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Molecular Cloning, Expression, and Chromosomal Localization of a Human Tubulointerstitial Nephritis Antigen

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Tubulointerstitial nephritis antigen (TIN-ag) is an extracellular matrix basement protein which was originally identified as a target antigen involved in anti-tubular basement membrane (TBM) antibody-mediated interstitial nephritis (TIN). Further investigations elucidated that TIN-ag plays a role in renal tubulogenesis and that TIN-ag is defected in hereditary tubulointerstitial disorder such as juvenile nephronophthisis. We previously isolated and characterized 54 kDa glycoprotein as TIN-ag. cDNA encoding rabbit and mouse TIN-ag has recently been identified. In the present study, the cDNA of the human homologue of TIN-ag was cloned and its nucleotide sequence was determined (Accession No. AB022277; the DDBJ nucleotide sequence database). Deduced amino acid sequence (476 aa) exhibited the presence of a signal peptide (1–18 aa), cysteine residues termed follistatin module, six potential glycosylation sites, and an ATP/GTP-binding site. Homology search revealed ~85% homology with both rabbit and mouse TIN-ag, and also some (~40%) similarity with *C. elegans*. Human TIN-ag contained a sequence similar to several classes of extracellular matrix molecules in amino terminal region and to cathepsin family of cysteine proteinases in the carboxyl terminal region. Northern blot analysis revealed exclusive expression of this molecule in human adult and fetal kidney tissues. Using a monoclonal antibody recognizing human TIN-ag, protein expression (~50 kDa) was identified in cultured COS-1 cells transfected with human TIN-ag cDNA. The human TIN-ag was mapped to chromosome 6p11.2-12 by fluorescence *in situ* hybridization. These results may provide further evidence for understanding TIN-ag molecule and clues for gene analysis of juvenile nephronophthisis. © 2000 Academic Press

The cDNA for human tubulointerstitial nephritis antigen reported in this paper have been deposited in the DNA Data Bank of Japan with Accession No. AB022277.

Autoimmune tubulointerstitial nephritis (TIN) is characterized by linear deposition of IgG with C3 along the renal tubular basement membrane (TBM) and tubulointerstitial inflammation (1). Animal models for TIN, based on immunization with heterologous TBM components, were developed in several species, including guinea pigs, mice, rats, and rabbits (2–4). The association between TIN and circulating anti-TBM antibodies has been described in human patients with drug-induced TIN, after kidney transplantation, and idiopathic form (1, 5). Several investigators have characterized the target antigens to which human anti-TBM antibody may be directed (6–9). Purification protocols using enzymatic digestion of human, rabbit or bovine TBM led to the identification of new glycoproteins, named TIN antigen (TIN-ag) (6, 10), 3M-1 (7, 8) or gp54 (9, 11, 12), which were similar in molecular mass (48–58 kDa) and showed an exclusive expression in TBM. cDNA encoding for rabbit TIN-ag has been cloned and sequenced by Nelson and co-workers (13). Recently, cDNA encoding mouse TIN-ag was also cloned, showing 80% identity with rabbit TIN-ag (14, 15).

Previous immunohistochemical studies suggested that patients with nephronophthisis, a hereditary progressive tubulointerstitial disorder, had a defect in TIN ag (16), which is analogous to type IV collagen in patients with Alport's syndrome (17).

In the present study, we isolated human TIN-ag cDNA. The chromosomal localization of human TIN-ag was also determined by fluorescence *in situ* hybridization (FISH). Knowledge of the primary structure of human TIN-ag may facilitate our understanding of the molecular structure of this novel basement membrane component, and may provide clues toward understanding its association with TBM abnormalities found in nephronophthisis.

MATERIALS AND METHODS

cDNA library screening. Primers, used in this study were: 5′-CTTAAGGGGTTGCTTAAATGCA-3′ (TIN-R1, antisense), 5′-TCT-

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CCCCATGACTTTCCCCA-3′ (TIN-R2, antisense), 5′-TCCATTTT-CCAAGGNCA(AG)-3′ (TIN-N1, sense), 5′-GTACCTGAACTTCAGA-GAATG-3′ (sense, TIN-N2), and 5′-TA(TC)CTTGCNAA(AG)GA(AG)-ATCTGGACA-3′ (sense, TIN-N3), derived from rabbit TIN antigen cDNA (GenBank Accession No. U24270); and 5′-GCCTGTACG-GAAGTGTTACT-3′ (sense, TIN-F1), 5′-CCTCAGTGGATGTTGCCT-TTAC-3′ (sense, TIN-F2), and 5′-AGAGGAGCACAAGGGCAGAA-3′ (sense, TIN-F3), derived from pT7 Blue vector (Novagen).

cDNA library, prepared from poly(A)$^+$ RNA from human kidney (Takara, Kusatsu, Japan), was screened by using primers TIN-R1 and F2. PCR reaction was performed as follows; denaturation at 94°C for 3 minutes, annealing at 55°C for 1 minute and extension at 72°C for 2 minutes; followed by 30 cycles. The first PCR product (1 μl) was used as a template for the second nested-PCR with primers TIN-R2 and F1. The second PCR products were gel purified and ligated into the plasmid vector (pT7 Blue vector). Nucleotide sequences were determined using a Dydeoxy Terminator Cycle Sequencing Kit (Perkin Elmer Japan Co., Urayasu, Chiba, Japan) and an Applied Biosystems Model 377 DNA Sequencer (Applied Biosystems, Foster City, CA). Based on the sequence obtained above, primers, 5′-ATGCCGTTTTCCTCGCCCATC-3′ (TIN-R3) and 5′-TCGCC-CATCAGACCTGCTTGC-3′ (TIN-R4) were constructed. The nested PCR extending NH3 and COOH termini was performed according to the conditions described above. The first PCR was performed using the human kidney cDNA library as template and the following primers; TIN-N2 and TIN-R5 for extending NH3 terminus and TIN-F2 and vector primer R1 for extending COOH-terminus. The primers for the second PCR were: TIN-N3 and TIN-R6 for NH3-terminus, and TIN-F3 and vector primer T3 for COOH terminus. Based on the sequence of the PCR products, primers, 5′-ATGTGGACCGGA-TATAAGATC-3′ (TIN-NN1), 5′-GCCATTTTCCAAGGGCAATAC-3′ (TIN-NN2), 5′-CCAGAGGGGAAAATAAGAAAATA-3′ (TIN-CC1), and 5′-TTATGGTTCATCAGAACTCGT-3′ (TIN-CC2), were constructed. The nested-PCR was performed using primers TIN-NN1 and TIN-CC1 for the first PCR and TIN-NN2 and TIN-CC2 for the second PCR to amplify full-length human TIN antigen cDNA. The PCR products were gel purified and ligated into the cloning vector pCR2. 1. The full-length clones were sequenced in both directions. The BLASTN database search program (National Center for Biotechnology Information) was used to identify homologies of the obtained sequences to those published.

cDNA and vectors. The 1.2 kb PCR fragment (nucleotide 161–1444) was initially ligated into pPCR II vector (Invitrogen, San Diego, CA) by using TA cloning kit (Invitrogen), and the BstXI digested fragment was ligated into an eukaryotic expression vector (pRc/CMV plasmid) (Invitrogen).

Transfection. Cultured COS-1 cells (American Type Culture Collection, Rockville, MD) were stably transfected by lipofectin (Life Technologies, Gaithersburg, MD) with hTIN-ag/pRc/CMV, and were selected by continuous growth in G418 (Geneticin, 400 μg/ml, Life Technology, U.S.A.). After 5 passages, 50 individual clones were isolated and screened for TIN expression by dot Northern blot analysis using ^{32}P-labeled EcoRI hTIN-ag cDNA fragment. Five clones expressing high levels of message were identified. Two of these, COS-hTIN1 and COS-hTIN2, were routinely utilized in the present study. As a control, wild COS-1 cells were also transfected with pRc/CMV plasmid alone and grown and maintained in G418.

Cell culture. Nontransfected, wild COS-1 (wt), empty vector transfected (COS^{vector}) and hTIN-ag transfected cells (COS^{TIN}) were routinely grown in RPMI 1640 (Sigma, St. Louis, MO), containing 10% fetal calf serum (FCS; Hyclone, Logan, UT) supplemented with 100 μg/ml ampicillin and 100 μg/ml streptomycin (Sigma). Transfected cos-1 cells were continuously grown in Geneticin (G418 sulfate, 400 μg/ml, Life Technology, NY).

RNA isolation and Northern blot analysis. Total RNA from each cells were isolated by using Isogen (Nippon Gene, Tokyo, Japan) and subjected to Northern blot analysis according to previously described

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TGAACTTCAGAGAATGTGGACCGGATATAAGATCTTAATCTTCTCTTATCTTACTACAGA   60
          M  W  T  G  Y  K  I  L  I  F  S  Y  L  T  T  E
AATCTGGATGGAGAAGCAGTATTTATCTCAAAGAGAAGTGGACCTAGAGGCTTATTTCAC  120
I  W  M  E  K  Q  Y  L  S  Q  R  E  V  D  L  E  A  Y  F  T
TAGGAATCACACCGTTTTGCAAGGTACTCGATTCAAAAGAGCCATTTTCCAAGGGCAATA  180
R  N  H  T  V  L  Q  G  T  R  F  K  R  A  I  F  Q  G  Q  Y
CTGTAGAAATTTTGGCTGTTGTGAAGACAGAGATGATGGCTGTGTCACTGAGTTCTATGC  240
C  R  N  F  G  C  C  E  D  R  D  D  G  C  V  T  E  F  Y  A
GGCGAATGCGTTGTGCTACTGTGATAAATTCTGTGACAGAGAAAATTCTGATTGCTGTCC  300
A  N  A  L  C  Y  C  D  K  F  C  D  R  E  N  S  D  C  C  P
TGACTACAAGTCCTTTTGCCGTGAAGAGAAAGAATGGCCTCCTCACACACAGCCTTGGTA  360
D  Y  K  S  F  C  R  E  E  K  E  W  P  P  H  T  Q  P  W  Y
TCCAGAAGGTTGCTTCAAAGATGGTCAACATTATGAAGAGGGATCAGTAATTAAAGAAAA  420
P  E  G  C  F  K  D  G  Q  H  Y  E  E  G  S  V  I  K  E  N
CTGCAACTCCTGCACATGCTCAGGACAGCAATGGAAATGTTCCCAGCATGTATGCCTTGT  480
C  N  S  C  T  C  S  G  Q  Q  W  K  C  S  Q  H  V  C  L  V
TCGTCCAGAATTAATTGAACAGGTCAATAAAGGAGACTATGGATGGACAGCACAGAATTA  540
R  P  E  L  I  E  Q  V  N  K  G  D  Y  G  W  T  A  Q  N  Y
CAGCCAATTTTGGGGAATGACTTTAGAAGATGGTTTTAAATTTCGCCTTGGCACTTTGCC  600
S  Q  F  W  G  M  T  L  E  D  G  F  K  F  R  L  G  T  L  P
ACCTAGTCTCATGCTCCTGAGCATGAATGAAATGACAGCTTCTTTACCTGCAACAACTGA  660
P  S  L  M  L  L  S  M  N  E  M  T  A  S  L  P  A  T  T  D
TCTTCCAGAGTTTTTGTTGCTTCTTATAAATGGCCTGGATGGACTCATGGCCCATTGGA  720
L  P  E  F  F  V  A  S  Y  K  W  P  G  W  T  H  G  P  L  D
TCAAAAAAATTGTGCTGCATCCTGGGCATTTTCCACTGCAAGTGTGGCTGCTGACCGAAT  780
Q  K  N  C  A  A  S  W  A  F  S  T  A  S  V  A  A  D  R  I
AGCAATTCAGTCTAAGGGTCGATACACGGCCAATCTATCCCCTCAGAATTTGATCTCTTG  840
A  I  Q  S  K  G  R  Y  T  A  N  L  S  P  Q  N  L  I  S  C
CTGTGCCAAGAACCGTCATGGATGCAATAGTGGAAGCATCGATAGGGCTTGGTGGTACCT  900
C  A  K  N  R  H  G  C  N  S  G  S  I  D  R  A  W  W  Y  L
GAGAAAACGTGGACTGGTATCCCACGCATGCTACCCACTTTTCAAAGACCAAAATGCTAC  960
R  K  R  G  L  V  S  H  A  C  Y  P  L  F  K  D  Q  N  A  T
CAACAATGGATGTGCCATGGCAAGCAGGTCTGATGGGCGAGGAAAACGGCATGCCACGAA 1020
N  N  G  C  A  M  A  S  R  S  D  G  R  G  K  R  H  A  T  K
GCCATGTCCCAACAACGTAGAAAAATCTAACAGGATCTATCAATGTTCTCCTCCATACAG 1080
P  C  P  N  N  V  E  K  S  N  R  I  Y  Q  C  S  P  P  Y  R
AGTCTCTTCCAACGAAACTGAGATAATGAAAGAAATCATGCAAAATGGACCAGTTCAAGC 1140
V  S  S  N  E  T  E  I  M  K  E  I  M  Q  N  G  P  V  Q  A
CATAATGCAAGTCCATGAAGATTTCTTCCATTATAAGACAGGGATATACAGACATGTTAC 1200
I  M  Q  V  H  E  D  F  F  H  Y  K  T  G  I  Y  R  H  V  T
CAGCACAAATAAAGAATCAGAAAAAATATCGAAAGCTTCAGACACATGCAGTCAAACTCAC 1260
S  T  N  K  E  S  E  K  Y  R  K  L  Q  T  H  A  V  K  L  T
TGGATGGGGCACACTGAGAGGAGCACAAGGGCAGAAAGAAAAATTTTGGATTGCTGCCAA 1320
G  W  G  T  L  R  G  A  Q  G  Q  K  E  K  F  W  I  A  A  N
TTCCTGGGGAAAGTCATGGGGAGAGAATGGCTATTTCAGGATTCTTCGAGGAGTAAATGA 1380
S  W  G  K  S  W  G  E  N  G  Y  F  R  I  L  R  G  V  N  E
GTCCGACATTGAAAAGTTGATTATCGCAGCTTGGGGCCAACTGACGAGTTCTGATGAACC 1440
S  D  I  E  K  L  I  I  A  A  W  G  Q  L  T  S  S  D  E  P
ATAACATATCATTAAATTTCCATAAGGTCATGCCTTTAAGTAACCCCCTAAATTGAAGTT 1500
TAGCAATATGACATTCTTGGTGACAGTGGAATCTTTGTCTCTTCACCGTGTTAACATAAT 1560
CTATCTATTTTCTTATTTTCCCCTCTGGA
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FIG. 1. Nucleotide and deduced amino acid sequence of human TIN-ag cDNA. The corresponding amino acids are listed below the nucleotide sequence.

methods. Aliquots of total RNA, each containing 15–20 μg, were subjected to electrophoresis, transferred to S & S Nytran nylon membranes (Schleicher & Schuell, Keene, NH), and fixed to the membrane by exposure to UV (CL-1000, Ultraviolet Crosslinker, Funakoshi Co., Tokyo, Japan). The membranes were prehybridized in a solution of 50% formamide, 5× SSC, 5× Denhardt's (Wako Pharmaceutical Co. Osaka, Japan), and 100 μg/ml sonicated salmon sperm DNA (Invitrogen) at 42°C. Following prehybridization, blots were hybridized with 0.5–1.0 cpm/ml of ^{32}P-labeled hTIN cDNA overnight at 42°C. After washing with 2× SSC for 15 minutes at room temperature twice, and at 65°C with 1× SSC/0.1% SDS for 15 minutes, and finally with 0.2% SSC/0.1% SDS for an additional 15 minutes, the membranes were exposed to Hyper ECL film (Amersham, Buckinghamshire, UK) at −70°C with an intensifying screen for 24–48 h. The blots were removed using strip solution (50 mM Tris (pH 8.0), 2 mM EDTA (pH 8.0), 0.5%/volume Na-pyrophosphate, 1× Denhardt solution) and reprobed with a human GAPDH cDNA (Clontech, Palo Alto, CA) under the conditions of labeling, hybridization, and washing as described above. The levels of mRNA were quantitated by densitometry and normalized to GAPDH expression.

Monoclonal antibody against human TIN-ag. A monoclonal antibody, H79 (12), showing immunoreactivity with human 54 kDa TIN-ag on Western blotting and immunolocalization to the proximal TBM, was used.

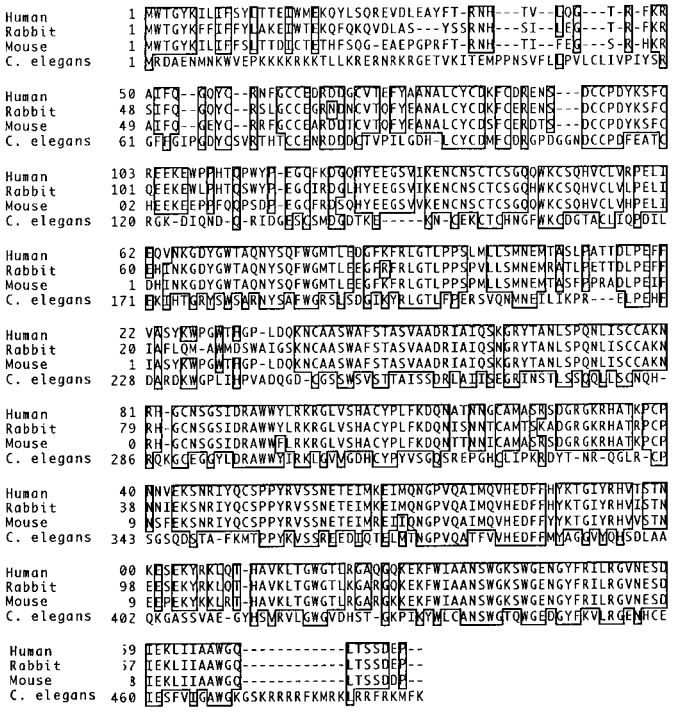

FIG. 2. Alignment of predicted amino acid sequence of human TIN-ag with rabbit and mouse TIN-ag, and *C. elegans.* GenBank Accession Nos. are AB022277, U24270, AF153366, Z81070-11 for human, rabbit, and mouse TIN-ag, and *C. elegans* F26E3 protein, respectively. Alignment was performed by Computer Software GENETYX-MAC Ver. 110 (Software Development Co., Tokyo, Japan), and visual inspection.

Immunoblotting. COS-1 cells were plated and cultured with RPMI 1640 containing 10% FCS in 10 cm plastic dishes and incubated for 48–72 h. After reaching confluence, cells were solublized in RIPA buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 50 mM NaF, 100 μM Na_3VO_4, 5 mM EDTA, 0.1% SDS, 0.5% sodium deoxycholate, and 1% aprotinin) after extensive washing with PBS. The protein concentration of the lysate was measured by the BCA protein assay kit (Pierce, Rockford, IL). Equal amounts of protein (300 μg/lane) were immunoprecipitated by anti-TIN monoclonal antibody (H79) coupled with PANSORBIN (Calbiochem, La Jolla, CA), separated on 10–20% gradient SDS-polyacrylamide gel electrophoresis, and transferred to Immobilon-P (Millipore Japan, Yonezaya) in transfer buffer (25 mM Tris-HCl (pH 8.5), 192 mM glycine, 20% methanol at 30 V for 16 h. The membranes were blocked by incubation with Tris-buffered saline (10 mM Tris-HCl (pH 7.4), 150 mM NaCl) containing 3% bovine serum albumin at room temperature and incubated with mouse anti-TIN antibody for 2 h at room temperature, followed by incubation with alkaline phosphatase-conjugated goat anti-mouse IgG for 2 h (EY Laboratories, San Mateo, CA). Immunoreactivity was visualized by a BCIP/NBT phosphatase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Immunofluorescence. Indirect immunofluorescent staining was carried out as previously described (11, 18). Each of the COS-1 cells were cultured in the 6-well plastic dishes coated with CELL-TAK (Collaborative Biomedical Products, Bedford, MA) until confluence, followed by incubation with primary antibody after washing 3 times with PBS, and then reacted with the goat anti-mouse IgG antibody labeled with fluorescein-isothianate (FITC) and examined under the immunofluorescent microscope. As controls, kidney sections or cultured COS-1 cells were incubated with nonimmune mouse sera or with unrelated mouse IgG monoclonal antibody, followed by incubation with FITC or secondary antibody alone. These controls were entirely negative.

Chromosomal localization. The human BAC genomic library (Genome Systems Inc., St. Louis, MO) was screened using human TIN-ag cDNA. A total of 4 positive clones were obtained. After sequencing using primers (TIN-cc2, TIN-R4, TIN-R4, and TIN-S2), a genomic clone (F916) was used for the fluorescent *in situ* hybridization (FISHJ) (Genome Systems, Inc.). DNA from clone F916 was labeled with digoxigenin dUTP by nick translation. Labeled probe was then combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from PHA stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate and 2× SSC. Specific hybridization signals were detected by incubating the hybridized slides in fluoresceinated anti-digoxigenin antibodies followed by counterstaining with DAPI. The initial experiment resulted in specific binding of the proximal short arm of a group C chromosome which was believed to be chromosome 6 on the basis of size, morphology, and banding pattern. A second experiment was conducted in which a genomic clone, which has been previously localized to 6q24 and confirmed by colocalization with chromosome 6 centromere specific probe, was cohybridized with clone F910. This experiment resulted in the specific labeling of the long and short arms of chromosome 6. A total of 80 metaphase cells were analyzed with 73 exhibiting specific labeling.

RESULTS

cDNA Cloning of Human TIN-ag

The nucleotide sequence for human TIN-ag (Accession No. AB022277; the DDBJ nucleotide sequence database) contained 1,589 bases with a 1,428 bases

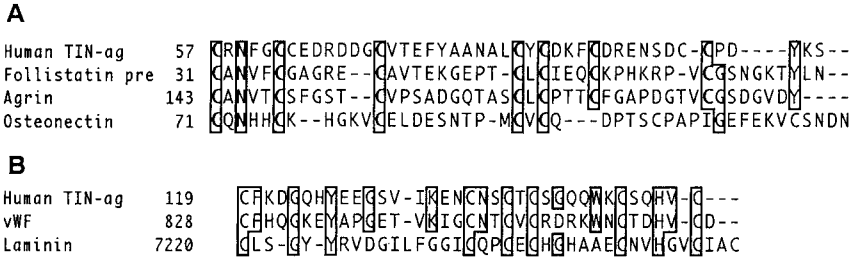

FIG. 3. Comparison of human TIN-ag protein with follistatin modules-containing proteins (A) and with extracellular matrix molecules (B). Sequences were taken from GenBank Accession Nos. Q12841 (follistatin precursor), P25304 (agrin), P07214 (osteonectin/SPARC), P04275 (von Willebrand factor), and P25391 (laminin α-1 chain). Abbreviation: vWF, von Willebrand factor.

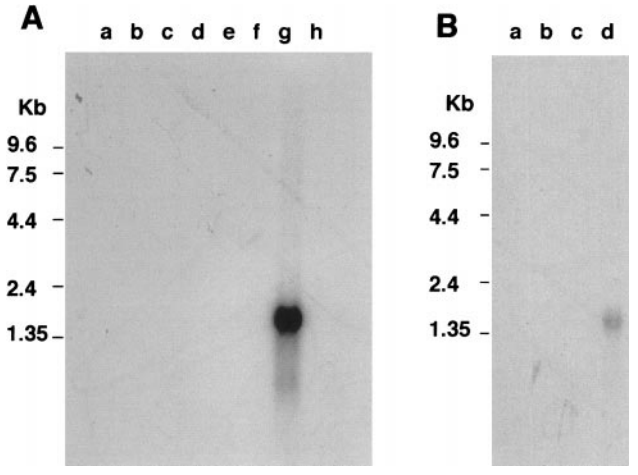

FIG. 4. Northern blot analysis. Human multiple tissue Northern blot (A) and human fetal multiple tissue Northern blot (B), containing 2 μg of poly(A)$^+$ RNA/lane, were probed with a ^{32}P-dCTP-labeled human TIN-ag cDNA. Transcript was exclusively present in kidney. The β-actin cDNA expression used as an internal control was similar in all the tissues (data not shown). (A) a, heart; b, brain; c, placenta; d, lung; e, liver; f, skeletal muscle; g, kidney; h, pancreas. (B) a, brain; b, lung; c, liver; d, kidney.

open reading frame encoding a protein of 476 amino acids (Fig. 1). The deduced amino acid sequence of human TIN-ag revealed ~85% homology with both rabbit and mouse TIN-ag, and also ~40% homology with C. elegans F26 (Fig. 2) (13, 15). Human TIN-ag protein contained six N-linked glycosylation sites (Asn-X-Ser/Thr) (19) and ATP/GTP binding site motif (AAN-SWGKS). Cysteine residues, clustered in the amino acids 57–95 regions, showed follistatin modules which is preserved in follistatin, agrin, osteonectin/SPARC, and SC1 (20) (Fig. 3A). There was no RGD sequence. Human TIN-ag contained sequence similar to von Willebrand factor precursor, laminin α-1 chain precursor, vitronectin precursor, and fibronectin receptor β subunit in the NH2 region (Fig. 3B) and to cathepsin B-like cysteine proteinase in the COOH terminal region. According to the rules of von Heijne (21), the putative signal peptide composed of the first 18 amino acids, as in the case of rabbit TIN-ag (13). Since the previously reported NH2-terminal region started on amino acid 50 (11), amino acid residues (amino acids 19–49) present between the signal peptide and the NH2-terminal region may represent a propeptide that is cleaved during the early stages following protein synthesis. The peptide cores of human TIN-ag at amino acids 50–476 were calculated to be 48.5 kDa with p*I* 7.5.

Northern Blot Analysis

A human multiple tissue Northern blot was probed with a ^{32}P-labeled PCR product (1.2 kb). Among tissues examined, the mRNA transcript expression was detected at a molecular size of 1.6 kb exclusively in both adult and fetus kidneys (Fig. 4).

Expression of Human TIN-ag

Using a monoclonal antibody (H79) recognizing human TIN-ag, cellular expression was detected in cultured COS-1 cells transfected with human TIN-ag cDNA (Fig. 5). Western blotting/SDS-PAGE of immunoprecipitates from culture media of the transformed COS-1 cells showed a major band of approximately 50 kDa (Fig. 6).

Chromosomal Localization

FISH using a Back clone (F910) labeled with digoxigenin dUTP resulted in the specific labeling of the short arms of chromosome 6. Observation of specifically labeled chromosome 6 demonstrated that F910 is located at a position which is immediately adjacent to the centromere, an area which corresponds to the band 6p11.2–12 (Fig. 7).

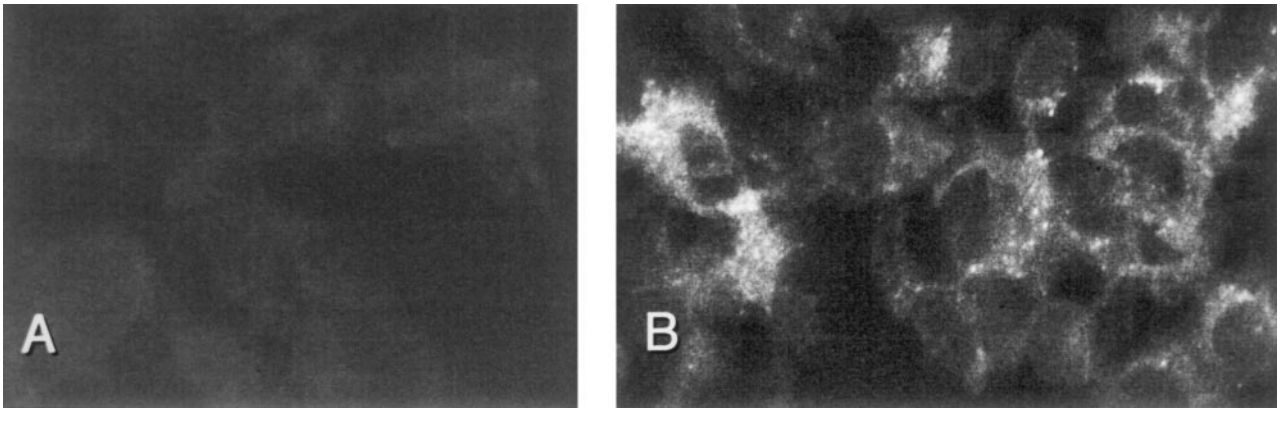

FIG. 5. Cellular localization of human TIN-ag in COS-1 cells. Cytoplasmic fluorescent signals were visualized in cells transfected with human TIN-ag cDNA (B) but not in cells transfected with vector alone (A).

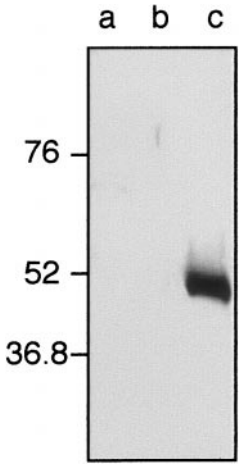

FIG. 6. Western blotting of immunoprecipitates from COS-1 cells transfected with human TIN-ag cDNA. Blotted membranes were incubated with mouse monoclonal antibody (H79) to TIN ag. The reaction product (~50 kDa) is seen in immunoprecipitates from COS-1 cells transfected with human TIN-ag cDNA (lane c), but not in those from wild COS-1 cells (lane a) or from COS-1 cells transfected with vector alone (lane b).

DISCUSSION

The present study describes the cloning and expression of human homologue of TIN-ag. The longest cDNA had 1589 nucleotides and contained a 5′ untranslated sequence of 13 nucleotide, a 3′ untranslated sequence of 148 nucleotides, and an open reading frame of 1,428 nucleotides coding for a 476 amino acid protein. An amino acid sequence of human TIN-ag showed a high (~85%) homology to rabbit and mouse TIN-ag, and also some (~40%) similarity with C. elegans. In the NH2 terminal regions, human TIN-ag contained cysteine residues showing the same spacing with follistatin modules. These modules have recently been described in several mosaic proteins, including follistatin, agrin, osteonectin/SPARC, and SC1 (20). Since follistatin and Osteonectin/SPARC bind the TGF-β family member, activin-A and platelet-derived growth factor, respectively. The presence of follistatine modules in human TIN-ag may be indicative of a role in the binding of molecules structurally related to TGF-β. Recently, follistatin-related protein was identified as a novel autoantigen in systemic rheumatic diseases (22). The follistatin modules present in human TIN-ag might be a target to which the aberrant immune response was directed in anti-TBM nephritis. Detailed epitopes are under investigation. Human TIN-ag also shared sequence homology with several classes of extracellular matrix adhesive glycoproteins in amino terminal region and with cathepsin family of cysteine proteinases in the carboxyl terminal region. Collectively, these results indicate that TIN-ag is a mosaic protein composed of follistatin modules, extracellular adhesion glycoprotein molecules and cysteine proteinase family protein.

Northern blot analysis showed that the mRNA transcript was exclusively expressed in human adult and fetal kidneys, which parallels previous immunohistochemical observations indicating a rather restricted distribution of this antigen (10–12). The function of TIN-ag in the kidney is still undetermined. However, TIN-ag is shown to interact with type IV collagen, laminin, and integrin $\alpha 3\beta 1$ and $\alpha v\beta 3$ (14, 23, 24), suggesting that this molecule is important for the maintenance of the integrity of the basement membranes in the renal tubules. Unlike other extracellular matrix proteins, TIN-ag lacks RGD sequence. Instead, TIN-ag possesses follistatin modules and ATP/GTP-binding motif, which may be important for the function of this molecule. Kanwar and co-workers (14, 15) revealed that TIN-ag was expressed in the developing kidney, selectively regulating tubulogenesis, but not glomerulogenesis. TIN-ag may play a role in renal development, facilitating cell-to-matrix interaction, in concert with other basement membrane proteins such as laminin, type IV collagen, and proteoglycans (25).

Nephronophthisis is characterized by early-onset chronic renal failure, sclerosing tubulointerstitilal in-

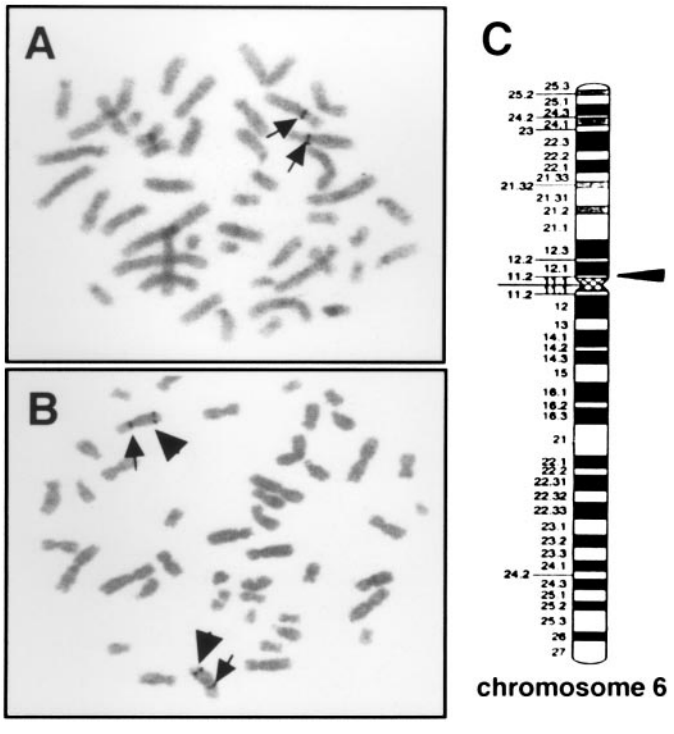

FIG. 7. FISH mapping of the human TIN-ag gene. (A) Hybridization signals (arrows) with a Back clone F910 (a probe for human TIN-ag) on chromosome 6. (B) Identification of chromosome 6 by cohybridization of clone F910 (arrows) with a genomic clone which has been previously localized to 6q24 (arrowheads). (C) Ideogram illustrating the chromosomal position of clone F910 at 6p11.2-12. The map position of human TIN-ag as indicated by arrowhead.

jury, and autosomal inheritance with frequent involvement of other organs, such as retinitis pigmentosa (26). Immunofluorescence studies using antibodies to human TIN-ag showed reduced or absent staining in the TBM of patients with this disorder (16). These findings suggest that fundamental defect in nephronophthisis might be abnormalities in the synthesis of TIN-ag. Using linkage analysis, a responsible gene (NPH1) for nephronophthisis without extrarenal manifestation was assigned to chromosome 2q (27). However, the frequent form of nephronophthisis accompanied with eye involvement did not map to the same chromosomal region as NPH1 (26). There must be another gene in human genome that gives rise to renal pathology identical found in NPH1. This study using FISH revealed that the human TIN-ag gene was mapped to chromosome 6p11.2-12. Thus, we speculate that chromosome 6p11.2-12 is a candidate as a second locus for nephronophthisis. Further investigations are obviously needed to confirm this speculation.

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