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Identification and Characteristics of a Novel E1 Like Gene *nUBE1L* in Human Testis

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Abstract A gene, presumably involved in spermatogenesis, was identified and characterized by using cDNA microarray. Hybridization intensity was 2.13 fold higher in adult testis than that in fetal testis. The full length of this gene was 4288 bp and it encoded a 578 amino acid protein. Conserved structure and amino acid sequence analysis revealed that the protein contained 1 Thif-domain, 2 UBACT-domains, and a functional active site cysteine lay upstream of UBACT domain, all of them also existed in ubiquitin-activating enzyme E1 and E1 like proteins. So we named this gene as a novel ubiquitin-activating enzyme E1 like gene (*nUBE1L*). Expression profile showed that nUBE1L was predominantly expressed in testis. Comparison of the expression of nUBE1L in different developmental stages of testis indicated that it was highly expressed in adult testis. In conclusion, *nUBE1L* is a novel human E1 like gene highly expressed in adult testis, which plays key role in ubiquitin system, and accordingly influences spermatogenesis and male fertility.

Key words ubiquitin-activating enzyme E1; ubiquitin; spermatogenesis

The selective degradation of many short-lived or abnormal proteins in eukaryotic cells is carried out by the ubiquitin system. In this pathway, proteins are targeted for degradation by covalent ligation to ubiquitin, a highly conserved 76-residue protein that is apparently abundant in all eukaryotic cells [1]. Ubiquitin-mediated degradation of regulatory proteins plays important roles in the control of numerous processes, and ubiquitination is likely one of the most versatile cellular regulatory mechanisms controlling physiological and pathological events [1–4]. In the past few years, we have witnessed the potential role of ubiquitin system in the male reproductive function. A variety of elements involved in the ubiquitin-dependent proteolysis system have been detected in the testis, epididymis and seminal plasma [5,6]. The activity of the ubiquitin system is relatively high during spermatogenesis [2,7].

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Covalent ligation of ubiquitin-protein requires the sequential action of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin carrier proteins (E2) and ubiquitin-protein ligases (E3). The initial reaction in this pathway involves the activation of ubiquitin by E1. E1 catalyzes the formation of a thiol ester bond between the C-terminal glycine of ubiquitin and a cysteine residue of E1. Then activated ubiquitin moiety is transferred to E2. E2 ligates ubiquitin directly to substrate proteins with or without the assistance of E3 [1]. As the first enzyme in the pathway, E1 has the potential to regulate the rate of ubiquitin conjugation, thus controls overall ubiquitin function [8].

Distinct El or E1 like genes have been isolated from mammals (including human, mouse, rabbit), yeast and plants [9]. Analysis of the predicated amino acid sequences of these genes shows that they all contain one or two Thif-domains and two UBACT-domains. Besides, a conserved cysteine lies upstream of the UBACT-domain, which probably involves the formation of thiol ester bond between ubiquitin and E1 [9]. Multiple forms of both the E1 protein and the E1 gene have been detected in plants and animals. Different El isozymes may have distinct

functions or patterns of expression [9,10].

On the basis of the adult testis cDNA microarray prepared in our laboratory, we compared the expression of genes in the fetal and adult human testis at a high throughput. A highly expressed novel human E1 like gene was found in adult testes. In this study, the characteristics and tissue distribution of this novel E1 like gene, its expression in different developmental stages of testis and its possible correlation with spermatogenesis are discussed.

Materials and Methods

Samples

Human adult testes from cadavers and fetal testes from accidentally aborted fetuses (Ca. six-month) were obtained after ethics approval and consent.

Preparation of human testis cDNA microarray

9216 positive phage clones were picked out randomly from Human Testis Insert λ Phage cDNA Library (Clontech, Hl5503U) and amplified by PCR. Then the PCR products were spotted on the membrane to make human testis cDNA microarray. The detailed methods were identical with those previously described [11].

Screening of genes differentially expressed in the fetal and adult testis

The human testis cDNA microarray was hybridized with the ^{33}P-labeled fetal testis and adult testis cDNA probes respectively. The hybridization intensity of corresponding dots in adult and fetus were compared. If the difference of spot intensity in adult and fetus was more than three fold, higher or lower, this clone was considered differentially expressed.

All differentially expressed cDNA plasmids were amplified, extracted and purified in mini-preps (QIAprep Spin Miniprep kit, Qiagen). The full insert lengths were sequenced with an ABI auto-sequencer (model No. 377) at Huada Gene Center (Beijing, China). The sequences were then blasted in GenBank (http://www.ncbi.nlm.nih.gov) by using the software Blast to determine the homology among various species and locations in chromosomes. The nucleic and deduced amino acid sequences were also analyzed by using Gene Runner and SMART (http://smart.embl-heidelberg.de/) software [12].

Tissue distribution of *nUBE1L* gene

After sequence identification and analysis, a gene highly expressed in adult testis, named *nUBE1L*, was identified. The expression profile of this gene was determined by using PCR screening. Multiple tissue cDNA panels were from commercial human Multiple tissue cDNA (MTC) k panel I and II kit (Cat#K1420-1 and K1421-1, Clontech), which includes sixteen kinds of human tissues (testis, skeletal muscle, liver, pancreas, brain, lung, kidney, heart, placenta, spleen, thymus, prostate, ovary, small intestine, colon, blood leukocytes). The forward primer is 5′-TTCTCACATTTAGTCATTGG-3′ (397–416 nt) and the reverse primer is 5′-TTCCTTTCTCTTTGCTTG-3′ (830–847 nt). The PCR product size was 451 bp. G3PDH was used as positive control of the cDNA templates. The reagents in 20 μl PCR reaction mixture were as follows: 2 μl 10×PCR buffer, 1.5 μl 25 mM Mg^{2+}, 0.15 μl 20 mM dNTPs, 0.15 μl *Taq* DNA polymerase (5 U/μl), 12.2 μl distilled water, 1 μl of each primer (5 pmol/μl), and 2 μl cDNA sample. PCR was performed with an initial denaturation temperature at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 1 min, and an additional extension at 72 °C for 7 min. The PCR products were analyzed by electrophoresis.

Expression of *nUBE1L* gene in different developmental stages of male testis

To compare the differential expression of *nUBE1L* gene in different development stages of testis, RT-PCR was used with corresponding primer synchronously. cDNA of male testes include adult (the prime of life) testis cDNA (n = 3) and fetal (6 month) testes cDNA (n = 3). The cDNAs were amplified with the sequence specific primers as described above and PCR products were resolved by electrophoresis. β-actin mRNA was also amplified as positive control.

Results

Hybridization with cDNA microarray identified *nUBE1L* gene

After hybridization and data analysis, genes differentially expressed in human adult and fetal testes were considered as testis development and/or spermatogenesis-related. A clone, named *nUBE1L*, was identified. This gene expressed highly in adult testis but lowly in fetal testes. The hybridization signal intensities in adult testes and fetal testes were 204.50 and 65.35 respectively. Fig. 1 showed

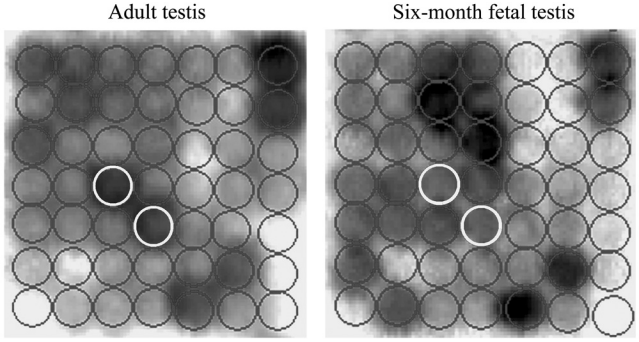

Fig. 1 Partial cDNA hybridization images showing differential expression of the *nUBE1L* gene in the adult and fetal testes

White rings indicate its cDNA, and the hybridization intensity in adult testis and fetal testis was 204.50 and 65.35 respectively. Hybridization intensity was 2.13 fold higher in adult than that was in fetal testis.

that the intensity of the gene expression in adult testis was approximately 2.13-fold higher than that in fetal testis.

The full-length cDNA of *nUBE1L* gene is 4288 bp and it contains an open reading frame (796–2532 nt) that encodes a protein with 578 amino acids. The methionine at 796–798 nt was the initiation site because there was an upstream stop code TAG at 475–477 nt (Fig. 2). The cDNA sequence of this clone was deposited with GenBank. The accession number was AY359880.

Blast search in the human genome database showed that the *nUBE1L* gene located in the human chromosome 4 (NT_022778.13|Hs4_22934). It is spliced by 20 exons and 19 introns, encompassing 32,408 bp genomic DNA in NT_022778.13. Blast search of the contig map showed that all exons were located within chromosome 4q13.2,

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ATTAATTGCAGTTTTTATTTACAACTTTCAACACAAACTGATAGCTTATCATTTTTAAGA    60
TCATTTTATTTTCCTTTATCATAGTTCAGGGTTTTTTCATAGTAGTTTTCATTAACAAAC   120
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TTCTTTGACAAAAACTATAAATGGTAGATATTCGAGTTAATTATAAATGATGCCCTAGAG   240
TGACATATAGAATCCAAAGACAAAAATGGGCAGATACTAGTAAAGGGAGACACTACTTAA   300
GGTAACACGCAAATGGGTCTGTTATCATGAAATTGGGACATTTTTAGAGATATTTAGTAG   360
GGGAACTGAATAGCTTTTTTAGAAGTTAAGTTCTAATTCTCACATTTAGTCATTGGAATT   420
TTAAGTGTACTGTGTAAATGCTGTTAATGTTATTGTCTTTTATGGCTTTTGTTTTAGCCT   480
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GCCATAGGCTGTGAA*ATG*TTGAAAAATTTTGCTTTACTTGGTGTTGGCACAAGCAAAGAG   840
                M  L  K  N  F  A  L  L  G  V  G  T  S  K  E
AAAGGAATGATTACAGATCCTGACTTGATAGAGAAATCCAACTTAAATAGACAG          900
  K  G  M  I  T  V  T  D  P  D  L  I  E  K  S  N  L  N  R  Q
TTCCTATTTCGTCCTCATCACATACAGAAACCTAAAAGCTACACTGCTGCTGATGCTACT    960
  F  L  F  R  P  H  H  I  Q  K  P  K  S  Y  T  A  A  D  A  T
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  L  K  I  N  S  Q  I  K  I  D  A  H  L  N  K  V  C  P  T  T
GAGACCATTTACAATGATGAGTTCTATACTAAACAAGATGTAATTATTACAGCATTAGAT   1080
  E  T  I  Y  N  D  E  F  Y  T  K  Q  D  V  I  I  T  A  L  D
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  N  V  E  A  R  R  Y  V  D  S  R  C  L  A  N  L  R  P  L  L
GATTCTGGAACAATGGGCACTAAGGGACACACTGAAGTTATTGTACCGCATTTGACTGAG   1200
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GCTTGGAATTTTAGAGGATAGGACTAATAAAAGGACTTTTGCAAAGAAAAAAAAAAAAAA   4260
AAAAAAAAAAAAAAAAAAAAAAAAAAAA                                   4320
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Fig. 2 The nucleotide sequence and deduced amino acid sequence of the cDNA for *nUBE1L*

Numbering of the nucleotide is shown on the right. The initiation and stop codons are in italic. Two UBACT domains are boxed and the Thif domain is underlined. PCR primers for the determination of expression profile are in bold. The upstream primer is located in the specific region of *nUBE1L*. The downstream primer is homologous with that of FLJ10808.

so this gene was mapped to chromosome 4q13.2.

Feature of nUBE1L peptide sequence

nUBE1L gene encoded a 578 amino acid protein with predicted molecular weight 65.67 kD and isoelectric point 7.5. Analysis of the amino acid sequence by using SMART software revealed that the protein had one Thif domain and two UBACT domains that all exist in E1 enzyme and E1 like enzyme. So we named this gene as a novel ubiquitin-activating enzyme E1 like gene (*nUBE1L*). The Thif domain was located at 1–129 a.a.. Two UBACT domains were at 272–335 a.a. and 374–446 a.a. (Fig. 2).

Previous studies showed that E1 and E1 like enzyme all contain one or two Thif-domains and two UBACT-domains. Besides, a conserved active site cysteine located at the upstream of the UBACT-domain, which was probably involved in the formation of thiol ester bond between ubiquitin and E1. Therefore, the amino acid sequence and conserved domain of nUBE1L, human E1, human E1 like protein, mouse E1, yeast E1 and wheat E1 were compared.

Conserved domain analysis showed that they all had one or two Thif domains and two UBACT domains (Fig. 3). Furthermore, we presented an alignment of amino acid sequences of them (Fig. 4). As a result, we found a conserved cysteine existed in every sequence lying upstream of UBACT domain. Sequence analysis showed a 40% identity and 57% similarity of nUBE1L to the human E1 at the amino acid level. So nUBE1L is probably a novel human E1 like protein.

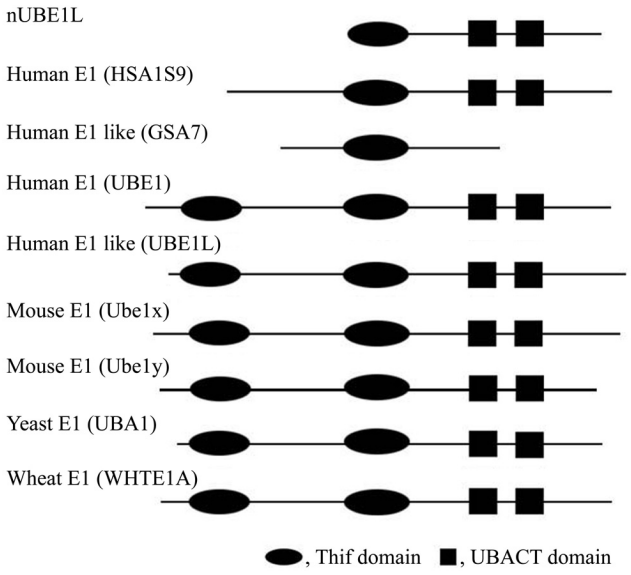

Fig. 3 Comparison of protein domains of nUBE1L with those of E1 and E1 like proteins

Homologous comparison of *nUBE1L* gene

Blast search found a splice variant mRNA of *nUBE1L*, *FLJ10808*, which is similar to human ubiquitin-activating enzyme E1 (GenBank accession No. NM_018227). The *FLJ10808* gene is also localized in human chromosome 4 (NT_022778.13|Hs4_22934). So they are spliced from identical gene. Splicing comparison of *nUBE1L* with *FLJ10808* gene showed that they both had 18 identical exons in the middle of cDNAs. The different exons were at the 5′ and 3′ of cDNA. *nUBE1L* gene lacked the initiative 13 exons of the homologous gene, and its first exon was longer than the fourteenth exon of the homologous gene. The last exon of *nUBE1L* gene was also longer than that of homologous gene (Fig. 5).

Expression profile of *nUBE1L* gene in different tissues

PCR and electrophoresis showed that this novel E1 like gene was predominantly expressed in testis, weakly in the pancreas, almost imperceptibly in other organs (Fig. 6).

Differential expression of *nUBE1L* in different development stages of male testis

RT-PCR showed *nUBE1L* was differentially expressed in human adult and fetal testes, which confirmed the hybridization result of cDNA microarray with stronger signal in adult than that in fetal testis (Fig. 7).

Discussion

In the present study, a human testis cDNA microarray constructed in our laboratory was used to identify the genes related to the development of human testis and spermatogenesis [11]. As a result, we found a new gene, *nUBE1L*, expressed more highly in human adult testis than in fetal testis.

This gene is 4288 bp in length and encodes a 578 a.a. protein. nUBE1L protein contains the characteristic domains of E1 and E1 like protein, one Thif domain and two UBACT domains. Comparison of amino acid sequence of nUBE1L with that of E1 and E1 like proteins indicates that there is a highly conserved cysteine lies upstream of UBACT-domain and probably participates in the formation of thiol ester bond between ubiquitin and E1. Hence, we consider nUBE1L protein as a novel human E1 like protein that plays key roles in ubiquitin system.

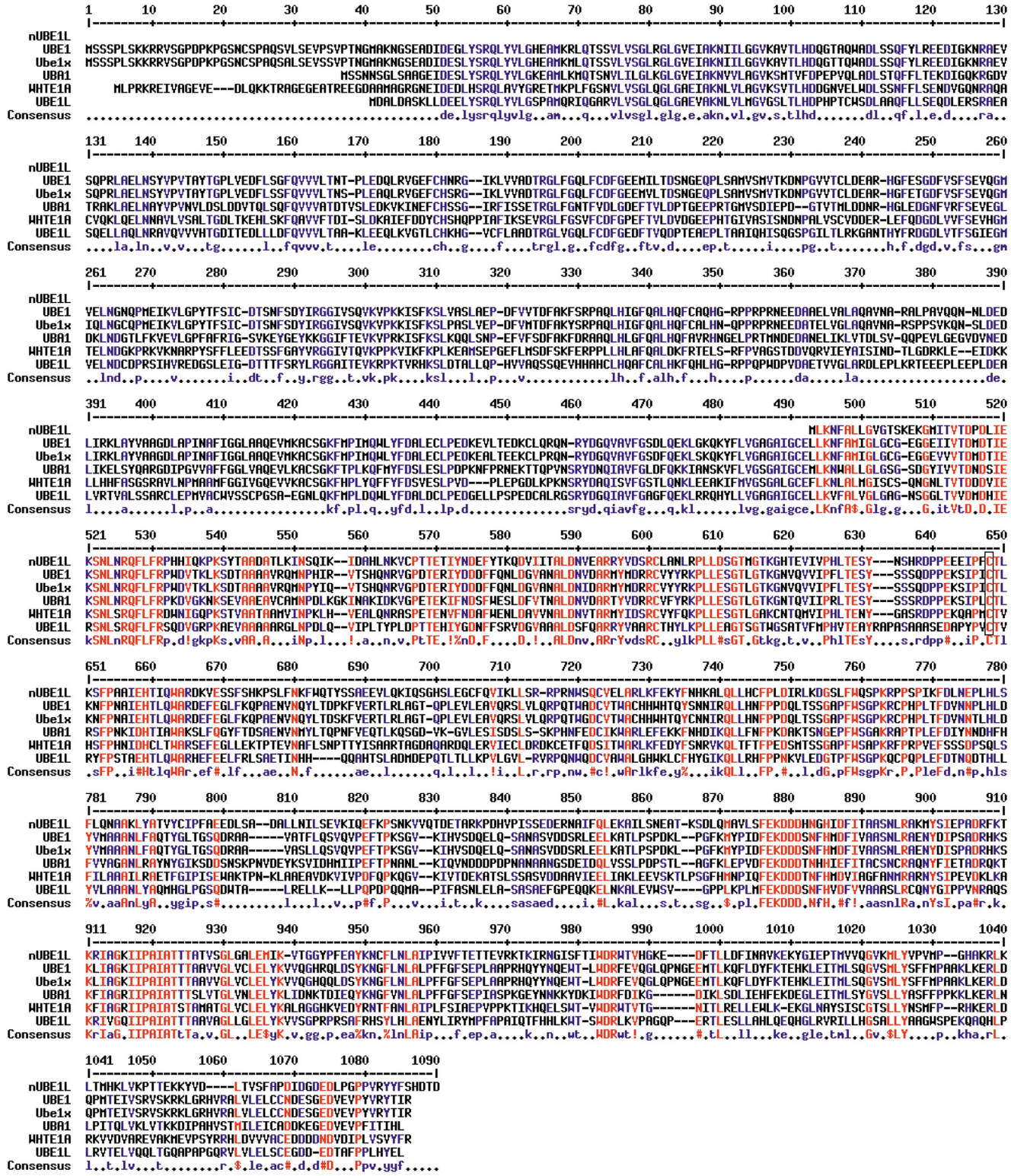

Fig. 4 Amino acid alignments of nUB-E1L with E1 and E1 like proteins

The sequences have the following database accession numbers: AY359880 (nUBE1L), NM_003334 (UBE1), NM_003335 (UBE1L), NM_009457 (Ube1x), X55386 (UBA1), M90663 (WHTE1A). Red, high consensus amino acids; blue, low consensus amino acids. Conserved active site cysteine that may be involved in the formation of thiol ester bond between ubiquitin and E1 are boxed and discussed in the text.

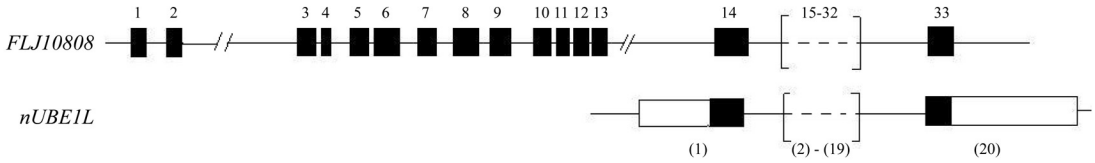

Fig. 5 Transcript and splicing comparison of *nUBE1L* with *FLJ10808*

18 identical exons in two sequences are boxed and omitted. Exons 1 to 13 only exist in *FLJ10808*. Exon (1) exists in *nUBE1L* which is longer than exon 14 of the homologous gene, and exon (20) is longer than exon 33 of *FLJ10808*.

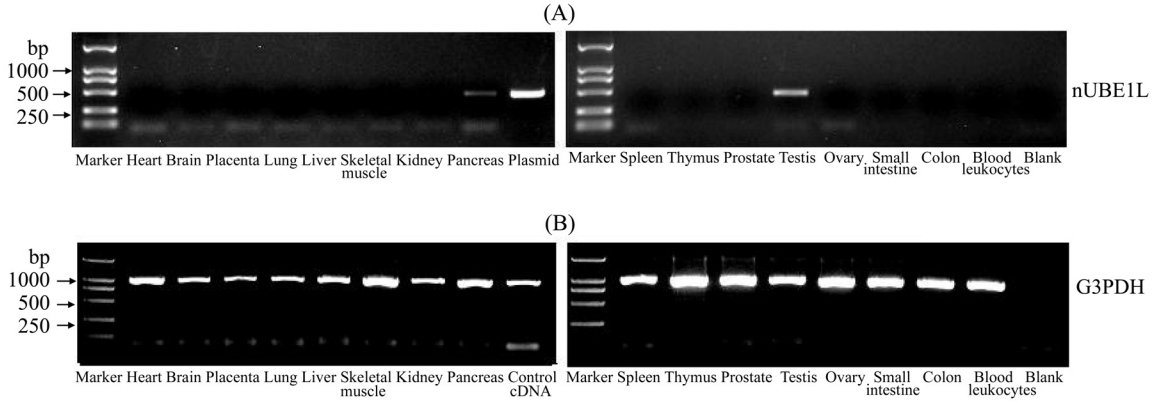

Fig. 6 RT-PCR analysis of tissue distribution of *nUBE1L* mRNA

Electrophoresis showed the expression profiles. (A) Amplification of *nUBE1L*. cDNAs of 16 kinds of tissues were amplified with nUBE1L sequence-specific primers. *nUBE1L* plasmid was used as positive control of PCR amplification. PCR product was 451 bp. (B) Amplification of *G3PDH* as control. *G3PDH* was expressed in all organs (983 bp).

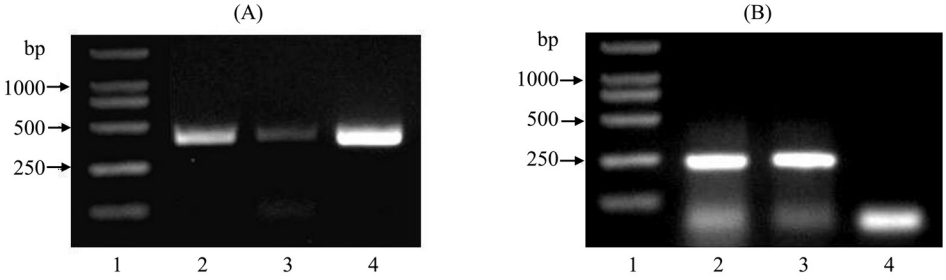

Fig. 7 Expression of *nUBE1L* in human adult and fetal testis (A) and β-actin as control (B)

nUBE1L was highly expressed in adult testis than that in fetal testis. 1, marker; 2, adult testis; 3, fetal testis. Lane 4 in (A) shows *nUBE1L* plasmid. Lane 4 in (B) shows blank.

El proteins and encoding genes have been isolated from humans [13], mice [14,15], yeast [16], wheat [8]. Besides, a few E1 like proteins also have been found [17–19]. In yeast, El is encoded by a single essential gene [16]. On the other hand, multiple forms of both the protein and the gene have been detected in plants and animals. The significance of El heterogeneity in more complex eukaryotes is unclear. However, different El isozymes may have distinct functions or patterns of expression. For example, in mice, an El gene essential for spermatogenesis has been isolated, which is distinct from an El gene expressed in most other tissues, indicating that there are differences in El expression [14,15].

In human, distinct E1 or E1 like genes have been cloned and confirmed to be expressed in several tissues, including placenta, colon, stomach, heart, brain, liver, kidney, pancreas and skeletal muscle [13,20]. Till now, no study has reported E1 or E1 like gene expressed in

human testis. The *nUBE1L* gene we identified in this study is probably a novel human E1 like gene that is predominantly expressed in testis. Previous studies in our lab have discovered the rule that 5′-terminal exon of most alternative splicings in testis was shorter than their alternative spliced counterparts. That is to say, majority of alternative splicings in testis used testis-specific and downstream promoter. In comparing with somatic cells, germ cells development is more complicated. To perform these functions, we speculate that mRNA of testis need not be transcribed from a new gene, it can use its specific and downstream promoter to transcribe from the same gene as the somatic cells. Thus RNA transcription is rather simple (unpublished paper). And in our study, the 5′-terminal exon of nUBE1L is just shorter than its alternative spliced counterparts, *FLJ10808*, an E1 similar gene expressed in monocytes. High expression of *nUBE1L* in adult testis indicates that it is probably involved in spermatogenesis and male fertility.

Spermatogenesis is a complex system leading to the formation of male gametes, which can be viewed as a cellular developmental process [21]. It occurs in successive mitotic, meiotic and post-meiotic phases regulated strictly by many intrinsic factors and extrinsic cues [22–24]. nUBE1L protein, as an E1 like protein, may regulate diverse events of spermatogenesis through ubiquitin pathway.

Ubiquitin system may play key role in spermatogonia proliferation. As we know, progression through the cell cycle depends on the specific proteolysis of cyclin. Different cyclins, specific for the G_1-, S-, or M-phase of the cell cycle, accumulate and activate cyclin-dependent kinases (Cdks) at appropriate time during the cell cycle, and then they are degraded, causing kinase inactivation, thus they conform the transition of each phase. All cyclins are degraded through the ubiquitin pathway [3]. So the role of ubiquitin system on cell-cycle control would influence mitosis of spermatogonia in testes. In adult testis, spermatogonia proliferate largely, so the synthesis and degradation of cyclins must be more efficient than that is in fetal testis. Accordingly ubiquitination is more efficient in adult testis. Our study showed that the novel E1 like gene just highly expressed in adult testis, which would start the ubiquitin pathway and advance the proliferation of spermatogonia.

On the other hand, ubiquitin system is also important in the process of spermatid metamorphosis. Ubiquitin and proteasomal subunits can be detected in the human sperm centrosome which undergoes dramatic reduction during spermatid elongation [2]. In addition, spermatid histones are ubiquitinated when they are transiently replaced by transitional proteins and permanently by protamines [25,26].

Besides, the normal structure and function of sperm are prerequisites for successful fertilization and embryonic development, so defective sperm during mammalian spermatogenesis must be eliminated [27]. It has proved that defective sperm become surface-ubiquitinated and subsequently phagocytosed by epididymal epithelial cells [28].

Spermatogenesis begins at puberty, so genes highly expressed in adult testis should be related to spermatogenesis. Ubiquitination is high in the process of spermatogenesis, which implies E1 or E1 like protein must be abundant in adult testes. *nUBE1L* gene we have identified is just an E1 like gene highly expressed in adult testis, so it would play key role in spermatogenesis and male fertility. Further study is required to provide more information and evidences for a better understanding about the exact role and mechanism of action of nUBE1L in spermatogenesis.

References

1 Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem, 1998, 67: 425–479

2 Sutovsky P. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: Killing three birds with one stone. Microsc Res Tech, 2003, 61(1): 88–102

3 Glotzer M, Murray AW, Kirschner MW. Cyclin is degraded by the ubiquitin pathway. Nature, 1991, 349(6305): 132–138

4 Diehl JA, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. Genes Dev, 1997, 11(8): 957–972

5 Baarends WM, Roest HP, Grootegoed JA. The ubiquitin system in gametogenesis. Mol Cell Endocrinol, 1999, 151(1-2): 5–16

6 Bebington C, Doherty FJ, Fleming SD. The possible biological and reproductive functions of ubiquitin. Hum Reprod Update, 2001, 7(1): 102–111

7 Oughtred R, Bedard N, Adegoke OA, Morales CR, Trasler J, Rajapurohitam V, Wing SS. Characterization of rat100, a 300-kilodalton ubiquitin-protein ligase induced in germ cells of the rat testis and similar to the Drosophila hyperplastic discs gene. Endocrinology, 2002, 143(10): 3740–3747

8 Hatfield PM, Callist J, Vierstra RD. Cloning of ubiquitin activating enzyme from Wheat and expression of a functional protein in Escherichia coli. J Biol Chem, 1990, 265(26): 15813–15817

9 Hatfield PM, Vierstra RD. Multiple forms of ubiquitin-activating enzyme E1 from wheat. Identification of an essential cysteine by in vitro mutagenesis. J Biol Chem, 1992, 267(21): 14799–14803

10 Cook JC, Chock PB. Isoforms of mammalian ubiquitin-activating enzyme. J Biol Chem, 1992, 267(34): 24315–24321

11 Sha J, Zhou Z, Li J, Yin L, Yang H, Hu G, Luo M *et al*. Identification of testis development and spermatogenesis-related genes in human and mouse testis using cDNA arrays. Mol Hum Reprod, 2002, 8(6): 511–517

12 Zhou ZM, Sha JH, Li JM, Lin M, Zhu H, Zhou YD, Wang LR *et al*.

Expression of a novel reticulon-like gene in human testis. Reproduction, 2002, 123: 227–234
13 Handley PM, Mueckler M, Siegel NR, Ciechanover A, Schwartz AL. Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. Proc Natl Acad Sci USA, 1991, 88 (16): 258–262
14 Mitchell MJ, Woods DR, Tucker PK, Opp JS, Bishop CE. Homology of a candidate spermatogenic gene from the mouse Y chromosome to the ubiquitin-activating enzyme E1. Nature, 1991, 354: 483–486
15 Kay GF, Ashworth A, Penny GD, Dunlop M, Swift S, Brockdorff N, Rastan S. A candidate spermatogenesis gene on the mouse Y chromosome is homologous to ubiquitin-activating enzyme E1. Nature, 1991, 354: 486–489
16 McGrath JP, Jentsch S, Varshavsky A. UBA 1: An essential yeast gene encoding ubiquitin-activating enzyme. EMBO J, 1991, 10(1): 227–236
17 Kok K, Hofstra R, Pilz A, van den Berg A, Terpstra P, Buys CH, Carritt B. A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitin-activating enzyme. Proc Natl Acad Sci USA, 1993, 90(13): 6071–6075
18 Yuan W, Stromhaug PE, Dunn Jr WA. Glucose-induced autophagy of peroxisomes in *Pichia pastoris* requires a unique E1-like protein. Mol Biol Cell, 1999, 10(5): 1353–1366
19 Carritt B, Kok K, van den Berg A, Osinga J, Pilz A, Hofstra RM, Davis MB *et al*. A gene from human chromosome region 3p21 with reduced expression in small cell lung cancer. Cancer Res, 1992, 52(6): 1536–1541
20 Gong L, Yeh ET. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J Biol Chem, 1999, 274(17): 12036–12042
21 McCarrey JR. Spermatogenesis as a model system for developmental analysis for regulatory mechanisms associated with tissue-specific gene expression. Semin Cell Dev Biol, 1998, 9(4): 459–466
22 Sutton KA. Molecular mechanisms involved in the differentiation of spermatogenic stem cells. Rev Reprod, 2000, 5(2): 93–98
23 Eddy EM. Regulation of gene expression during spermatogenesis. Semin Cell Dev Biol, 1998, 9(4): 451–457
24 Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill J eds. *The Physiology of Reproduction*. 2nd ed. New York: Raven Press. 1994, 1363–1434
25 Baarends WM, Hoogerbrugge JW, Roest HP, Ooms M, Vreeburg J, Hoeijmakers JH, Grootegoed JA. Histone ubiquitination and chromatin remodeling in mouse spermatogenesis. Dev Biol, 1999, 207(2): 322–333
26 Chen HY, Sun JM, Zhang Y, Davie JR, Meistrich ML. Ubiquitination of histone H3 in elongating spermatids of rat testes. J Biol Chem, 1998, 273(21): 13165–13169
27 Rajapurohitam V, Bedard N, Wing SS. Control of ubiquitination of proteins in rat tissues by ubiquitin-conjugating enzymes and isopeptidases. Am J Physiol Endocrinol Metab, 2002, 282(4): E739–E745
28 Sutovsky P, Moreno R, Ramalho-Santos J, Dominko T, Thompson WE, Schatten G. A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. J Cell Sci, 2001, 114(9): 1665–1675

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