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Short sequence-paper

Identification and characterization of UEV3, a human cDNA with similarities to inactive E2 ubiquitin-conjugating enzymes☆

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Abstract

Recent studies have shown that ubiquitination is an essential factor in endosomal sorting and virus assembly. The human TSG101 gene has been demonstrated to belong to a group of genes coding for apparently inactive E2 ubiquitin-conjugating enzymes, which exert regulatory effects on E2 activity in cellular ubiquitination processes. In this study, a novel human cDNA (UEV3) encoding a putative protein of 379 amino acids was isolated from a human placenta library that may represent a partial paralogue of human TSG101. The predicted protein contains an N-terminal domain homologous to the catalytic domain of ubiquitin-conjugating enzymes (Ubc), which is fused to a sequence showing significant homology to members of the lactate dehydrogenase protein family. The UEV3 gene is located on chromosome 11 closely adjacent to TSG101 and LDH-C. Northern blot and UEV3-specific reverse transcription/polymerase chain reaction (RT/PCR) analyses of various colon carcinoma cell lines as well as both normal and tumor samples from colon revealed an expression of the UEV3 cDNA in all tested samples.

Keywords: TSG101; E2 ubiquitin-conjugating enzyme; Lactate dehydrogenase

The murine tumor susceptibility gene tsg101 has been identified by a random gene knock-out approach in which homozygous inactivation of the tsg101 gene resulted in cellular transformation of NIH3T3 fibroblasts. Their inoculation into nude mice led to metastatic tumor formation [1]. The human TSG101 homologue was cloned and mapped to chromosome 11p15.1-p15.2, a genomic locus frequently affected by allelic loss in various tumor entities [2]. Occurrence of truncated TSG101 transcripts has been variously reported in several tumor and normal tissue samples [3,4] initially suspected to be caused by genomic deletions [2]. However, subsequent analyses failed to detect any genomic alterations in tumor samples that presented TSG101 truncated transcripts.

Detailed analyses of the murine and human TSG101 protein sequences suggested a close relationship to a class of apparently inactive variants of ubiquitin-conjugating enzymes E2 (UEV) that might influence E2-activity in a dominant negative way [5,6]. Interaction of TSG101 with MDM2 and p53 has been demonstrated [7]. TSG101 inhibits the ubiquitination and degradation of MDM2 and is thus directly involved in cell cycle control by regulation of cellular p53 levels. Recent studies indicated that TSG101 is essential for HIV-1 budding by interaction with the Gag protein of HIV type 1 [8–10], supporting the earlier notion that the Ub system has a crucial role in virus assembly and vacuolar protein sorting [11]. Two human E2 homologues with 90% amino acid identity among each other in the N-terminal region, UEV1 and UEV2, have been isolated previously

Abbreviations: aa, amino acid(s); kb, kilobase(s); LOH, loss of heterozygosity; nt, nucleotide(s); ORF, open reading frame; RACE, rapid amplification of cDNA ends; RT/PCR, reverse transcription/polymerase chain reaction; Ub, ubiquitin; Ubc, ubiquitin-conjugating; UEV, ubiquitin conjugating enzyme E2 variant

☆ The nucleotide sequence in this paper has been submitted to the DDJB/EMBL/GenBank databases under the accession number AF503350.

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                                                CAGAAGGTCCGGGGGCTGGAGTCCTGGGACCTAGCTCGGGACCGGCCTGGAG   52

ATG GAG TTC GAC TGC GAG GGC CTG AGA CGG CTG CTT GGC AAG TAC AAG TTC AGG GAC CTA   112
M  E  F  D  C  E  G  L  R  R  L  L  G  K  Y  K  F  R  D  L                      20

ACT GTG GAA GAA CTA AGG AAT GTA AAT GTA TTT TTC CCA CAT TTC AAA TAT TCC ATG GAC   172
T  V  E  E  L  R  N  V  N  V  F  F  P  H  F  K  Y  S  M  D                      40

ACC TAT GTT TTT AAA GAT AGT TCT CAG AAA GAC CTG CTG AAT TTT ACT GGC ACA ATT CCT   232
T  Y  V  F  K  D  S  S  Q  K  D  L  L  N  F  T  G  T  I  P                      60

GTG ATG TAT CAG GGT AAT ACA TAT AAC ATA CCA ATT CGT TTC TGG ATT TTG GAT TCT CAC   292
V  M  Y  Q  G  N  T  Y  N  I  P  I  R  F  W  I  L  D  S  H                      80

CCT TTC GCT CCC CCT ATT TGC TTC TTG AAG CCA ACT GCA AAT ATG GGA ATC TTA GTC GGA   352
P  F  A  P  P  I  C  F  L  K  P  T  A  N  M  G  I  L  V  G                     100

AAA CAT GTG GAT GCT CAA GGC AGA ATA TAT TTG CCC TAT CTC CAA AAC TGG AGC CAT CCT   412
K  H  V  D  A  Q  G  R  I  Y  L  P  Y  L  Q  N  W  S  H  P                     120

AAA TCT GTC ATT GTT GGA TTA ATT AAA GAA ATG ATT GCC AAG TTT CAA GAG GAA CTT CCC   472
K  S  V  I  V  G  L  I  K  E  M  I  A  K  F  Q  E  E  L  P                     140

ATG TAT TCT CTA TCA TCA TCT GAT GAG GCA CGG CAG GTA GAC TTG CTA GCC TAT ATT GCA   532
M  Y  S  L  S  S  S  D  E  A  R  Q  V  D  L  L  A  Y  I  A                     160

AAA ATC ACT GAA GGT GTT TCA GAT ACA AAT TCA AAG AGC TGG GCA AAT CAT GAG AAT AAA   592
K  I  T  E  G  V  S  D  T  N  S  K  S  W  A  N  H  E  N  K                     180

ACA GTC AAT AAA ATT ACT GTG GTT GGA GGT GGA GAA CTC GGT ATT GCC TGC ACA TTA GCA   652
T  V  N  K  I  T  V  V  G  G  G  E  L  G  I  A  C  T  L  A                     200

ATT TCA GCA AAG GGT ATT GCA GAC AGG CTT GTC CTC TTA GAC CTC TCA GAA GGG ACT AAA   712
I  S  A  K  G  I  A  D  R  L  V  L  L  D  L  S  E  G  T  K                     220

GGA GCC ACG ATG GAC CTT GAA ATC TTC AAC CTT CCT AAT GTG GAG ATC AGC AAA GAT TTG   772
G  A  T  M  D  L  E  I  F  N  L  P  N  V  E  I  S  K  D  L                     240

TCT GCC TCT GCT CAT TCC AAG GTG GTG ATC TTC ACA GTC AAC TCT TTG GGT AGT TCT CAG   832
S  A  S  A  H  S  K  V  V  I  F  T  V  N  S  L  G  S  S  Q                     260

TCG TAC CTT GAT GTG GTA CAG AGC AAT GTG GAT ATG TTC AGA GCC CTT GTC CCA GCT CTG   892
S  Y  L  D  V  V  Q  S  N  V  D  M  F  R  A  L  V  P  A  L                     280

GGA CAT TAT AGT CAA CAC AGT GTC CTG CTC GTT GCA TCT CAA CCA GTG GAA ATC ATG ACC   952
G  H  Y  S  Q  H  S  V  L  L  V  A  S  Q  P  V  E  I  M  T                     300

TAT GTA ACA TGG AAA CTG AGT ACA TTT CCT GCA AAT CGA GTG ATC GGA ATT GGA TGT AAT  1012
Y  V  T  W  K  L  S  T  F  P  A  N  R  V  I  G  I  G  C  N                     320

CTG GAT TCA CAG AGA TTA CAG TAT ATT ATT ACA AAT GTT TTG AAG GCA CAG ACT TCA GGC  1072
L  D  S  Q  R  L  Q  Y  I  I  T  N  V  L  K  A  Q  T  S  G                     340

AAA GAA GTA TGG GTT ATT GGC GAG CAA GGA GAA GAC AAA GTG CTC ACA TGG AGT GGC CAA  1132
K  E  V  W  V  I  G  E  Q  G  E  D  K  V  L  T  W  S  G  Q                     360

GAA GAA GTA GTG AGT CTT ACC TCT CAA GTG CAG CTG TCC AAC AGG GAT ATT ATG ATA TAA  1192
E  E  V  V  S  L  T  S  Q  V  Q  L  S  N  R  D  I  M  I  *                     380

ATAGTGAAGTGTTTTTAAGTTTGCCTTGCATCCTTGGAACCAATGGAGTATCTGAAGTTATCAAAACCACACTGAAAGA  1271

AGATACAGTTACTGAGAAACTCCAAAGCAGTGCATCCTCAATCCACAGTCTCCAACAACAGTTAAAACTTTGATTCTCA  1350

AATGCAATTTGAGAGGCTGGACTTCTACCTAAAGGGAAAAGTCATTTAATTTTACCTATATATAGGTTTGAGGATTTCT  1429

GTATCCTGCTACTTACTTTTACAAACTGCTTGGTTAAAGTAGAGGGTTTCTTGATTAGCTTTGTGATGTAAATCCTTAA  1508

GGAGTTATACAAGGAGGGGAAAAATTAATTTTATTTGGGGTTCTTGAGATATCTATGCTGTTCTTTAAATCTACAGCAG  1587

GGGTAAACATTCATCTGCAGTGTGCATCAATTTAAATCATATATCCTAAACTAAAAGCACAATTCATACTTCGGGAATA  1666

TTTTATAAGTAATATATCTTTAAAAGAAAATTACCCTTTGACTTTTATAATCAACATAAGTTCCAGGCCCAGTATGGAT  1745

TTACAAAATCTGTGTCAGTTGTACATTCACAGGATCCACAGCTTAAGTTACTAATGTTTCTTGTGTAAAATCCTGTTGG  1824

TAGTAATAGTAAAGCATTGTATTTCCCTTCTTCAAATTAATTACCTACCAAAAAATGGAAAAGAATTTTACATGCACTT  1903

TAAAATAGTAAAATGGAAAGTGAATTTTTAAAATATATGCATTAAAAGTTTACTTTAATTTCCAAAAAAAAAAAAAAAA  1982
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Fig. 1. Nucleotide sequence of UEV3 cDNA and deduced amino acid sequence of the corresponding protein. A human placenta cDNA library ligated into the λ gt10 phage vector (Human Placenta 5′-STRETCH PLUS cDNA Library, Clontech) was used as template for the PCR amplification of the UEV3 cDNA using sense primers m35for 5′-CTGACCATGGAGTTCGACTG-3′ (first PCR) and m66for 5′-GACGGCTGCTAGGCAAGTACAAGTTC-3′ (nested PCR) in combination with λ gt10 vector specific primers 5′-CGAGCTGCTCTATAGACTGCTG-3′ (first PCR) and 5′-AGCAAGTTCAGCCTGGTTAAG-3′ (nested PCR), respectively. Nucleotides and amino acids are numbered on the right. Amino acid sequence is shown in bold letters. The translation stop codon is indicated by an asterisk. The putative polyadenylation signal is underlined. A 5′-RACE protocol (Roche Diagnostics) with antisense primer t102-63.rev 5′-ATTTACATTCCGTAGTTCTTCCACAG-3′ was used for the isolation of the nucleotide sequence in the 5′-region of UEV3 cDNA.

[12,13]. Recent studies in ciliates indicated the high conservation of the UEV family in eukaryotic evolution [14].

In the present study, database search was performed to identify proteins homologous to TSG101 possibly exerting similar functions in the regulation of proteasome-mediated protein degradation and vacuolar protein sorting. A murine cDNA sequence (mus musculus signaling molecule ATTP, accession NM_016855) was detected, which displayed strong homology to putative inactive ubiquitin conjugating enzymes E2 [6]. We designed primers based on the mouse ATTP signaling molecule, which also amplified a 350-bp cDNA fragment from human lymphocyte RNA samples in reverse transcription/polymerase chain reaction (RT/PCR). The human-derived PCR product we obtained showed 88% nucleotide identity to the corresponding mouse cDNA, suggesting high conservation during mammalian evolution. The partial human cDNA sequence was then used to design primers specific for the human gene. Using a combination of vector- and gene-specific primers, we were able to amplify the 3′ end of the full-length cDNA from a human placenta cDNA library. The resulting sequence was confirmed by rapid amplification of cDNA ends (3′-RACE) using cDNA of the liposarcoma cell line 1955/91 [15] as a template. The sequence from nt 1 to 139 with a 93-bp overlap from nt 47 to the previously amplified product was obtained by 5′-RACE-PCR on 1955/91. However, attempts at cloning the full-length 5′ sequence were not successful. The assembled cDNA sequence, which we named UEV3, was 1982 nt in

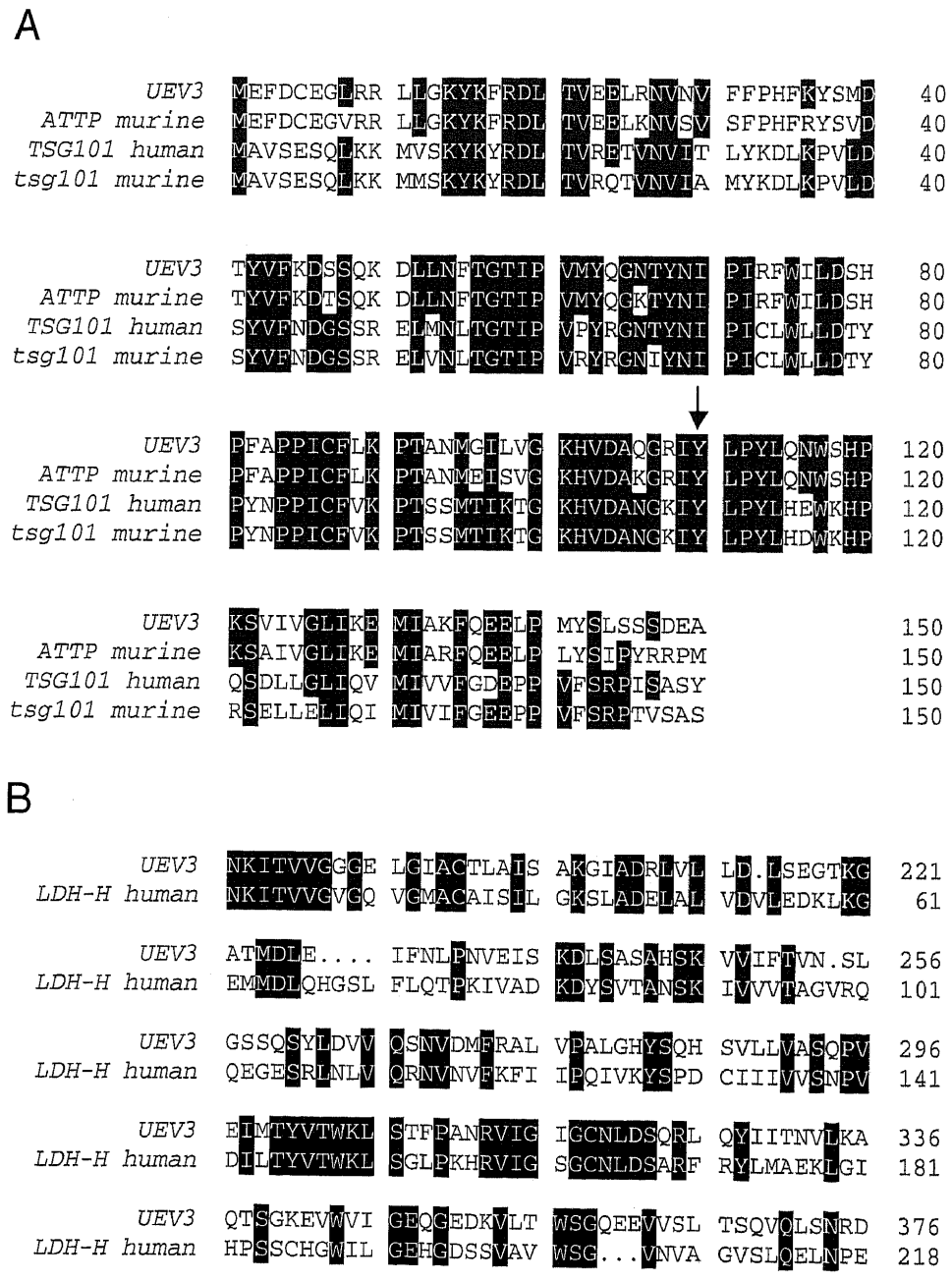


Fig. 2. Sequence alignment of UEV3 with related proteins. (A) The amino acid sequence of UEV3 is compared to mouse signaling molecule ATTP (NM_016855), human TSG101 (NM_006292), and mouse tsg101 (NM_021884). The tyrosine residue replacing the active site cystein of E2 enzymes is marked by an arrow. (B) Comparison of UEV3 and protein sequence of human LDH-B (NP_002291).

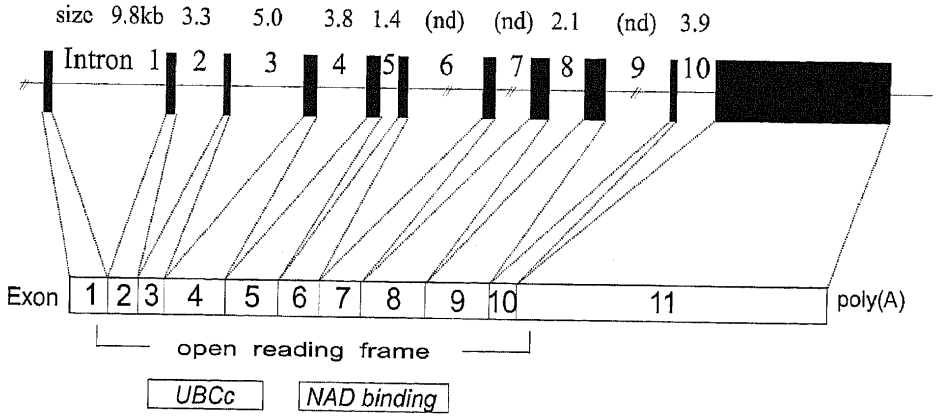
length and presented a 379 amino acid open reading frame (ORF) with a putative translational start codon at nt 53 and a stop codon at nt 1190 (Fig. 1). The ATG codon appears in a strong context (conserved purine in position −3 and a G residue following in position +4), the GC-rich upstream region lacking additional start codons provides further evidence for an effective translation initiation at the marked ATG codon [16]. The predicted UEV3 protein has a calculated mass of 42 kDa and a theoretical p*I* of 5.94.

UEV3 has a high sequence similarity to the Ub binding domain of E2 ubiquitin conjugating enzymes in the amino terminal region from position 51 to 140 of the amino acid sequence. As in TSG101, a cysteine residue required for ubiquitin conjugase activity is missing and replaced by a tyrosine residue (aa position 110, marked in Fig. 2), thus inactivating the Ub binding domain. Homology to TSG101 was restricted to the N-terminal region from amino acid position 1–150. At the C-terminus, UEV3 shows significant similarity to dehydrogenase sequences presenting a conserved NAD binding domain from position aa 183 (Fig. 2). No homology to dehydrogenases was seen in the N-terminal region. Closest relationship was found to rabbit lactate dehydrogenase M chain (P13491) with 44% identical aa, alignments with different lactate dehydrogenase isozymes from several other species revealed similar scores.

By comparison to human genome working draft sequences, we were able to deduce the genomic organization of UEV3. As shown in Fig. 3, the UEV3 gene is composed of 11 exons ranging in size between 66 nt (exon 3) and 708 nt (exon 11). Consensus signals (GT and AG) are present in all splice acceptor and splice donor sites. The UEV3 gene encompasses a genomic region approximately 56 kb in size. To exclude the possibility that the UEV3 cDNA is derived from a processed pseudogene, intron 5 was amplified by genomic PCR using primers in the surrounding exons. Only the PCR product of the expected size was obtained and specificity was confirmed by sequencing.

The UEV3 gene could be assigned to a single locus on chromosome 11p15.1-2 using the genomic BLAST program (http://www.ncbi.nlm.nih.gov). Interestingly, TSG101 and LDH-C, which seem to be partial paralogues of UEV3, are localized in close proximity in the same region of

A



B

Exon (size)	cDNA position	Donor site	Acceptor site
1 (94bp)	1 - 94	GCTTGGCAAG gtgcgggcag	ttctcttttcag TACAAGTTCA
2 (85)	95 - 179	GACACCTATG gtaagaatca	tttttcttctag TTTTTAAAGA
3 (66)	180 - 245	ATGTATCAGG gtaagtgtgg	tgtctcaaatag GTAATACATA
4 (154)	246 - 409	CTGGAGCCAT gtaagatcaa	tgtattctacag CCTAAATCTG
5 (136)	410 - 545	ATCACTGAAG gtttgtatat	ctttctttctag GTGTTTCAGA
6 (119)	546 - 664	TTCAGCAAAG gtatgtaaac	cttactgtacag GGTATTGCAG
7 (103)	665 - 767	ATCAGCAAAG gtctgtttat	ttcattcactag ATTTGTCTGC
8 (171)	768 - 938	TCTCAACCAG gtaaaatgct	tgcaatatttag TGGAAATCAT
9 (174)	939 - 1112	GAAGACAAAG gtaagaagta	atctaattttag TGCTCACATG
10 (64)	1113 - 1176	TGTCCAACAG gtaacactga	ttctcatttcag GGATATTATG
11 (708)	1177 - 1984		

Fig. 3. Putative genomic structure of the UEV3 gene, predicted by comparison with htgs database sequences (accession numbers AC027544, AC025620, AC016750). (A) Schematic diagram representing the structural features of UEV3. Solid lines represent introns; exons are indicated by solid boxes. Distances of DNA segments are not drawn to scale. Intron length is indicated by numbers above the respective intron. 'nd' means that intron length has not been determined. Interruptions within introns 6, 7, and 9 indicate that introns have not been completely sequenced. The bottom of the figure presents a schematic diagram of UEV3 mRNA indicating the contributions of each exon and the position of the coding region. Localization of the predicted catalytic domain of ubiquitin-conjugating enzymes E2 (UBCc) and the NAD-binding region is illustrated by boxes below. (B) Exon/intron organization of the UEV3 gene. Exon sequences are represented by capital letters, intron sequences by lowercase letters.

chromosome 11. The genomic organization and the existence of an ORF of 379 aa in length point out that UEV3 is not a pseudogene variant of TSG101 or LDH-C.

Loss of heterozygosity (LOH) at the chromosomal locus of the UEV3 gene, 11p15, has been variously observed in several different cancers including carcinomas of the digestive tract [17–19]. An LOH frequency of 17% has been reported in adenocarcinomas of the colon [20]. We analyzed the expression of the UEV3 gene in colon carcinomas and normal colon epithelium. A Northern blot loaded with mRNA samples of several colon carcinoma cell lines was hybridized using the cDNA fragment comprising nucleotides 78–567 as a probe. Two different transcripts, approximately 2.5 and 4.5 kb in length, were detected in all tested cell lines at similar levels (Fig. 4A). Hybridizations were repeated with a highly specific cDNA probe from the 3′ untranslated region of UEV3 and presented the same transcriptional pattern (not shown), indicating that both transcripts are specific for UEV3 and may represent two different 5′ variant transcripts. In addition, RNA samples from colon carcinomas and corresponding normal colon mucosa were tested for UEV3 expression by RT-PCR with oligonucleotide primers in exon 1 and 11 (Fig. 4B). No significant differences in expression were detected. An additional alternatively spliced transcript with exon 3 missing was observed in all tumor and normal tissue samples. Carcinomas of the uterine cervix, matched normal cervical epithelium, and peripheral blood leucocytes were examined by UEV3-specific RT-PCR with primers covering different regions of the cDNA sequence. Expression of UEV3 was

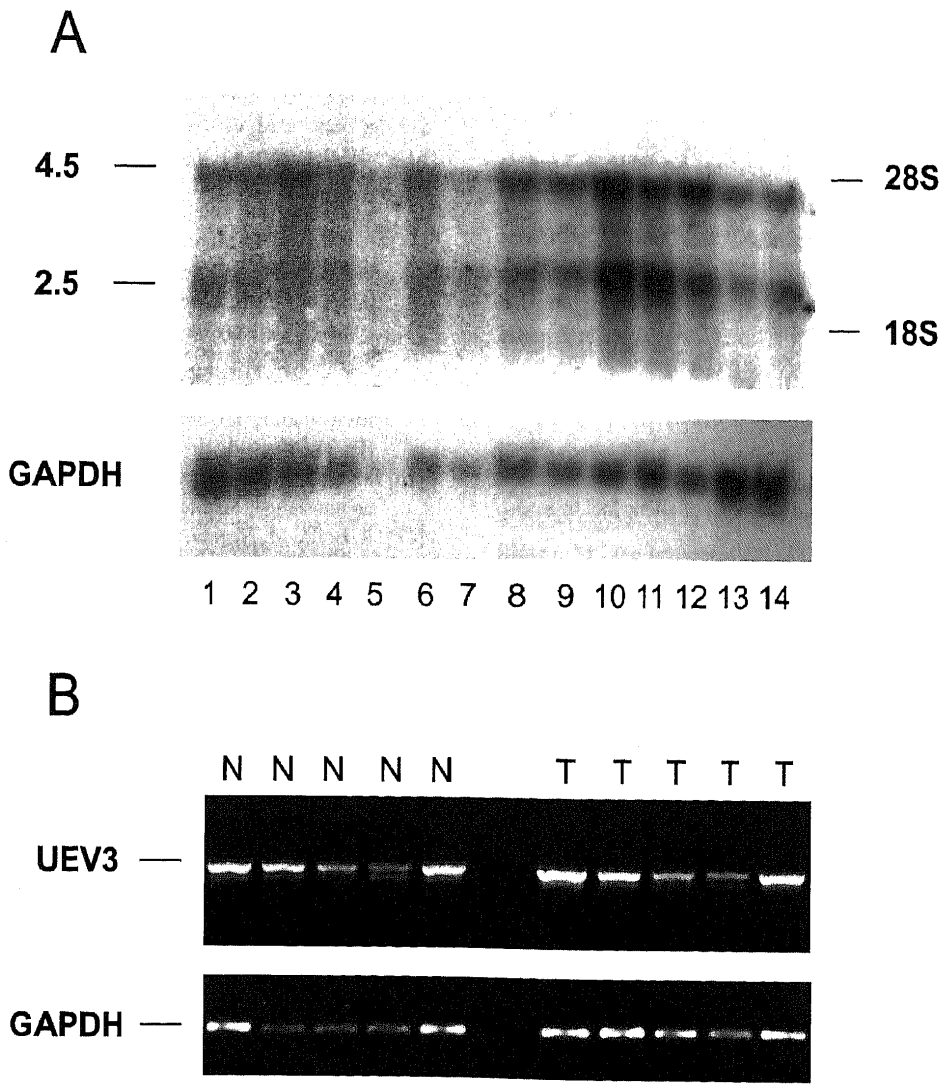


Fig. 4. Expression analysis of UEV3. (A) Two micrograms poly A^+ RNA from various colon carcinoma cell lines was separated by agarose gel electrophoresis and blotted onto a Hybond N+ membrane (Amersham Life Science). The following colon carcinoma cell lines were tested: lane 1, KM12; lane 2, SW707; lane 3, SW480; lane 4, SW948; lane 5, SW48; lane 6, LS174T; lane 7, LS180; lane 8, CX-2; lane 9, CXF 94; lane 10, HT-29; lane 11, Caco-2; lane 12, COLO 320; lane 13, LoVo; lane 14, HCT116. The blot was hybridized with a 491 nt ^{32}P-labeled UEV3 cDNA probe (top panel) or a ^{32}P-labeled GAPDH cDNA probe (bottom panel) using a Random Priming DNA labeling kit (MBI Fermentas, Vilnius, Lithuania). Membrane filters were hybridized and washed as described. (B) UEV3 specific RT-PCR. Normal colon mucosa (N) and colon carcinomas from five patients were tested for UEV3 expression by RT-PCR using primers P1 (exon 1, sense, 5′-CCTGGAGATGGAGTTCGACTG-3′) and P2 (exon 11, antisense, 5′-GGCAAGTACAAGTTCAGGGACC-3′). Reaction mixtures were subjected to 35 cycles using the following parameters: denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 1 min. GAPDH was used as a control (amplified with primers 5′-GACCACAGTCCATGCCATCACT-3′ and 5′-TCCACCACCCTGTTGCTGTAG-3′).

demonstrated in all tested samples at similar levels (data not shown).

In summary, UEV3 cDNA encodes a novel putative member of the human UEV protein family which is characterized by a Cys residue exchange resulting in inactivation of the Ub binding domain. This suggests possible negative regulatory effects on polyubiquitination. However, as supposed for TSG101, UEV3 may still be able to interact with ubiquitin E3 ligase and thus be actively involved in ubiquitination processes due to the conservation of the protein binding motif surrounding the Tyr residue of the Ubc domain. In addition, the highly conserved NADH binding domain within the C-terminal region suggests a possible dehydrogenase activity of UEV3. Further work has to elucidate the enzymatic activities of UEV3 and its functional interaction with the cellular ubiquitination system.

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