discussed.

Plasminogen activation; uPA receptor; Alternative splicing; PCR; Soluble; 3′ RACE

1. INTRODUCTION

The urokinase pathway of plasminogen activation is involved in tissue remodelling under normal and pathological conditions, including cancer invasion [1]. A specific cell surface receptor for uPA (uPAR) has been identified on human monocytes and on a variety of cultured cell lines of neoplastic origin [2–4]. Receptor binding of uPA strongly enhances plasminogen activation and uPAR plays a crucial role in cell surface proteolysis [5]. The receptor was recently characterized in details: its 1,4 kb mRNA [6] is encoded by a gene located on the long arm of chromosome 19 [7] and is translated into a protein consisting of 313 amino acid residues and a signal peptide. Based on alignment of cysteines, the receptor has been shown to consist of three homologous repeats of which the amino-terminal constitutes the ligand-binding domain [8]. uPAR is a highly glycosylated protein, which is linked carboxy-terminally to the cell membrane by a glycosylphosphatidyl inositol (GPI) lipid anchor [9]. In the mouse, evidence has been provided for the existence of a uPA receptor homologous to human uPAR (muPAR1) [10,11], as well as a mouse uPAR mRNA variant (muPAR2) produced by alternative splicing in the middle of the second repeat and encoding a molecule identical to muPAR1 in the uPA binding domain but lacking the GPI anchoring, and probably representing a soluble form of muPAR1 [10]. We now report the identification of a variant form of human uPAR mRNA (uPAR2) likely to represent a human functional analog of mouse uPAR2, but arising by alternative splicing in the middle of the third domain.

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Abbreviations: uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; muPAR, mouse uPAR; RACE, rapid amplification of cDNA ends; GPI, glycosylphosphatidyl inositol; PCR, polymerase chain reaction.

2. MATERIALS AND METHODS

2.1. *Materials*

pBluescriptKS(+) plasmid vector, competent *E. coli* strain JM109 and a human genomic cosmid library were from Stratagene, La Jolla, CA. All other materials were those previously described [10]. RNA from human tissues and cultured cells was prepared as described [10,12].

2.2. *Primers*

The following synthetic oligonucleotide primers were used for RACE amplification and for generating PCR products (prefix 'c' designates a complementary primer). (i) Primers common to both uPAR1 and uPAR2 (numbers in brackets refer to Roldan et al. [6]): 90-8 (386–405), c90-9 (576–597), 91-02 (625–645), 91-07 (113–130), 92-13 (1–22). (ii) uPAR1-specific primers (numbers in brackets refer to Roldan et al. [6]): c91-03 (933–955), 91-04 (901–924), c91-05 (1255–1276), 92-305 (1287–1309). (iii) uPAR2-specific primers (numbers in brackets refer to Fig. 2): c91-101 (1025–1045), c92-306 (921–933), 92-311 (1042–1062), c92-304 (816–842). (iv) RACE downstream primers: dT_{17}-adapter: 5′GACTCGAGTCGACATCGA-dT_{17}; adapter: 5′GACTCGAGTCGACATCGA.

2.3. *RACE amplification of 3′ ends*

For RACE amplification of total RNA extracts [13] the dT_{17}-adapter primer was used for reverse transcription and this adapter primer, together with the uPAR cDNA-specific primer, 91-02, was used for PCR-amplification. The PCR reaction mixture was then analysed on a 1% agarose gel containing ethidium bromide.

2.4. *Sequencing RACE products*

DNA from the RACE amplification contained in agarose gel pieces was purified (Geneclean II, Bio 101 Inc., La Jolla, CA), cut with restriction enzymes *Apa*I and *Cla*I and subcloned into pBluescriptKS(+) plasmid vector using standard techniques [14]. 10 μg DNA from the clones pIa and pIIb was used for sequencing by the dideoxy method [15]. Sequence homology searching was performed on the EMBL and Swissprot databases (updated 14 April 1993) using the FastA/TFastA method [16].

2.5. *PCR for uPAR2 mRNA*

DNase I-treated purified RNA from human cells and tissues was used for reverse transcription performed as the RACE first strand synthesis, except that the RACE primer was substituted by primer c91-101. In the following PCR amplification step, 1/20 of the reaction mixture was used with primers c91-101 and 91-07.

2.6. *In vitro translation*

For generation of synthetic RNA corresponding to uPAR1 and uPAR2 mRNA, the respective cDNA coding sequences were cloned into pBluescriptKS(+). uPAR1 cDNA for this purpose was prepared by PCR amplification of full-length uPAR cDNA (clone p-uPAR-1 [6]) using primers 92-13 and 91-05. uPAR2 cDNA was prepared as follows. First, the 5′ end was prepared by cutting uPAR cDNA with NcoI (cutting uPAR cDNA at position 718) and isolating the 718 bp DNA fragment. Next, the 3′ end was made by PCR amplification of pIIb using primers 91-02 and 91-101, cutting the resulting product with *Nco*I and isolating the fragment of 321 bp. Finally the two ends were ligated with T4 DNA ligase. Both uPAR1 and uPAR2 cDNA was then blunt-end cloned into pBluescriptKS(+)[14]. Purified plasmid DNA of both constructs was verified by dideoxy sequencing using T3 and T7 primers. For in vitro translation, unlabelled sense RNA was prepared and used for translation in rabbit reticulocyte lysate as described [10]. 1/20 of each reaction mixture was analysed by SDS-PAGE and fluorography.

2.7. *Sequencing selected regions of the uPAR gene*

500,000 *E. coli* colonies of a human genomic cosmid library were screened on Genescreen nylon filters using a ^{32}P-labelled PCR fragment covering the entire coding region of uPAR. The hybridization and washing conditions were as recommended by the manufacturer. Two positive clones were obtained: CosHUR07 and CosHUR10.

DNA from cosmid clone CosHUR07 was used for PCR amplification with primers 92-305 and c92-306. The resulting approximately 750 bp product was blunt-end cloned into pBluescriptKS(+) and sequencing was performed as above. Inverse PCR of flanking regions [17] was performed on DNA from CosHUR07. In brief, 10 μg of purified DNA was cut with *Pst*I, self-ligated with T4 DNA ligase and amplified with the uPAR2-specific primers c92-304 and 92-311. A resulting ≈900 bp PCR fragment was blunt-end cloned into pBluescriptKS(+) plasmid and sequenced as above.

Genomic human DNA from human umbilical cord endothelial cell line, HUVEC, was used for sequencing the region amplified with primers 90–8 and c90–9, corresponding to the region in the mouse genome where the muPAR2-specific coding sequence is located [10].

2.8. *RNase protection assay*

Plasmid pIIb (see section 2.4.) was linearized with *Apa*I and a radiolabelled RNA antisense probe was prepared by transcription using [^{32}P]UTP and T7 polymerase as described [18], except that unlabeled UTP was added in a 10-times molar excess over [^{32}P]UTP. Two non-radiactive control RNAs to serve as size markers were prepared by transcription in the sense direction of pIIb and pHUR06 (a 584 bp *Bam*HI–*Bam*HI uPAR1 cDNA fragment [18]). RNase protection assay, using 2.5 μg ethanol precipitated and DNase I-treated poly(A) RNA from A549 cells grown with or without PMA was performed as described [14]. Protected mRNA regions were analysed on a denaturing polyacrylamide gel and autoradiography.

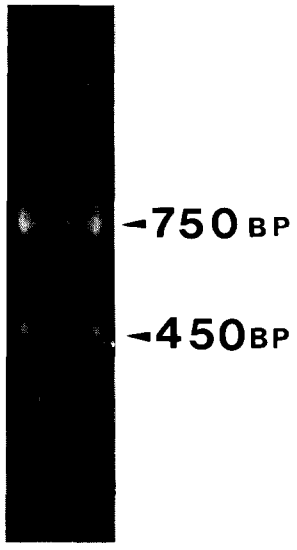

Fig. 1. 3′ end RACE amplification of RNA from HT1080 cells with a uPAR specific primer. Total RNA from HT1080 cells was amplified with the RACE technique using an upstream primer corresponding to nucleotide 625–645 [6] in uPAR cDNA. The amplification products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. Two bands of approximately 450 and 750 bp are seen.

3. RESULTS

3.1. *Identification of a variant of uPAR mRNA by RACE amplification*

RACE amplification of cDNA corresponding to the 3′ end of uPAR mRNA was performed with a primer corresponding to nucleotides 625–645 [6] on total RNA purified from HT1080 cells. Analysis of the RACE products on a ethidium bromide-stained agarose gel revealed two bands of approximately 450 and 750 bp (Fig. 1). Both products were subcloned into a sequencing plasmid vector and the nucleotide sequence was determined on clones pIa and pIIb, containing the 750 bp and the 450 bp inserts, respectively. The 750 bp product was, as expected, identified as the 3′ end of uPAR cDNA [6]. The 450 bp product consisted of 153 bp showing complete sequence homology with uPAR cDNA (position 650–803) followed by a 268 bp sequence unrelated to uPAR cDNA and ending in a poly(A) sequence (Fig. 2). We concluded that this represented the 3′ end of a 1,071 bp-long nucleotide variant of uPAR cDNA. This new cDNA variant was termed uPAR2, and the previously cloned variant uPAR1. uPAR2 contains an open reading frame with a translation stop codon located in position 891-93 and is predicted to yield a protein identical to uPAR1 in amino acid residues 1–252 but differing from uPAR1 in the carboxy-terminal 29 residues (Fig. 2). No homologies of the uPAR2-specific sequence to any nucleotide or protein sequences were found by a search in the EMBL and Swissprot data bank. A hydrophobicity plot of the uPAR2-specific carboxy-terminal by the algoritm of

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uPAR1 cDNA          GGTAGCCACCGGCACTCACGAACCGAAAAACCAAAGCTATATGGTAAGAGGCTGTGCAAC   840
uPAR2 cDNA          ..................AACGCTCACTCTGGGGAAGCTGGTTGCCATGTAAAAGTAC    -
uPAR1 protein       ValAlaThrGlyThrHisGluProLysAsnGlnSerTyrMetValArgGlyCysAlaThr   243
uPAR2 protein        - - - - - - - - ArgSerLeuTrpGlySerTrpLeuProCysLysSerThr       -

uPAR1 cDNA          CGCCTCAATGTGCCAACATGCCCACCTGGGTGACGCCTTCAGCATGAACCACATTGATGT   900
uPAR2 cDNA          TACTGCCCTGAGACCACCATGCTGTGAGGAAGCCCAAGCTACTCATGTATAAATGCCATG    -
uPAR1 protein       AlaSerMetCysGlnHisAlaHisLeuGlyAspAlaPheSerMetAsnHisIleAspVal   263
uPAR2 protein       ThrAlaLeuArgProProCysCysGluGluAlaGlnAlaThrHisValEnd            259

uPAR1 cDNA          CTCCTGCTGTACTAAAAGTGGCTGTAACCACCCAGACCTGGATGTCCAGTACCGCAGTGG   960
uPAR2 cDNA          TGGAGATAGAGCCCCAGATGTTTCAGCCATCTCAGCCCAGGCACCAGACAAGTGGGTGAA    -
uPAR1 protein       SerCysCysThrLysSerGlyCysAsnHisProAspLeuAspValGlnTyrArgSerGly   283

uPAR1 cDNA          GGCTGCTCCTCAGCCTGGCCCTGCCCATCTCAGCCTCACCATCACCCTGCTAATGACTGC  1020
uPAR2 cDNA          GAAGCCACCTTGGACATGTAGCCCCACGAGATGTGATATAGAGAAGAAACAGGAAACTTG    -
uPAR1 protein       AlaAlaProGlnProGlyProAlaHisLeuSerLeuThrIleThrLeuLeuMetThrAla   303

uPAR1 cDNA          CAGACTGTGGGGAGGCACTCTCCTCTGGACCTAAACCTGAAATCCCCCTCTCTGCCCTGG  1080
uPAR2 cDNA          GCTATATTAGTTTCCTAGGGCTGCCTGTGATAAATTATTACGAACTTTATAAAAAAAAAA    -
uPAR1 protein       ArgLeuTrpGlyGlyThrLeuLeuTrpThrEnd                              313

uPAR1 cDNA          CTGGATCCGGGGGACCCCTTTGCCCTTCCCTCGGCTCCCAGCCCTACAGACTTGCTGTGT  1140[1]
uPAR2 cDNA          AAAAAAAAAAAAAAA                                               1097
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[1]The total number of nucleotides in uPAR1 cDNA is 1368.

Fig. 2. Sequence of the 3′ ends of the two uPAR cDNAs identified in Fig. 1 and the corresponding deduced amino acid sequences. The data are aligned with and numbered according to the previously reported uPAR cDNA and protein sequences [6,8]. (−) and (.) designates the parts of uPAR2 that are identical to uPAR1.

Kyte and Doolittle [19] yielded values close to the neutrality line, indicating that the putative uPAR2 protein is a soluble form of uPAR. No potential glycosylation sites are present in the 29 residue carboxy-terminal end of the uPAR2 protein.

3.2. *Gene organization*

To study whether the two uPAR variants were the result of alternative splicing, a genomic human uPAR DNA clone was isolated from a cosmid library, by screening with a cDNA probe covering the entire coding region of uPAR1 cDNA, PCR amplification with 2 primers specific for uPAR1 and uPAR2, respectively, and inverse PCR amplification of the regions flanking the uPAR2-specific sequence. Sequencing of a part of this genomic clone revealed the presence of a 72 bp sequence identical to the 3′ end of uPAR1 mRNA and a 268 bp sequence identical to the 3′ end of uPAR2 mRNA. These two sequences are separated by a 578 bp sequence which is not found in any of the two uPAR mRNA variants and which contains an *alu* sequence. Splice junction consensus sequences are present at a position ≈10 bp downstream from the polyadenylation site in uPAR1 mRNA, at the junction between the new sequence and the uPAR2-specific sequence, and at a position ≈10 bp downstream from the polyadenylation site in the uPAR2-specific sequence (Fig. 3). These findings indicate that the two uPAR mRNA variants arise by alternative splicing of two mutually exclusive 3′ end exons, each containing a polyadenylation site and a carboxy-terminal coding sequence (Fig. 3).

In the murine system the uPAR mRNA variants, 1 and 2, arise by alternative splicing at nucleotide 480 in domain 2 [10]. Human genomic uPAR DNA from the region corresponding to this position was also isolated by PCR techniques. Sequencing of this DNA revealed the presence of an approximately 900 bp-long intron after nucleotide 516. No open reading frame sequence analogous to that found for muPAR2 was detected, however, indicating that this region in the human uPAR gene is not involved in a similar RNA splicing event (results not shown).

3.3. *In vitro translation of uPAR mRNA variants*

Full-length sense RNA for uPAR1 and uPAR2 was prepared and translated in rabbit reticulocyte lysates. SDS-PAGE and fluorography showed generation of proteins with molecular weights of ≈ 35,000 and ≈ 31,000 as expected for uPAR1 and uPAR2, respectively (Fig. 4).

A.

92-305

genomic clone CTGTTGTGTTGTTGTTATTAATTAATATTCATATTATTTATTTTATCTTACATAAAGATTT 60
uPAR1 cDNA 1352
P ↓
genomic clone TTGTACCAGTGGACAAGGCCAGGTATGCCCTTCTTTGTAGTGTCTATCTTTGGGAGGATAA 120
uPAR1 cDNA .....AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1400

genomic clone ATGGTGGGGGACACCTTTTCACAGGCTCCTGGAATCAGGGGTCATGTTTGAGGCCCCTGCC 180

genomic clone TAGGTTGGAAGTCCTCCTGGGGGCTGCCTCCACATCTATTTCTGGACTCCAAATGCTGACA 240

genomic clone GACAGGAGCACTGCCATCTTGAACAAACACCACCATTCTTAGTTCCTCTTTGATTAAAATC 300
→

genomic clone GGCTAAATCCGGACGTGGTGGTGGGTGACTGTAACTCAGCTACCGGAGGGCTGAGGCAGGA 360

genomic clone GATCGCTTGAACCAGAGAGAGTGTAGTGAGCAAGATCACCACTGCTAGAACCGTGAGACAT 420

genomic clone TCCAAAAAAAAAAAAAAAAGACTCTCATGATTGGGCATGAGGACAGTCTGTTAGGCTTCTC 480
←

genomic clone TAATGACATATCACACTTGCACGTAGTGGCTTAAAGAATGGAGGTCTGTACTTCTGCCTCT 540

genomic clone TGATATGAGTGACTCTGTGACTGCTTTGACCAGTAGATGGTGGAAGTTTTGCTGTGCTATG 600
↓

genomic clone TTCTGGAACCAAGTTTTAAGAAACTTTCATTTTTCACTTTCTGAATCTTAGAACGCTCACT 660
uPAR2 cDNA 810

c92-304

genomic clone CTGGGGAAGCTGGTTGCCATGTAAAAGTACTACTGCCCTGAGACCACCATGCTGTGAGGAA 720
uPAR2 cDNA .....----------..... 870

92-311; c92-306

genomic clone GCCCAAGCTACTCAGATAAAGCCAAGTGGAGTATAGAGCCCCAGATGTTTCAGCCATCTCA 780
uPAR2 cDNA .....---------- 930

genomic clone GCCCAGGCACCAGACAAGTGGGTGAAGAAGCCACCTTGGACATGTAGCCCCACGAGATGTG 840
uPAR2 cDNA ....----------..... 990

genomic clone ATATAGAGAAGAAACAGGAAACTTGGCTATATTAGTTTCCTAGGGCTGCCTGTGATAAATT 900
uPAR2 cDNA 1050
P ↓

genomic clone ATTACGAACTTTATAACTAACACATTGTGTGTCCTATATCAAAACATCATGGAAGGACAGG 960
uPAR2 cDNA .....AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1097

genomic clone CACAGTGGCTCATCCTGTAGTCCTAGCACTTC 992

B

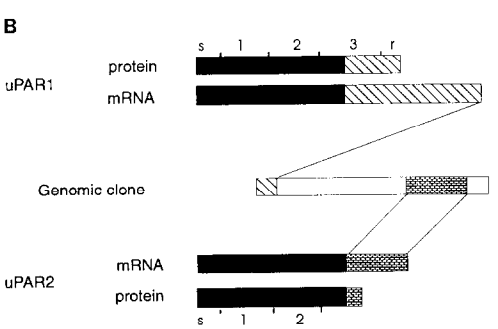

Fig. 3. (A) Sequence of part of the uPAR gene and the corresponding parts of uPAR1 and uPAR2 cDNA. Sequence 1–786 in the genomic DNA is obtained from a PCR fragment produced by conventional amplification of DNA from cosmid clone CosHUR07 using primers 92-305 (located in the far 3′ end of uPAR1) and c92-306 (located in the uPAR2-specific 3′ end). The remainder of the sequence is derived from a fragment obtained by inverse PCR on the same clone using the uPAR2-specific primers, c92-304 and 92-311. (.) indicates sequences in uPAR1 and uPAR2 cDNA identical to the genomic sequence, numbered according to Fig. 2. Primers used for generation of PCR-fragments are underlined. (↓) indicates splice junctions. (→) and (←) indicate the start and end of an *alu* sequence. P indicates polyadenylation sites. (B) Schematic representation of the relationship between the partial genomic uPAR sequence, the uPAR1 and uPAR2 mRNAs, and the deduced protein sequences. Hatched bars = uPAR1-specific sequences uPAR1; tiled bars = uPAR2-specific sequences; black bars = sequences common to uPAR1 and uPAR2; white bars = intron sequences spliced out in both uPAR1 and uPAR2. In the proteins, 's' indicates the signal peptide, '1–3' the three homologous repeats, and 'r' a peptide removed during post-translational attachment of a glycolipid anchor.

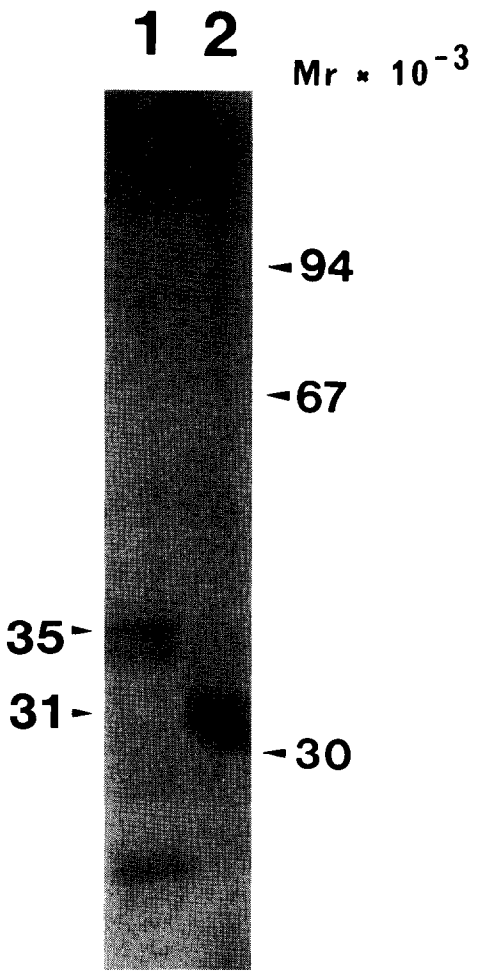

Fig. 4. In vitro translation products of uPAR1 and uPAR2 synthetic RNA using rabbit reticulocyte lysate and [^{35}S]cysteine. Analysis by SDS-PAGE and fluorography. Protein bands of the expected sizes are generated by uPAR1 (lane 1) and uPAR2 RNA (lane 2). The position of protein size markers are indicated on the right and the apparent molecular weight of the translation products on the left. The additional bands of lower molecular weight are probably caused by incomplete terminations. No microsomes were added to the translation reactions.

3.4. *Detection of uPAR mRNA variants by PCR and RNase protection*

By PCR with a primer common to uPAR1 and uPAR2 (91-07) and a primer specific for uPAR2 mRNA (c91-101), uPAR2 mRNA was detected in the following human cells and tissues: U937 monocyte-like cells, HepG2 hepatocarcinoma cells, A549 lung carcinoma cells, alveolar macrophages, leukocytes and colon tissue (results not shown).

Expression of the uPAR mRNA variants was also studied by RNase protection assay, using a radiolabeled antisense RNA probe containing a sequence common for uPAR1 and uPAR2, and a sequence specific for uPAR2. Analysis of poly(A)-purified RNA from A549 cells cultured in the presence or absence of the phorbol ester PMA, showed that uPAR1, as well as uPAR2 mRNA, was present in both preparations and that the PMA-treated cells clearly contained increased amounts of both mRNA variants in comparison with unstimulated cells (Fig. 5). RNase protection analysis of poly(A) RNA from HT1080 cells also confirmed the presence of both mRNA variants in these cells (results not shown).

4. DISCUSSION

The present study demonstrates the existence of a new variant of human uPAR mRNA, uPAR2. This variant and the previously identified uPAR1 mRNA variant arise by alternative splicing of two mutually exclusive 3′ end exons, each containing both a translation stop codon and a polyadenylation site.

The deduced amino acid sequence of uPAR2 mRNA comprises a protein which, in addition to a signal peptide, consists of 259 amino acid residues, the 230 residue amino-terminal sequence being identical to that of uPAR1, while the 29 residue carboxy-terminal sequence is unique for uPAR2. We have previously found that the 89 amino-terminal amino acid residues of uPAR1 constitutes the uPA binding domain [8] and that mature uPAR1 is linked to the cell surface by a GPI anchor attached at amino acid residue 282, 283 or 284 [9]. It is therefore likely that the putative uPAR2 protein has retained uPA binding activity and is a water-soluble secreted protein.

uPAR2 mRNA is expressed in several cell types and tissues, as demonstrated by PCR and RNase protection analysis. In PMA-stimulated A549 lung carcinoma cells, uPAR2 mRNA of the expected size has also been detected by Northern blotting analysis (unpublished results). Recombinant uPAR2 protein was produced by in vitro translation and showed the predicted molecular weight, thus confirming the presence of a translation stop codon. The expression of native uPAR2 protein remains to be investigated.

In the mouse, two uPAR mRNA variants arise by alternative splicing of an intron with two different donor sites giving rise to a muPAR1 mRNA homologous to the human uPAR1 mRNA, and a muPAR2 mRNA that encodes a 199 amino acid residue protein [10]. The first 133 residues are identical to muPAR1 and include the uPA binding domain, while the last 66 residues are unique for muPAR2. There are no homologies between the sequences unique for human and mouse uPAR2, but both lack potential GPI anchoring sites and both are hydrophilic. In spite of the different sites of and mechanisms of alternative splicing, the human and mouse uPAR2 proteins probably have two important properties in common: an intact uPA binding domain and hydrophilicity. It is noteworthy that although different alternative splicing mechanisms are involved in the generation of the human and mouse uPAR2 mRNA variants, the predicted donor splicing site involved in the human system (nucleotide 800, see Fig. 2)

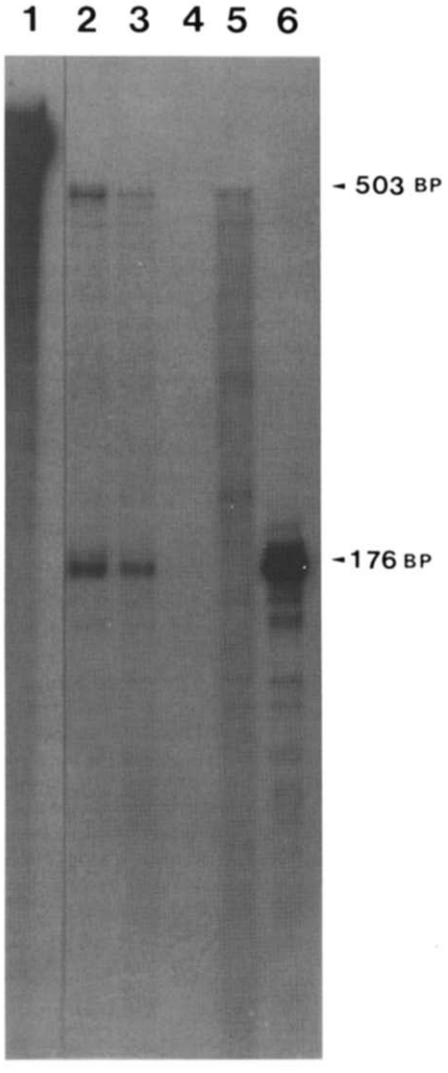

Fig. 5. RNase protection assay of poly(A) RNA from cultured human A549 lung carcinoma cells. An ^{32}P-labelled uPAR2 antisense RNA probe (lane 1) was hybridized with poly(A)-enriched RNA from A549 cells grown with (lane 2) or without (lane 3) PMA; 10 μg tRNA (lane 4); uPAR2 sense RNA (503 bp of protected region of pIIb cDNA) (lane 5); and uPAR1 sense RNA (176 bp of protected region of a uPAR1 cDNA fragment) (lane 6).

is located in the third repeat of uPAR1 at a position which exactly corresponds to the alternative splicing site (nucleotide 480) in the homologous second repeat in the murine receptor sequence [8,10].

The possible function of the uPAR2 molecules remain to be investigated. Binding of uPA and the zymogen, pro-uPA, to uPAR1 plays a crucial role in cell surface plasminogen activation [5]. Recent studies have shown that monoclonal uPAR1 antibodies that inhibit uPA-binding also inhibit plasmin generation [20]. The putative uPA-binding, water-soluble and secreted uPAR2 molecules may therefore serve a physiological function as inhibitors of cell surface proteolysis by inhibition of the binding of uPA to uPAR1. Alternatively uPAR2 may have a function in inducing plasminogen activation at sites other than cell surfaces. Recent studies suggest that uPAR1 enhances plasminogen activation by participating in a formation of complexes between pro-uPA and plasminogen at cell surfaces [21]. It is possible that the uPAR2 molecules serve a similar function at certain extracellular sites and that the uPAR2-specific sequences serve to locate the uPAR2 molecules at these sites.

Acknowledgements: The excellent technical assistance of Britt Nagel, Jette Mandelbaum and Marianne Vallø Nielsen is gratefully acknowledged. This work was supported financially by the Danish Biotechnology programme, the Danish Cancer Society and the Danish Medical Research Council.

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