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Cloning, characterization and subcellular localization of a gene encoding a human ubiquitin-conjugating enzyme (E2) homologous to the *Arabidopsis thaliana* UBC-16 gene product

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1. ABSTRACT

Ubiquitin charging and activation of class III E2 enzymes has been directly linked to their nuclear import. It has not been published whether other classes E2s also abide by this mechanism. During the large-scale sequencing analysis of a human fetal brain cDNA library, we isolated a cDNA clone that is 2252 base pair in length, encoding a putative 162 amino acid protein, which shares high homology to *Arabidopsis thaliana* ubiquitin-conjugating enzyme 16 (Accession number NP_565110, 51% identity and 71% similarity) at protein level. Bioinformatics analysis revealed that the gene is composed of 7 exons, located on human chromosome 8q13-8q21.1, and that the predicted protein of the gene is a class I E2, for only composed of a conserved ~150-amino acid catalytic core, ubiquitin-conjugating enzyme E2 domain (UBC domain). In the C-terminal of the UBC domain sequence, there are two nuclear localization signals (NLSs). RT-PCR showed that this gene is ubiquitously expressed in 16 kinds of normal human tissues, but expression level is very low, unless in human heart, brain, liver, and pancreas. The subcellular localizations of the new human Ubiquitin conjugating enzyme E2 and its mutation were also examined, which showed that the nuclear localization of hUBC16 depended on two conditions: It has NLS, and at the same time, has enzyme active site, too, at least in HEK293 cells.

2. INTRODUCTION

Ubiquitination, the covalent attachment of the protein ubiquitin (Ub) to other cellular proteins, has been implicated in a number of important physiological processes including selective protein degradation, DNA repair, cell cycle control, organelle biosynthesis (1), ribosome biogenesis, the inflammatory response(2), cellular signaling in response to stress and to extracellular signals, morphogenesis, the secretory pathway (3, 4), neural and muscular degeneration (5), transcriptional regulation and programmed cell death (3). Formation of ubiquitin-protein conjugates is generally through a multi-enzyme cascade: Firstly, ubiquitin is activated by ubiquitin-activating enzyme (UBA or E1) (6), and then transferred to a specific cysteine residue of ubiquitin-conjugating enzyme (UBC or E2). In the final step, the E2 enzyme donates ubiquitin to an ε-amino group of the target protein's lysine residue, either directly or with the assistance of ubiquitin-protein ligase (UBL or E3) (3, 7-10). Although E3 is usually required for substrate specificity (11), some E2s can play a critical role in specifying the function of ubiquitin(12).

Ubiquitination can regulate many nuclear proteins, which, especially, predestined to degradation by the proteasome (8). The nuclear localization of these ubiquitin-targeted proteins implies that enzymes involved in conjugating Ub may need to be imported into the nucleus

from cytoplasm. Several examples have been described in which the nuclear localization of E3 ligases are regulated to control ubiquitination (13, 14). For example, MDM2, an E3-type Ub-ligase that ubiquitylates the tumor suppressor protein, p53, shuttles in and out of the nucleus (15). In contrast to the wealth of information concerning how interactions between E3 ligases and their substrates are regulated, much less was known about potential regulatory mechanisms involving E2 conjugating enzymes previously. At present, Plaflker *et al.* reveal a new mechanism through which the subcellular localization of the class-III E2s is in fact regulated and linked to their catalytic activity (16), in other words that the nuclear import of these E2 enzymes depends on the possession of the wild type cysteine, the active site of these enzymes.

The E2s exist as a family of proteins which differ with respect to size, structure and function (17), and are present in the eukaryotic genome as a multigene family. All E2s known so far contain a conserved domain of about 16kDa, called ubiquitin conjugating enzyme E2 domain (UBC domain), which is a conserved ~150-amino acid catalytic core (18). The UBC domain includes a highly conserved cysteine residue which functions as an active site for the thiol ester formation with ubiquitin (19). E2s can be further subclassified into four groups. Some E2s, containing only this core region, belong to class □ enzymes, which may need E3 for substrate recognition. While other E2s, with C-terminal (class □) or N-terminal (class □) or with C- and N-terminal (class □) extensions have been suggested to be involved in substrate recognition (16).

During our large-scale sequencing analysis of human cDNA libraries, we cloned a full-length cDNA whose nucleotide sequence has been submitted to the Genbank/EMBL Database with accession number AY948289. The cDNA encodes a putative protein of 162 amino acid residues, which shares 71% homology with *Arabidopsis thaliana* UBC-16. We termed this gene hUBC-16 (human Ubiquitin conjugating enzyme 16), whose putative protein is a new member of the class □-E2s. In order to explore the subcellular localization of hUBC16 in living cells, defferent hUBC16-green fluorescence protein (GFP) constructs were developed and used for transient transfection of HEK293 cells.

3. MATERIALS AND METHODS

3.1. cDNA library construction

A high-quality cDNA library was constructed in a modified pBluescript II SK (+) vector with the human fetal brain mRNA purchased from Clontech. The modified vector was constructed by introducing 2 Sfi I recognition sites, i.e. Sfi I A (5'-GGCCATTATGGCC-3') and Sfi I B (5'-GGCCGCCTCGGCC-3'), between the EcoR I and Not I sites of pBluescript II SK (+) (Stratagene, La Jolla, CA, USA). Double-stranded cDNAs were synthesized using SMART™ cDNA Library Construction Kit (Clontech, Palo Alto, CA, USA) following manufacturer's instructions.

3.2. Template preparation and sequencing

The 96-well REAL plasmid kit(QIAGEN, Chatsworth CA) was used to prepare double-stranded plasmids. The cDNA inserts were sequenced on ABI PRISM™ 377 DNA sequencer (Perkin-Elmer, San Francisco, CA, USA) using the BigDye Terminator Cycle Sequencing Kit and BigDye Primer Cycle Sequencing Kit (Perkin-Elmer) with -21M13 primer and M13Rev primer. Synthetic internal walking-primers were designed according to the obtained cDNA sequence fragments. Each part of the insert was sequenced at least three times bi-directionally. Subsequent editing and assembly of all the sequences from one clone was performed using Acembly (Sanger's Center).

3.3. Bioinformatics analysis

DNA and protein sequence homology searches and comparisons were carried out using BLAST-N and BLAST-X algorithms on the National Center for Biotechnology Information Web server (http://www.ncbi.nlm.nih.gov/BLAST). ScanProsite was done at the The ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) (http://us.expasy.org/cgi-bin/scanprosite). Protein alignment was performed by GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml). The prediction of nuclear localization signals (NLSs) was performed with Prediction of Protein Sorting Signals II (PSORTII) (http://psort.nibb.ac.jp/form2.html).

3.4. Assessment of hUBC16 mRNA tissue distribution by RT-PCR

Adult multiple tissue cDNA (MTC) panels (Clontech) were used as PCR template according to the manufacturer's protocol. Each PCR primer-set of the hUBC16 and GAPDH gene was shown as follows, hUBC16: sense primer (5'- gaaggagggtggaagtatggtttc-3' corresponding to nucleotides 47bp-70nt). antisense primer (5'- caaaagcttaaaaaaataaaaataaaaaatgg -3' corresponding to nucleotides 763bp-794nt). PCR product size is 748bp. The expression of GAPDH was analyzed as a control: sense primer (5'-tgaaggtcggagtcaacggatttggt-3'). antisense primer (5'-catgtgggccatgaggtccaccac-3'). Twenty-four cycles (for GAPDH) and thirty-six cycles (for hUBC16) of the amplification (30s at 94ºC, 30s at 65ºC, 1 min at 72ºC) were performed using ELONGASE DNA polymerase (Gibco Brl, Gaithersburg, MD, USA). The PCR products were then resolved on 1.5% Metaphor agarose gel (FMC, Philadelphia, PA, USA).

3.5. Strains, media, plasmids and cell line

E.coli strain DH5a was grown in Luria Broth medium in the presence of antibiotics as required for plasmid. Plasmids, pEGFP-C1 was purchased from Clontech. HEK293 cell line (ATCC number is CRL-1573) was purchased from Chinese Academic of Science, cultured and stored in our lab. Media and reagents for cell culture were purchased from Invitrogen.

3.6. Plasmid construction

The full length open reading frame (ORF) of the cDNA encoding hUBC16 was obtained by PCR amplification from the recombinant clone constructed by our lab and was cloned into pEGFP-C1 (Clontech), which is w-hUBC16-GFP. Deletion mutant hUBC16-GFP/-NLSs

Table 1. Primers used for GFP-fussion proteins

Primers	Sequence
GFP XhoI sense w-hUBC16	5' ccctcgagctatggcgtcaatgcagaccacag 3′
GFP EcoRI antisence w-hUBC16	5′ ggaattcgatcaacaagtatcatcatgataccac 3′
GFP XhoI sense hUBC16-NLSs	5' ccctcgagctatggcgtcaatgcagaccacag 3′
GFP BamHI antisence hUBC16-NLSs	5′ cgggatccttccttgcagctggaaagcatgcta 3′
GFP internal antisense C102A-hUBC16	5′ gaatggataa***agc***gatatgaccattgctata 3′
GFP internal sense C102A-hUBC16	5′ atggtcatatc***gct***ttatccattctaacaga 3′

Restriction enzyme recognition sites were underlined, and C102A codon was shown as bold and italics

was constructed by excluding the two NLSs in C-termal of hUBC16. The hUBC16 cDNA was altered by overlap-extension PCR, using appropriate mutator oligonucleotides (listed below), so as to introduce amino acid substitutions at C102 (C102A, pEGFP-C1), which is C102A-hUBC16-GFP. The primers and restriction enzymes of all expression constructs are listed in Table 1.

3.7. Cell culture and liposome-mediated transfection

HEK293 cells were grown in DMEM (Dulbecco's modified Eagle's medium) containing 10% FBS (Fetal bull serum) and maintained at 37^{o}C in 5% CO_2 atmosphere. On the day before transfection, cells were plated on the coverslips in the 6-well tissue culture plates. Cells were transfected at the 60%-80% confluence with the indicated expression vectors, using Lipofectamine 2000 (Invitrogen) according to the instructions supplied by the manufacturer. 32h~48h after transfection, cells were washed with 1× PBS three times, fixed with 4% paraformaldehydeand 15 min, washed with 1× PBS three times, counterstained with DAPI (4',6'-diamidino-2-phenylindole) 10 min, washed with ddH_2O two times. Then the samples were viewed using R2100 Confocal Microscope (BioRad) with appropriate filters and the photos were captured with software Leica DC viewer. The overlays were processed with software Photoshop 6.0.

3.8. Subcellular localization of hUBC16 and its mutants in HEK293 cells

2 μg plasmid construction of pEGFP-C1, w-hUBC16-GFP, hUBC16-GFP/-NLSs, or C102A-hUBC16-GFP was mixed in 250μl DMEM (no serum) respectively, and 5 μl Lipofectamine 2000 was also mixed gently in 250μl DMEM (no serum); after 5 min, they were mixed together for 20min. Then these mixtures were transfected individually into HEK293 cells cultured on the coverslips in 6-well plates. All cell culture, transfection and cells processing procedures were mentioned above.

4. RESULTS

4.1. Cloning and sequence analysis of hUBC16

By means of large-scale sequencing analysis of human cDNA libraries, we cloned a novel cDNA encoding the human Ubiquitin-conjugating enzyme 16. The nucleotide sequence has been submitted to the GenBank/EMBL database with accession number AY948289. The 2252bp cDNA spans an open reading frame (ORF) from nucleotide 25 to 513, encoding a protein of 162 amino acids. The cDNA might be full length for a cozak sequence (ATCATGG) near the start codon and two polyA signals (AATAAA) after the ORF (Figure 1A). The molecular mass of the putative protein human UBC16 is 17.6kD and the isoelectric point is 8.07. Bioinformatics analysis using BLASTx revealed that hUBC16 shared high homology with *Arabidopsis thaliana* ubiquitin-conjugating enzyme 16 (aUBC16, Accession number NP_565110, 51% identity and 71% similarity) (Figure 1B). For the distant sources of hUBC16 and aUBC16, high homology implies that the function of hUBC16 is very conserved and impartant. Based on NCBI conserved domain search (CD-search) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), we found a conserved ~150-amino acid catalytic core, UBC domain (17-159 amino acid residues of hUBC16) which is 97.9% similarity with consensus sequence of classical UBC domain of NCBI conserved domain database, and which suggests that hUBC16 is a class I E2.

4.2. Prediction of the enzyme active site cysteine and the two NLSs

ScanProsite was done at the The ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB)(http://us.expasy.org/cgi-bin/scanprosite) which showed that the enzyme active site cysteine residue was at the 102 amino acid of putative protein hUBC16, which was consistent with the result of alignment between the new member of class I E2 with other class I E2s (human E2-A, E2-B, E2-C and E2-D; accession number NP_003327, P63146, NP_861515 and NP_003329). Though the similarity of them is low, the sequences, nearby the active site cysteine, share very high homology with each other (Figure 2A). Utilizing PSORTII sequence analyses, we determined two putative nuclear localization signals (^{133}KRRP^{136} and ^{150}PKKTKWW^{156}) within the C-terminal of UBC domain of hUBC16 (Figure 2B).

4.3. Tissue-specific distribution of hUBC16 gene

The tissue distribution of hUBC16 gene transcript was determined by cycle-limited reverse transcription polynerase chain reaction (RT-PCR). The expected length of PCR product was 748 bp. Template cDNAs from 16 human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte) were purchased from Clotech. As shown in Figure 3, expression of hUBC16 was detected, ubiquitoursly, but in human lung, skeletal muscle, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, the expression level was much lower.

4.4. Gene structure and chromosome location of hUBC16 gene

By using the international human genome

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gggtcttgggggggatggttccatcatggcgtcaatgcagaccacaggaaggagggtggaagtatggtttccaaaacgactacag      84
                        M  A  S  M  Q  T  T  G  R  R  V  E  V  W  F  P  K  R  L  Q       20
aaagaactgttggctttgcaaaatgacccacctcctggaatgaccttaaatgagaagagtgttcaaaattcaatttacacagtgg     168
 K  E  L  L  A  L  Q  N  D  P  P  P  G  M  T  L  N  E  K  S  V  Q  N  S  I  T  Q  W       48
attgtagacatggaaggtgcaccaggtaccttatatgaaggggaaaaatttcaacttctatttaaatttagtagtcgatatcct     252
 I  V  D  M  E  G  A  P  G  T  L  Y  E  G  E  K  F  Q  L  L  F  K  F  S  S  R  Y  P       76
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 F  D  S  P  Q  V  M  F  T  G  E  N  I  P  V  H  P  H  V  Y  S  N  G  H  I  C  L  S       104
attctaacagaagactggtccccagcgctctcagtccaatcagtttgtcttagcattattagcatgctttccagctgcaaggaa     420
 I  L  T  E  D  W  S  P  A  L  S  V  Q  S  V  C  L  S  I  I  S  M  L  S  S  C  K  E       132
aagagacgaccaccggataattcttttttatgtgcgaacatgtaacaagaatccaaagaaaacaaaatggtggtatcatgatgat     504
 K  R  R  P  P  D  N  S  F  Y  V  R  T  C  N  K  N  P  K  K  T  K  W  W  Y  H  D  D       160
acttgttgatgccactgctatcatcctcctagcagaagatagtcctactgagaaaatgagcactttgatcattcagtctttgaa     588
 T  C  *                                                                                  162
ctttaacctttgactggaagtgacctataggcaatgaagactacttccttttactgcatttttactcgtgtgcattctgggcgc     672
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ataatattttaaaattgtttttaatctgtatagatgacattttgtagctttgtacatgttgttaattaagggcatataatttta    2184
cactcaaagtataattgctgaactcaggggtgggtagacttcaaaaatatgtctgctatagaaataac                    2252
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A

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hUBC16    -MASMQTTGRRVEVWFPKRLQKELLALQNDPPPGMTLNEKSVQNSITQWIVDMEGAPGTL     59
aUBC16    MSSSGAPSRKTLSKIATNRLQKELVEWQMNPPTGFKHK---VTDNLQRWIIEVIGAPGTL     57

hUBC16    YEGEKFQLLFKFSSRYPFDSPQVMFTGENIPVHPHVYSNGHICLSILTEDWSPALSVQSV     119
aUBC16    YANDTYQLQVDFPEHYPMESPQVIFLHP-APLHPHIYSNGHICLDILYDSWSPAMTVSSI     116

hUBC16    CLSIISMLSSCKEKRRPPDNSFYVRTCN--KNPKKTKWWYHDDTC                   162
aUBC16    CISILSMLSSSTEKQRPTDNDRYVKNCKNGRSPKETRWWFHDDKV                   161
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B

Figure 1. A. Nucleotide and deduced amino acid sequences of hUBC16 gene (GenBank accession number AY948289) . The nucleotide sequence of the 2252bp cDNA was shown in the top lines, and its predicted amino acid sequence was shown below in single-letter code. Numbers on the right referred to the nucleotide and the acid amino in each corresponding line. The ORF extended from nucleotide 25 to 513 and encoded a protein of 162 amino acids. The start codon bordering the ORF is marked in boldface, and the cozak sequence is boxed. Two nuclear localization signals (NLSs) are underlined. The UBC domain is dark shading highlighted. An asterisk indicates the terminator in the protein sequence. Two PolyA signals are marked in italic type. B. Alignment of human UBC16 protein to its homologous proteins.hUBC16 [*Homo sapiens*](accession number AY948289) and aUBC16 [*Arabidopsis thaliana*] (accession number NP_565110). The alignment was performed by GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml): Black (100%similarity); grey (80-90%similarity); light grey (60-70%similarity).

database on NCBI, we compared hUBC16 with human genomic sequence data. hUBC16 gene was mapped to contig NT_008183.18, which was localized on chromosome 8q13-8q21.1. It spans more than 8.6 kb of the genome squence and consisted of 7 exons and 6 introns (Figure 4). All sequences at the exon-intron junctions are

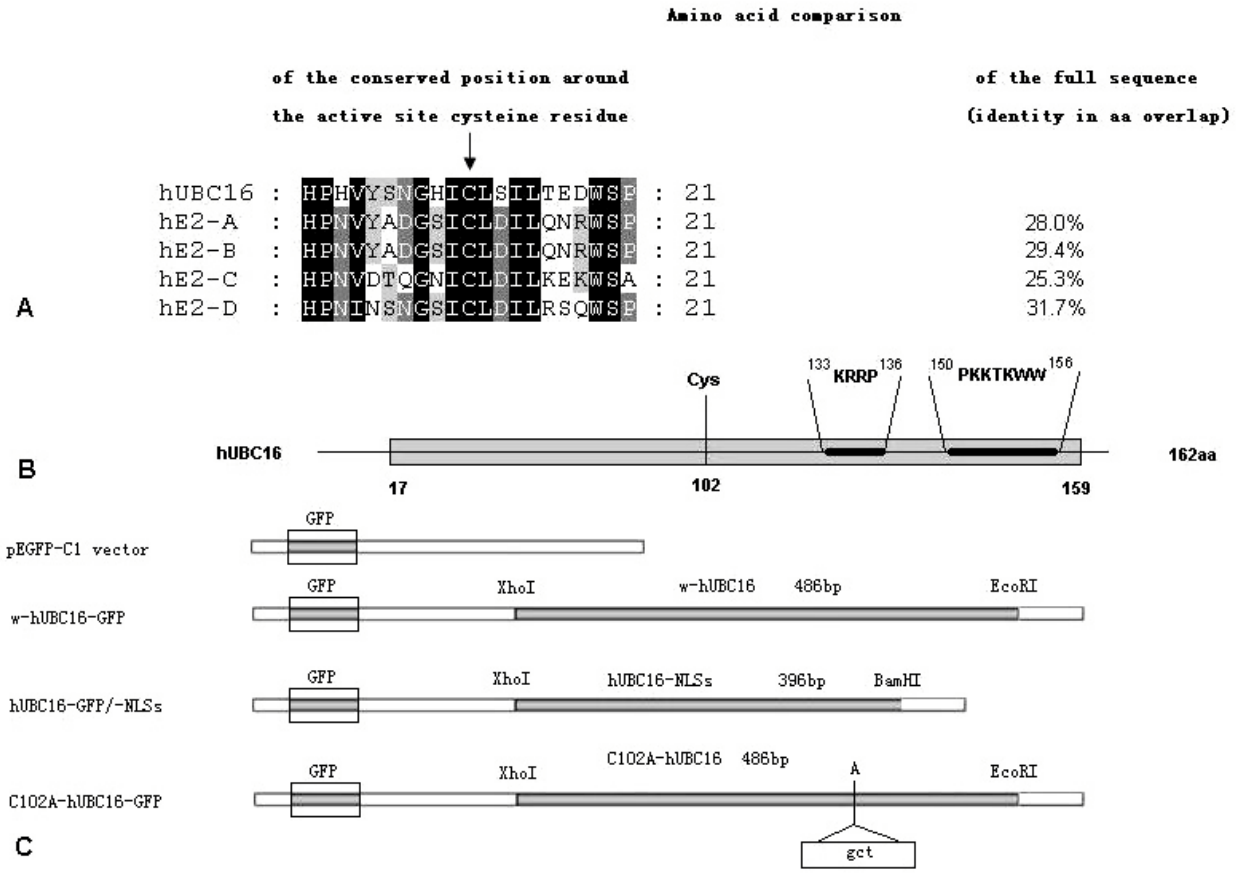

Figure 2. A. Amino acid comparison of the conserved position around the active site cysteine residue and of the full sequence. The arrow points to the active site cysteine residue of amino acid sequence of UBC domain. B.The protein structures of hUBC16. The numbers indicate the locations of the conserved motifs in the protein. Light grey box denotes the UBC domain; Black lines show the NLSs. C. Schematic diagram of the plasmid construction for hUBC16-GFP fusion proteins. Primers, which were listed in Table 1, containing EcoRI, BamHI, XhoI restriction enzymes were used to isolate the respective DNA fragments and to clone them into the multiple cloning site of pEGFP-C1 vector.

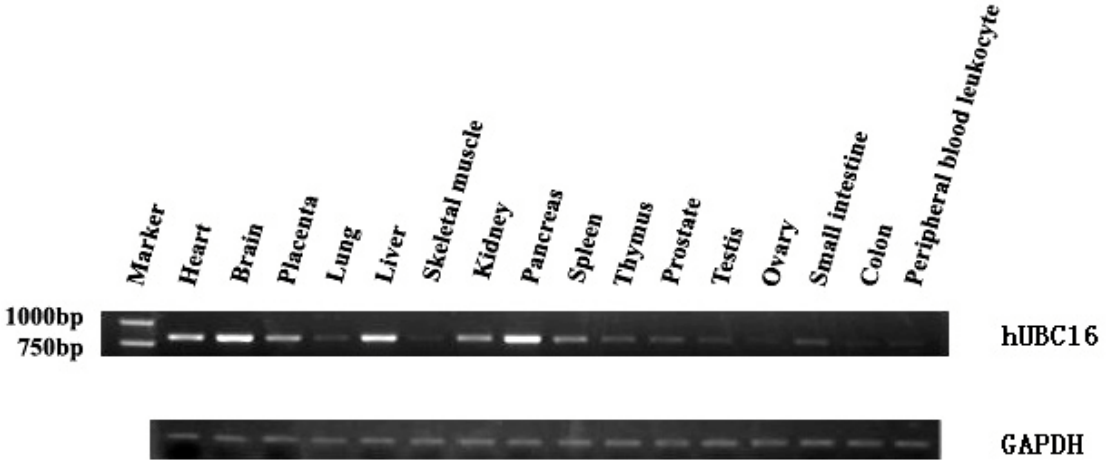

Figure 3. Expression of hUBC16 gene in 16 adult human tissues. RT-PCR analysis of human cDNA for hUBC16 and GAPDH (as a control). Prenormalized cDNAs from sixteen human adult tissues were purchased from CLONTECH and employed as templates in PCR reactions containing human hUBC16 and GAPDH-specific primer as described in Materials and Methods.

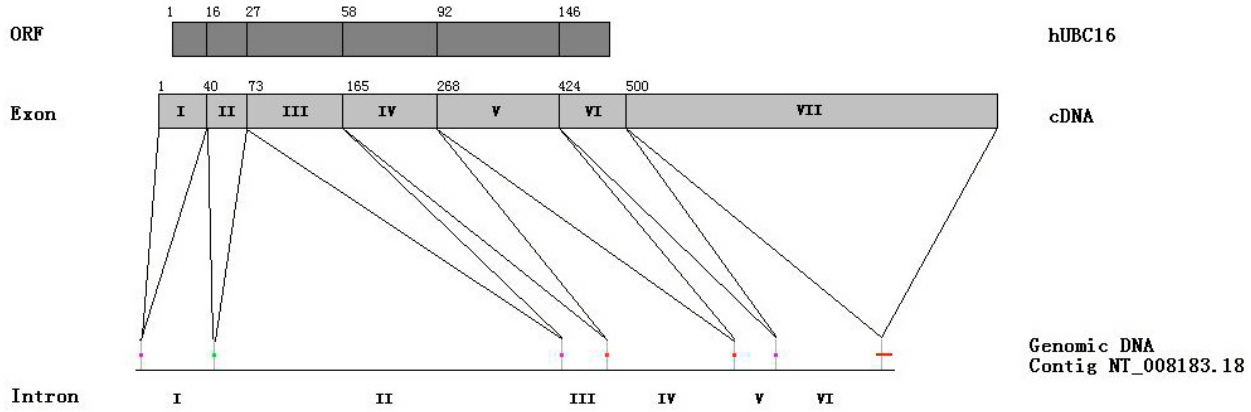

Figure 4. Genomic structure of hUBC16 gene.

Table 2. Nucleotide sequence of exon-intron junctions of the hUBC16 gene

3' Splice acceptor	Exon	Size (bp)	5' Splice donor	Intron	Size(bp)
cDNA end GGGTCTTGGGGG	1	39	GCGTCAATGCAG***gt***gagaggagtt	1	8506
ttgtattttc***ag***ACCACAGGAAGG	2	33	GTATGGTTTCCA***gt***aagtgtgtgt	2	39796
gttcttttct***ag***AAACGACTACAG	3	92	TTCAATTACACA***gt***aagtatttaa	3	5131
tttctattct***ag***GTGGATTGTAGA	4	103	GACTCTCCTCAG***gt***aattgccttg	4	14517
attctctctt***ag***GTCATGTTTACT	5	156	TGCAAGGAAAAG***gt***aagacttttc	5	4747
tttttgtttc***ag***AGACGACCACCG	6	76	GGTGGTATCATG***gt***aagcagtttg	6	11535
cttttcttaa***ag***ATGATACTTGTT	7	1753	TATAGAAATAAC		

The intron sequence is shown in lowercase letters and the exon sequence is shown in uppercase letters.

consistent with the AG-GT rule (Table 2).

4.5. Subcellular localization of hUBC16 and its mutants

To determine the elements of hUBC16 that are responsible for the differential subcellular localization of the protein in 293 cells, expression vectors encoding GFP-tagged hUBC16 were constructed (Figure 2C). The w-hUBC16-GFP is found predominantly in the nucleus (Figure 5d), whereas hUBC16-GFP/-NLSs and C102A-hUBC16-GFP are distributed in cytoplasm and nucleus(Figure 5g and 5j), same as pEGFP-C1 vetor (Figure 5a).

5. DISCUSSION

Post-translational modifications which include ubiquitination, acetylation, phosphorylation, methylation, ADP-ribosylation and glycosylation (5,20-22). Protein phosphorylation is a time-honored mechanism of signal transduction, while recently, other protein modifications, including ubiquitination, glycosylation, methylation and acetylation have also come to be recognized as biologically relevant signals. Many advances in ubiquitin-mediated signaling show that besides signaling proteolysis, ubiquitination can be a signal for trafficking, kinase activation, and other nonproteolytic fates (23). Ubiquitination is exquisitely regulated through a series of enzymatic steps, which is a proceeding including three enzymes: E1, E2, and E3.

There are two kinds of ubiquitin modifications to substrates: polyubiquitin and monoubiquitin. A polyubiquitin chain is formed when ubiquitin is attached to a lysine within ubiquitin itself, and this process is repeated. Monoubiquitin, the attachment of a single ubiquitin polypeptide to a substrate, serves as an important regulatory modification, which acts as a sorting signal throughout the endocytic pathway and regulates diverse proteins including histones, endocytic machinery, and transcription factors (11, 24). E1-Ub and E2-Ub, intermediate products in the proceeding of formation of ubiquitin-protein, could be also regard as monoubiquitin modification, which was also described as a signal for intranuclear trafficking (27). It was well known that Interactions between E3 ligases and substrates are regulated by a multiplicity of mechanisms that include post-translational modifications, interactions with regulatory factors and control of substrate or E3 localization (25,26), based on most attention has been given to the last step—regulation of substrate recognition by the various E3 ligases, while much less is known about potential regulatory mechanisms involving E2 conjugating enzymes. Recently, it was reported that the activesite cysteine residue of UbcM2 was required for its interaction with importin-11, a member of the karyopherin family of nuclear transport receptors, and that the nuclear import of UbcM2 (a class-III E2 enzyme) is linked to their ubiquitin charging and activation in the cytoplasm, which is the first example indicating that similar regulation can occur at the level of E2 enzymes (16, 28).

Nuclear transport receptors can recognize specific nuclear localization signals (NLSs) in proteins and facilitate their transport between the cytoplasm and the nucleus by mediating translocation through nuclear pore complexes (NPCs). The recognition of NLSs by karyopherins family of nuclear transport receptors is often regulated, and the selective trafficking of proteins between

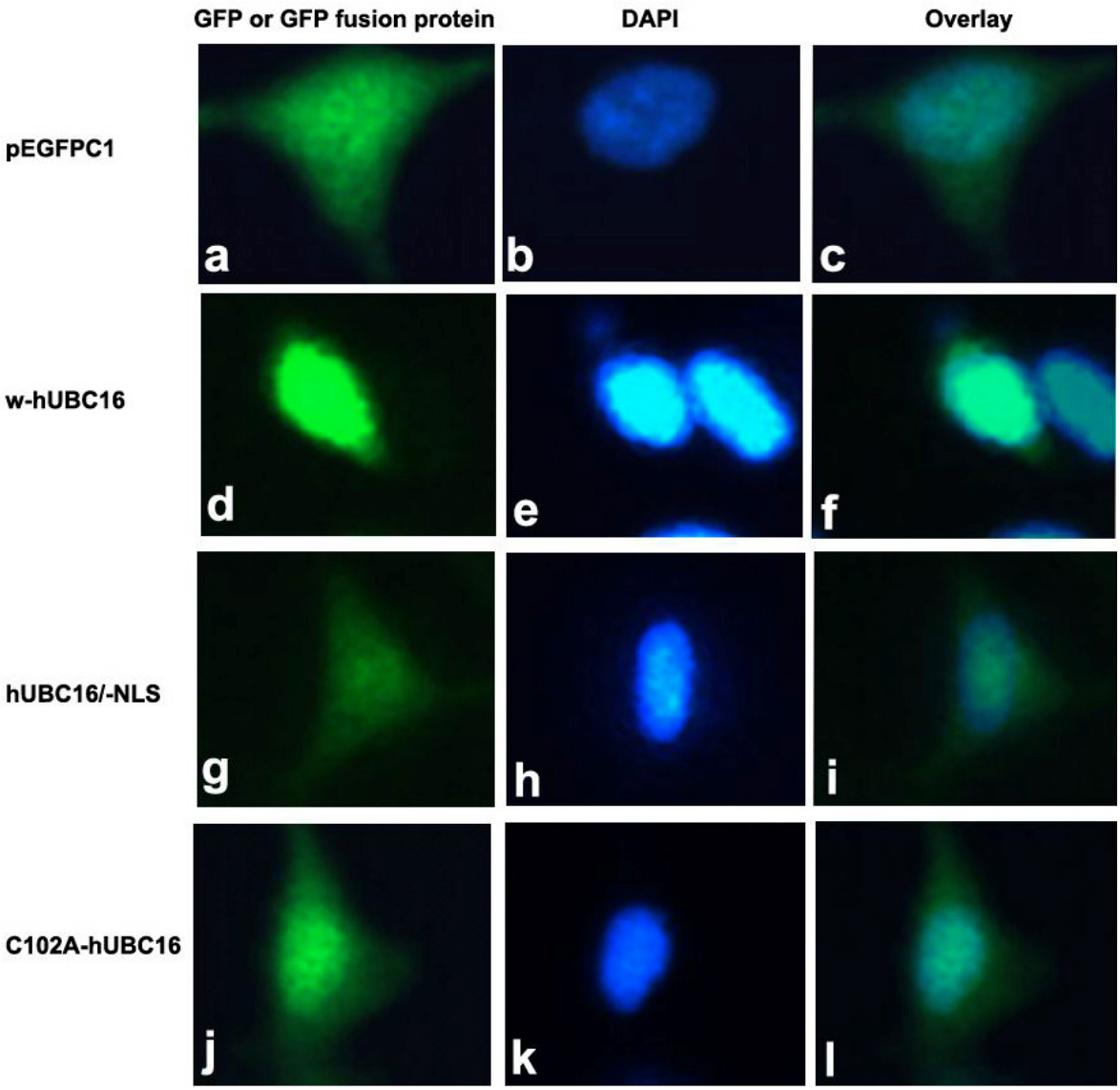

Figure 5. Subcellular localization of hUBC16 and its mutants. The fluorescent signals of (a), (d), (g) and (j) were analyzed by fluorescence microscopy. (b), (e), (h) and (k) were stain by DAPI. The overlay images, (c), (f), (i) and (l) were produced by merging the two kinds of signals from GFP fusion proteins and nucleuses stained by DAPI.

the cytoplasm and the nucleus can therefore function as an important mechanism for controlling protein localization and function (25, 29).

We cloned a novel gene hUBC16, which is a class I E2. Wild type of hUBC16 is found in nucleus, whereas one mutant, the activesite cysteine replaced by alanine, is distributed in cytoplasm and nucleus, which is same as the distribution of the other mutant, the two NLSs deletion hUBC16. The results show that the distribution of hUBC16 does not follow the mechanism about the nuclear localization of class III E2. Although the class III E2 enzymes are theoretically small enough to freely diffuse between the nucleus and cytoplasm, previous studies indicate that their nuclear import depends on a specific transport receptor (28), which can load them as cargo when the class III E2 charged by ubiquitin. We do not know if the nuclear localization of hUBC16 is concerned with nuclear transport receptors, however from Figure 5, we can see that the nuclear localization of the new member of the class I E2 (hUBC16) dependeds on hUBC16 containing NLS and the enzyme active site cysteine, at least in HEK293 cells. It is far away from opening the mechanism regulating the subcellular localization of the class I E2, and it needs further research to do.

6. ACKNOWLEDGMENT

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Abbreviations: E2/Ubc, ubiquitin-conjugating enzyme; E3/Ubr, ubiquitin-protein ligase; E1/Uba, ubiquitin activating enzyme; GFP, green flaorescent protein; DAPI, 4',6'-diamidino-2'-phenylindole; Ub, ubiquitin; NLS, Nuclear Localization Signal

Footnote: The nucleotide sequence reported in this paper has been submitted to GenBank under accession number: AY948289

Key Words Ubiquitin Conjugating Enzyme (UBC or E2), class I, Subcellular Localization, Nuclear Localization Signal, NLS, Reverse transcription, Polymerase chain reaction, RT-PCR, Chromosome, 8q13, 8q21.1

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