Identification of RPE65 in transformed kidney cells[1]

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Abstract The protein RPE65 has an important role in retinoid processing and/or retinoid transport in the eye. Retinoids are involved in cell differentiation, embryogenesis and carcinogenesis. Since the kidney is known as an important site for retinoid metabolism, the expression of RPE65 in normal kidney and transformed kidney cells has been examined. The RPE65 mRNA was detected in transformed kidney cell lines including the human embryonic kidney cell line HEK293 and the African green monkey kidney cell lines COS-1 and COS-7 by reverse transcription PCR. In contrast, it was not detected in human primary kidney cells or monkey kidney tissues under the same PCR conditions. The RPE65 protein was also identified in COS-7 and HEK293 cells by Western blot analysis using a monoclonal antibody to RPE65, but not in the primary kidney cells or kidney tissues. The RPE65 cDNA containing the full-length encoding region was amplified from HEK293 and COS-7 cells. DNA sequencing showed that the RPE65 cDNA from HEK293 cells is identical to the RPE65 cDNA from the human retinal pigment epithelium. The RPE65 from COS-7 cells shares 98 and 99% sequence identity with human RPE65 at the nucleotide and amino acid levels, respectively. Moreover, the RPE65 mRNA was detected in three out of four renal tumor cultures analyzed including congenital mesoblastic nephroma and clear cell sarcoma of the kidney. These results demonstrated that transformed kidney cells express this retinoid processing protein, suggesting that these transformed cells may have an alternative retinoid metabolism not present in normal kidney cells.

Key words: COS cell; Kidney; Retinal pigment epithelium; Retinoid; RPE65; Tumor

1. Introduction

RPE65 (p63) was identified and purified from the retinal pigment epithelium (RPE) [1,2]. It was originally reported to be specifically expressed in the RPE, preferentially in the microsomal fraction. It has been reported that RPE65 associates with the plasma retinol-binding protein (RBP) and thus, it was proposed to be the RBP receptor in the RPE [1,3]. It is known that the RPE is an important site for retinoid metabolism in the vision process [4–9] and RPE65 forms a complex with 11-*cis* retinal dehydrogenase [10]. The RPE65 knockout mouse model has an abnormal rhodopsin generation in the retina and disturbed retinoid profile in the RPE, suggesting a disrupted retinoid processing [11]. Recently, a number of mutations in the RPE65 gene have been shown to be linked to Leber's congenital amaurosis (LCA), autosomal recessive childhood onset severe retinal dystrophies (arCSRDs) and retinitis pigmentosa [12–14]. These findings suggest that RPE65 may be involved in retinoid processing and that a functional RPE65 is required for the physiological function of the RPE and normal vision.

Retinoids play important roles in cell proliferation and differentiation [15–17]. They also regulate animal development, embryogenesis and carcinogenesis via regulating gene expression [17–19]. It has been suggested that some cancer cells have altered retinoid processing and signaling pathways as they have altered expression of retinoid receptors, binding proteins and processing enzymes [20–24]. It has been shown that when a mouse cell line was transformed by the bovine papilloma virus, cellular retinoid acid-binding protein levels were markedly increased [20]. In contrast, serum levels of RBP are significantly lower in patients with malignant tumors of the head and neck region [25]. The altered retinoid metabolism may contribute to carcinogenesis in tumor tissues.

The kidney is an important site for retinoid metabolism [26–29]. RBP, a carrier transporting retinol to target tissues, has been shown to be cleared from the circulation by the kidney [29]. The kidney contains the highest retinal oxidase activity and may play a major role in the oxidative metabolism of retinol [26]. Retinoids have important functions in the regulation of gene expression, embryogenesis and morphogenesis in the kidney [18,19,30,31]. Retinoic acid (RA), interacting with growth factors, promotes tubulogenesis in cultured adult renal proximal tubule cells in a manner reminiscent of inductive embryonic kidney morphogenesis [31]. Retinoid X receptor (RXR) is known to form a heterodimer with 1,25-dihydroxyvitamin D3 (VD) receptor (VDR) and plays an important role in the VD-regulated transactivation in tubular cells [32].

We have examined the expression of RPE65 in transformed kidney cells, primary cultures of renal tumors and normal kidney tissues and report here that the RPE65 is expressed in transformed kidney cells, but not in normal kidney tissue.

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[1] The nucleotide sequence reported in this paper has been deposited into GenBank under accession number AF093455.

Abbreviations: arCSRD, autosomal recessive childhood onset severe retinal dystrophy; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LCA, Leber's congenital amaurosis; RBP, retinol-binding protein; RPE, retinal pigment epithelium; RT-PCR, reverse transcription and polymerase chain reaction

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2. Materials and methods

2.1. RNA isolation from cultures of kidney proximal tubular cells, transformed kidney cells and renal tumors

Transformed kidney cell lines COS-1, COS-7 and HEK293 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Human renal cell cultures were derived from normal kidney tissues (proximal tubular cell) and two pediatric renal neoplasms including congenital mesoblastic nephroma (CMN-1 and CMN-2) and clear cell sarcoma of the kidney (CCSK-2 and CCSK-BG1). Tumor cell lines were established, propagated and characterized by previously published procedures [33,34]. Total RNA was isolated from kidney tissues and cells either by the guanidinium isothiocyanate-CsCl gradient centrifugation method [35] or the guanidinium isothiocyanate-phenol/chloroform extraction method [36].

2.2. Reverse transcription (RT-) PCR and Southern blot analysis

The RT reaction mixture (20 μl) contained 1 μg total RNA, 100 pmol pd(N)6 random hexamer (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 4 μl of 5×first strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 2 μl 0.1 M DTT, 3 μl 2 mM dNTP and 10 U Moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA). The RT mixture was incubated at 37°C for 1 h followed by 95°C for 5 min to inactivate the reverse transcriptase. The RT products were used as templates for PCR separately to amplify the RPE65 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR mixture included 1 μl of the RT products, 10 pmol of each primer, 2.5 μl 10×PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.1% gelatin), 3 μl 2 mM dNTP, 2.5 U AmpliTaq (Perkin Elmer Cetus, Foster City, CA, USA). For the RPE65 PCR, the 5′ primer had a sequence of 5′-GTTTCTGATTGTGGATCTC-3′ and the 3′ primer was 5′-GGGATGTTAATCTCCACTTC-3′. For GAPDH PCR, the 5′ and 3′ primers were 5′-GTGAACCATGAGAAG(A)TATGA-3′ and 5′-TTGAAGTCAG(C)T(A)GGAGACAACC-3′, respectively. The PCR was carried out for 30 cycles (94°C, 1 min; 55°C, 1 min and 72°C, 3 min) in a Robocycler (Stratagene, La Jolla, CA, USA). 5 μl of each PCR product was probed with a ^{32}P-labelled nested primer in a Southern blot analysis as described previously [37].

2.3. Western blot analysis

COS-7, HEK293 and cultured primary human proximal tubular cells and monkey kidney tissues were washed with PBS and homogenized in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.5% NP-40) containing protease inhibitors: 2 μg/ml pepstatin, 2 μM E64, 0.5 mM PMSF and Protease Inhibitor Cocktail Tablets (Boehringer Mannheim) 1 tablet/10 ml buffer. The cell debris were removed by centrifugation at 14 000×g at 4°C for 10 min. The protein concentration in the supernatant was measured with a Bio-Rad protein assay. 100 μg of protein from each sample was loaded for a Western blot analysis using the ECL (enhanced chemiluminescence) Western blotting kit (Amersham International). Proteins were resolved with SDS-PAGE (8–16% acrylamide gel) and then electrotransferred onto a Hybond-ECL nitrocellulose membrane (Amersham International) (25 V, 2 h). The membrane was blocked by incubation with 5% (w/v) BLOTTO in TBST (20 mM Tris-Cl, pH 7.6, 137 mM NaCl, 0.1% Tween 20) for 2 h at room temperature. The membrane was then blotted with a monoclonal antibody specific to bovine RPE65 (1:2000 dilution) (a generous gift from Dr John Saari at the University of Washington) overnight at 4°C. The membrane was washed three times with the BLOTTO solution, 15 min each, at room temperature. A rabbit anti-mouse antibody labelled with horseradish peroxidase (Amersham International) was diluted 1:10 000 in the BLOTTO solution and incubated with the membrane for 3 h at room temperature. The membrane was washed four times in TBST to remove free antibody. Two detection solutions provided by the ECL kit were mixed and added onto the surface of the membrane followed by 1 min incubation. The excess detection solution was drained off and the membrane was covered with SaranWrap and exposed to a Kodak X-Omat film.

2.4. DNA sequence analysis

PCR products were purified by agarose gel electrophoresis and sequenced on both strands by Sanger's dideoxynucleotide chain termination method using the dsDNA Cycling Sequencing System (Gibco BRL). All sequences were verified by sequencing PCR products from another independent PCR. The DNA sequence was analyzed by the Wisconsin Genetics Computer Group (GCG) UNIX System. The amino acid sequence was deduced using the GCG Translate program.

3. Results

3.1. Identification of RPE65 mRNA in transformed kidney cell lines

Using primers specific to the consensus sequences of the human and bovine RPE65, RT-PCR amplified a single band of RPE65 fragment from the transformed African green monkey kidney cell lines COS-1 and COS-7 and the transformed human kidney cell line HEK293. The PCR products were hybridized with a nested oligonucleotide probe specific for human RPE65 cDNA, which confirmed the specificity of the PCR amplification (Fig. 1A). The PCR products showed an expected length of 600 bp as estimated based on the human and bovine RPE65 cDNA sequences. The size of 600 bp excluded that the PCR product was amplified from genomic DNA, because the PCR primers span two large introns which are more than 6 kb in length according to the human RPE65 gene [38]. Under the same PCR conditions, however, the RPE65 mRNA was not detected in cultured primary kidney proximal tubular cells or normal kidney tissue (Fig. 1A). Amplification of the house-keeping gene mRNA GAPDH was used to assess the RNA integrity and efficiency of reactions. Using the same RT products as templates, a PCR of GAPDH yielded comparable levels of products from all of the samples, demonstrating that each reaction received an equal amount of RNA (Fig. 1B). This indicated that the absence of RPE65 in normal kidney cells and tissue was not a false negative result.

3.2. Identification of RPE65 protein in transformed kidney cell lines

The presence of the RPE65 at the protein level was confirmed by Western blot analysis with a monoclonal antibody specific to RPE65. A single band of 65 kDa was recognized by the monoclonal antibody in the RPE, COS-7 and HEK293

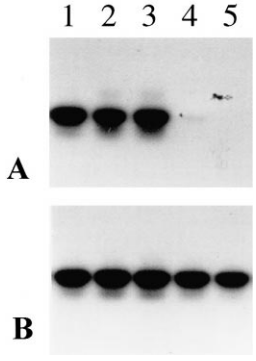

Fig. 1. RT-PCR and Southern blot analysis of RPE65 in transformed kidney cell lines. RNAs from cells or tissues were used as templates for RT-PCR using primers specific to RPE65 (A) and GAPDH (B) mRNA. The PCR products were subjected to Southern blot analysis using nested oligonucleotides as probes. A: PCR amplified a specific band of the RPE65 cDNA (600 bp) from COS-1 (lane 1), COS-7 (lane 2) and HEK293 (lane 3) cells, but not from primary human proximal tubular cells (lane 4) or monkey kidney tissues (lane 5) under the same conditions. B: the PCR amplified a GAPDH cDNA fragment (460 bp) from all the cells and tissues analyzed.

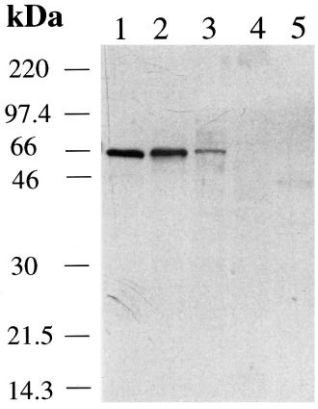

Fig. 2. Western blot analysis of RPE65 in transformed kidney cells. Western blot analysis was performed as described in Section 2. The RPE65 monoclonal antibody recognizes a single band of 65 kDa in the RPE (lane 1), COS-7 (lane 2) and HEK293 (lane 3) cells, but not in the primary kidney cells (lane 4) or normal kidney tissue (lane 5).

cells. However, no RPE65 was observed in the primary proximal tubular cells or normal kidney tissue (Fig. 2). This result demonstrated that RPE65 protein is also expressed in the transformed kidney cells but not in the benign kidney tissue.

3.3. Sequence analysis of RPE65 from COS-7 and HEK293 cells

To determine whether the RPE65 mRNA in the transformed kidney cells is from the legitimate RPE65 gene or from another homologous gene, the full-length RPE65 cDNAs were synthesized from COS-7 and HEK293 cells. The primers were designed according to the consensus sequence of the human and bovine RPE65 and span the full-length encoding region. To exclude potential artefacts introduced by PCR, PCR products from three independent PCRs were purified and sequenced. The RPE65 amplified from HEK293 cells showed an identical sequence to the human RPE65 cDNA from the RPE while the RPE65 cDNA from COS-7 cells did not match any existing sequences in the GenBank (Release 107.0, June 1998). The sequence from COS-7 cells is 1677 bp in length and contains a full-length open read-

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1                                                   M  S  I  Q  V  E  H  P  A  G  G  Y    12
1    CAGTTGGTGCCAGAACTCTGGATCCTGAACTGGAAGAAAATGTCTATCCAGGTCGAGCATCCTGCTGGTGGTTAC    75

13    K  K  L  F  E  T  V  E  E  L  S  S  P  L  T  A  H  V  T  G  R  I  P  L  W    37
76   AAGAAACTGTTTGAAACTGTGGAGGAACTGTCCTCGCCGCTCACAGCTCATGTAACAGGCAGGATCCCCCTGTGG   150

38    L  T  G  S  L  L  R  C  G  P  G  L  F  E  V  G  S  E  P  F  Y  H  L  F  D    62
151  CTCACCGGCAGTCTCCTTCGATGTGGGCCAGGACTCTTTGAAGTTGGATCTGAGCCATTTTACCACCTGTTTGAT   225

63    G  Q  A  L  L  H  K  F  D  F  K  E  G  H  V  T  Y  H  R  R  F  I  R  T  D    87
226  GGGCAAGCCCTTCTGCACAAGTTTGACTTTAAAGAAGGACACGTCACATACCACAGAAGGTTCATCCGCACTGAT   300

88    A  Y  V  R  A  M  T  E  K  R  I  V  I  T  E  F  G  T  C  A  F  P  D  P  C   112
301  GCTTACGTACGGGCAATGACTGAGAAAAGGATCGTCATAACAGAATTTGGCACCTGTGCTTTCCCAGATCCCTGC   375

113   K  N  I  F  S  R  F  F  S  Y  F  R  G  V  E  V  T  D  N  A  L  V  N  V  Y   137
376  AAGAATATATTTTCCAGGTTTTTTTCTTACTTTCGAGGAGTGGAGGTTACCGACAATGCCCTTGTTAATGTCTAC   450

138   P  V  G  E  D  Y  Y  A  C  T  E  T  N  F  I  T  K  I  N  P  E  T  L  E  T   162
451  CCAGTGGGGGAAGATTACTACGCTTGCACAGAGACCAACTTTATTACAAAGATTAATCCAGAGACCTTGGAGACA   525

163   I  K  Q  V  D  L  C  N  Y  V  S  V  N  G  A  T  A  H  P  H  I  E  N  D  G   187
526  ATTAAGCAGGTTGATCTTTGCAACTATGTCTCTGTCAATGGGGCCACTGCTCACCCCCACATTGAAAATGATGGA   600

188   T  V  Y  N  I  G  N  C  F  G  K  N  F  S  I  A  Y  N  I  V  K  I  P  P  L   212
601  ACCGTTTACAATATTGGTAATTGCTTTGGAAAAAATTTTTCAATTGCCTACAACATTGTAAAGATCCCACCACTG   675

213   Q  A  D  K  E  D  P  I  S  K  S  E  I  V  V  Q  F  P  C  S  D  R  F  K  P   237
676  CAAGCAGACAAGGAAGATCCAATAAGCAAGTCAGAGATCGTTGTACAATTCCCCTGCAGTGACCGATTCAAGCCA   750

238   S  Y  V  H  S  F  G  L  T  P  N  Y  I  V  F  V  E  T  P  V  K  I  N  L  F   262
751  TCTTACGTTCATAGTTTTGGTCTGACTCCCAACTATATCGTTTTTGTGGAGACACCAGTCAAAATTAACCTGTTC   825

263   K  F  L  S  S  W  S  L  W  G  A  N  Y  M  D  C  F  E  S  N  E  T  M  G  V   287
826  AAGTTCCTTTCTTCATGGAGTCTTTGGGGAGCCAACTACATGGATTGTTTTGAGTCCAATGAAACCATGGGGGTT   900

288   W  L  H  I  A  D  K  K  R  K  K  Y  L  N  N  K  Y  R  T  S  P  F  N  L  F   312
901  TGGCTTCATATTGCTGACAAAAAAAGGAAAAAGTACCTCAATAATAAATACAGAACTTCTCCTTTCAACCTCTTC   975

313   H  H  I  N  T  Y  E  D  N  G  F  L  I  V  D  L  C  C  W  K  G  F  E  F  V   337
976  CATCACATCAACACCTATGAAGACAATGGGTTTCTGATTGTGGATCTCTGCTGCTGGAAAGGATTTGAGTTTGTT  1050

338   Y  N  Y  L  Y  L  A  N  L  R  E  N  W  E  E  V  K  K  N  A  R  K  A  P  Q   362
1051 TATAATTACTTATATTTAGCCAATTTACGTGAGAACTGGGAAGAGGTGAAAAAAAATGCCAGAAAGGCTCCCCAA  1125

363   P  E  V  R  R  Y  V  L  P  L  N  I  D  K  A  D  T  G  K  N  L  I  T  L  P   387
1126 CCTGAAGTTAGGAGATATGTACTTCCTTTGAATATTGACAAGGCTGACACAGGCAAGAATTTAATCACGCTCCCC  1200

388   N  T  T  A  T  A  I  L  C  S  E  E  T  I  W  L  E  P  E  V  L  F  S  G  P   412
1201 AATACAACTGCCACTGCAATTCTGTGCAGTGAGGAGACAATCTGGCTGGAGCCTGAGGTTCTCTTTTCAGGGCCT  1275

413   R  Q  A  F  E  F  P  Q  I  N  Y  Q  K  Y  C  G  K  P  Y  T  Y  A  Y  G  L   437
1276 CGCCAAGCGTTTGAGTTTCCTCAAATCAATTACCAGAAGTATTGTGGGAAACCTTACACATACGCATATGGACTT  1350

438   G  L  N  H  F  V  P  D  R  L  C  K  L  N  V  K  T  K  E  T  W  V  W  Q  E   462
1351 GGCTTGAATCACTTTGTTCCAGATAGGCTCTGTAAGCTGAATGTCAAAACTAAAGAAACTTGGGTTTGGCAAGAG  1425

463   P  D  S  Y  P  S  E  P  I  F  V  S  H  P  D  A  L  E  E  D  D  G  V  V  L   487
1426 CCTGATTCATACCCATCAGAACCCATCTTTGTTTCTCACCCAGATGCCTTGGAAGAAGATGATGGTGTAGTTCTG  1500

488   S  V  V  V  S  P  G  A  G  Q  K  P  A  Y  L  L  I  L  N  A  K  D  L  S  E   512
1501 AGTGTGGTGGTGAGCCCAGGAGCAGGACAAAAGCCTGCTTATCTCCTGATTCTGAATGCCAAGGACTTAAGTGAA  1575

513   V  A  R  A  E  V  E  I  N  I  P  V  T  F  H  G  L  F  K  K  S  *            533
1576 GTTGCCCGGGCTGAAGTGGAGATTAACATCCCTGTCACCTTTCACGGACTGTTCAAAAAATCTTGAGCATACTCT  1650

1651 AGCAGGATATGGTTTTGGTAGCAAAAC  1677
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Fig. 3. Nucleotide and amino acid sequence of the RPE65 expressed in COS-7 cells. The lower line represents the nucleotide sequence and the upper one is the deduced amino acid sequence. The stop codon is indicated by an asterisk. The potential glycosylation sites are in bold. This sequence has been deposited into GenBank under accession number AF093455.

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Human  MSIQVEHPAGGYKKLFETVEELSSPLTAHVTGRIPLWLTGSLLRCGPGLFEVGSEPFYHL  60
COS-7  ------------------------------------------------------------
Bovine --S---------------------------------------------------------
Salam. -TNR-D-----------ST---VA-V--Q------V-S-----------------Q----

Human  FDGQALLHKFDFKEGHVTYHRRFIRTDAYVRAMTEKRIVITEFGTCAFPDPCKNIFSRFF 120
COS-7  ------------------------------------------------------------
Bovine ------------------------------------------------------------
Salam. ----------E--G---I---------T-----------------F-------------L

Huamn  SYFRGVEVTDNALVNVYPVGEDYYACTETNFITKINPETLETIKQVDLCNYVSVNGATAH 180
COS-7  ------------------------------------------------------------
Bovine ---------------I------------------V--------------------------
Salam. ---Q-L------------------------Y-----------V-K--------I--V---

Human  PHIENDGTVYNIGNCFGKNFSIAYNIVKIPPLQADKEDPISKSEIVVQFPCSDRFKPSYV 240
COS-7  ------------------------------------------------------------
Bovine ------------------------------------------------------------
Salam. ----H-------------H-AF-----------------N-AKV-------E-------

Human  HSFGLTPNYIVFVETPVKINLFKFLSSWSLWGANYMDCFESNETMGVWLHIADKKRKKYL 300
COS-7  ------------------------------------------------------------
Bovine ------------------------------------------------------------I
Salam. --------------Q--------------I-----------H------M-V-E-HTGE--

Human  NNKYRTSPFNLFHHINTYEDNGFLIVDLCCWKGFEFVYNYLYLANLRENWEEVKKNARKA 360
COS-7  ------------------------------------------------------------
Bovine --------------------HE-----------------S-------------------
Salam. -I-----A------------H--------------------------------RS-E-P

Human  PQPEVRRYVLPLNIDKADTGKNLVTLPNTTATAILCSDETIWLEPEVLFSGPRQAFEFPQ 420
COS-7  ------------------------I-------------E----------------------
Bovine ------------------------------------------------------------
Salam. ------------D-H-V-------N--Y-----V-R------------------------

Human  INYQKYCGKPYTYAYGLGLNHFVPDRLCKLNVKTKETWVWQEPDSYPSEPIFVSHPDALE 480
COS-7  ------------------------------------------------------------
Bovine ------G-----------------------------------------------------
Salam. ---K-HG--D------V----------S----------------T---------Q---I-

Human  EDDGVVLSVVVSPGAGQKPAYLLILNAKDLSEVARAEVEINIPVTFHGLFKKS        533
COS-7  ----------------------------------------------------
Bovine ----------------------------------------------------
Salam. ----------I---E-----F--------M--I-----DS--------M---A
```

Fig. 4. RPE65 cDNA sequence comparison between human, COS-7 cells, bovine and salamander RPE65. The deduced amino acid sequence of the COS-7 RPE65 was aligned with the human, bovine and salamander (salam.) RPE65 using GCG (version 9.1). The human RPE65 is used as the template and only the amino acids different from the human RPE65 are indicated in COS cells, bovine and salamander RPE65.

ing frame. The translation start codon ATG is located at position 40 in a Kozak context with an A at the −3 position [39]. The stop codon is located at position 1639 (Fig. 3). The cDNA encodes a protein of 533 residues and with a predicted molecular weight of 60 991 and calculated p*I* of 6.35. Hydropathy analysis indicates that it does not contain any typical hydrophobic transmembrane helix. The deduced protein sequence contains three potential glycosylation sites (Asn-199, Asn-282 and Asn-388) with the sequence of Asn-X-Ser/Thr (Fig. 3).

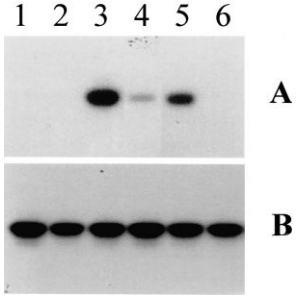

Fig. 5. RT-PCR and Southern blot analysis of RPE65 in renal tumors. Total RNA from normal human kidney tissue and renal tumors was used for RT-PCR and Southern blot analysis. A: PCR amplified a 600 bp fragment of the RPE65 cDNA from a clear cell sarcoma (CCSK-2) (lane 3) and two congenital mesoblastic nephromas (CMN-1 and CMN-2) (lanes 4 and 5), but not from another case of clear cell sarcoma (CCSK-BG1) (lane 6), normal human kidney tissue (lane 1) or primary human proximal tubular cells (lane 2) under the same conditions. B: PCR amplified a GAPDH cDNA fragment (460 bp) from all the cells and tissues analyzed.

A global GenBank search showed that the RPE65 cDNA from COS-7 cells has a high sequence homology to the human and bovine RPE65 isolated from the RPE. It shares 99 and 98% amino acid sequence identity to the human and bovine RPE65, respectively. There are only two residues different from that of human RPE65 (Fig. 4), indicating that RPE65 is highly conserved across species.

3.4. RPE65 mRNA in human renal tumor cells

In order to determine the expression of the RPE65 gene in renal tumors, RNA from primary cultures of four renal tumors was used for RT-PCR. Under the same conditions as described above, the RPE65 mRNA was detected in three of the four cultures analyzed, including a clear cell sarcoma (CCSK-2) and two congenital mesoblastic nephromas (CMN-1 and CMN-2). One case of clear cell sarcoma of the kidney (CCSK-BG1) was devoid of RPE65 mRNA (Fig. 5A). The specificity of PCR products was confirmed by Southern blot analysis and DNA sequencing (Fig. 5A). The RPE65 mRNA was not detected in normal human kidney tissues or cultured proximal tubular cells (Fig. 5A). However, the GAPDH mRNA was detected in all of the samples at comparable levels (Fig. 5B), indicating that the lack of RPE65

mRNA in normal kidney tissues, proximal tubular cells and CCSK-BG1 cells did not result from RNA degradation.

4. Discussion

RPE65, a protein originally found in the RPE, has been suggested to play a role in the retinoid processing or transport [1,40,41]. Recently, it was identified immunochemically in yolk sac and was speculated to be responsible for the vitamin A transport [42]. It was also identified in cone photoreceptor cells but not in rods and thus, it may contribute to the distinct retinoid processing pathways in cone photoreceptor cells [43]. The present study has identified RPE65 expression in some transformed kidney cell lines but not in normal kidney tissues or primary kidney cultures. This finding suggests that transformed cells may have an altered retinoid metabolic pathway and may provide clues for elucidating the distinct retinoid processing machinery in transformed kidney cells.

COS-1 and COS-7 cells are SV40-transformed African green monkey kidney cell lines [44]. HEK293 cells are a human embryonic cell line transformed by adenovirus [45]. All of these cells show a transformed growth and phenotypes. The present study demonstrated that RPE65 is expressed in these transformed kidney cells but not in normal kidney tissues or cultures derived from normal kidney tissues. The mechanism of activation of the RPE65 gene is unclear at this time. COS-1, COS-7 and HEK293 cells were transformed by different viruses and thus, the activated RPE65 expression cannot arise from a specific virus transformation. Because of the endogenous expression of RPE65, these cell lines are not suitable hosts for transfection and expression of exogenous RPE65. The RPE65 mRNA was also detected in some types, but not all of the renal tumors analyzed, suggesting that activation of the RPE65 gene transcription is dependent on the tumor case. The expression of RPE65 in these renal tumors remains to be confirmed at the protein level.

The RPE65 sequence isolated from the transformed kidney cells is identical to that from the RPE. The sequence comparison also confirmed that this protein is highly conserved during evolution, as RPE65 from COS cells differs from the human RPE65 only by two amino acids. Similarly, it does not contain a hydrophobic transmembrane domain and thus, it is unlikely to be a typical transmembrane protein. Sequence conservation across species underscores its functional significance.

The physiological function of RPE65 protein is currently uncertain, nevertheless, several lines of evidence have linked it with retinoid metabolism or transport [10,11,41]. It was demonstrated that RPE65 forms a complex with 11-*cis* retinol dehydrogenase and may function as an enzyme complex in the RPE [10]. In COS-7 cells, however, the mRNA of 11-*cis* retinol dehydrogenase was not detected by RT-PCR (data not shown), indicating that RPE65 does not complex with this retinoid processing enzyme in COS cells. This finding suggests that the function of RPE65 in COS cells may not be necessarily identical to that in the RPE. As retinoid processing and transport have been found to be important for cell differentiation, embryogenesis and carcinogenesis [17–19,46], the activated expression of this retinoid processing protein in transformed cells may contribute to the distinct retinoid metabolism and signaling in transformed kidney cells. The role of RPE65 expression in the retinoid metabolism in the transformed cells remains to be studied.

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