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·Original Article·

Expression of a novel beta adaptin subunit mRNA splice variant in human testes

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Abstract

Aim: To identify a novel isoform of adaptin 2 beta subunit (named Ap2β-NY) and to investigate its relationship with testicular development and spermatogenesis. **Methods:** Using a human testis cDNA microarray, a clone (Ap2β-NY), which was strongly expressed in adult testes but weakly expressed in embryo testes, was sequenced and analyzed. Using polymerase chain reaction (PCR), the tissue distribution and expression time pattern of Ap2β-NY were determined. **Results:** Ap2β-NY was identified and has been deposited in the GenBank (AY341427). The expression level of Ap2β-NY in the adult testis was about 3-fold higher than that in the embryo testis. PCR analysis using multi-tissue cDNA indicated that Ap2β-NY was highly expressed in the testis, spleen, thymus, prostate, ovary, blood leukocyte and brain, but not in the heart, placenta, lung, liver, skeletal muscle, kidney and pancreas. In addition, Ap2β-NY was variably expressed in the testes of patients with spermatogenesis-disturbance and spermatogenesis-arrest but not expressed in those of Sertoli-cell-only syndrome, which implied that, in the testis, Ap2β-NY was restrictively expressed in germ cells. **Conclusion:** Ap2β-NY is an isoform of Ap2β and may be involved in regulating the process of spermatogenesis and testis development. *(Asian J Androl 2005 Jun; 7: 179–188)*

Keywords: alternative splicing; adaptin beta subunit; spermatogenesis

1 Introduction

Adaptin 2 beta subunit (Ap2β-NY) is a part of the adaptin protein 2 (AP2) coat assembly protein complex which links clathrin to receptors in the coated vesicles [1]. Previously, the AP2 adaptor was reported as a complex composed of two large ~110 kDa subunits (and β2), one medium ~50 kDa subunit (μ2) and one small ~17 kDa subunit (σ2) [2, 3]. Each large subunit can be divided into a 60-70 kDa trunk domain separated from a 25-30 kDa appendage domain (sometimes referred to as an "ear" domain) by a ~100-residue, protease-sensitive linker [4]. AP2 links the endocytic cargo (ligand and receptor) to the clathrin coat and the internalization occurs through forming clathrin-coated vesicles (CCVS). The α subunit interacts with CCV formation regulatory protein or accessory proteins such as AP180, auxilin, amphiphysin, eps15 and epsin via its appendage domain [5, 6]. The β2 subunit binds to clathrin via the canonical clathrin box motif (LΦxΦD/E, where Φ is a bulky hydrophobic amino acid and x represents any amino acid) [7, 8] in its linker or hinge region [9]. In addition, AP2 adaptor binds to the short linear internalization motifs on

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cargo molecules, YxxΦ via the μ2 subunit [10, 11] and D/ExxxLL probably via the β2 trunk domain [12]. It was confirmed by Yao *et al*. that AP2/transforming growth factor beta (TGF-β) receptor binding was mediated by a direct interaction between the β2 subunit N-terminal trunk domain and the cytoplasmic tails of the receptors [13]. AP2 adaptor is one of the intriguing proteins playing central roles in clathrin-mediated endocytosis. Clathrin-mediated endocytosis is involved in many cellular processes including the internalization and subsequent down-regulation of activated growth factor receptors, regulating the number and type of small molecule receptors, channels and transporters in the plasma membrane, recycling of synaptic vesicles and the maintenance of membrane identity.

On the basis of the adult testis cDNA microarrays prepared by this laboratory, we compared the genes expressed in the adult testis and embryo testis at a high throughput [14]. Here we report a gene coding a novel isoform of adaptin 2β subunit (Ap2β), named Ap2β-NY. The structure analysis results and distribution pattern of Ap2β-NY in the testis indicated its presumable functions in the regulation of testicular development and spermatogenesis by affecting protein transportation through CCV conformation changing.

2 Materials and methods

2.1 Construction of human cDNA microarrays

A total of 9216 positive phage clones were picked out randomly from Human Testis Insert λ phage cDNA library (clontech, Hl5503U) and amplified by po;ymerase chain reaction (PCR). Then the PCR products were spotted on the nylon membrane to make human testis cDNA microarray. Protocol for cDNA microarray preparation has been described in detail recently [14].

2.2 Array scanning and analyzing clones of interest

After being hybridized with the 33P-labeled embryo testis and adult testis cDNA probes respectively, the arrays were scanned and read out using the array gauge software (Fuji Photo Film, Tokyo, Japan). After subtraction of background, clones with intensities >10 were considered positive signals. Then interested clones were sequenced using the ABI 377 automatic sequencing machine and analyzed (see also in Sha *et al.* and Wang *et al.* [14, 15]).

2.3 Multiple tissue distribution of Ap2β-NY

To determine the tissue distribution of Ap2β-NY, specific primers overlapping an intron were designed to amplify cDNAs from sixteen kinds of human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon and blood leukocytes), purchased from Clontech (K1420-1 and K1421-1). The primers specific to Ap2β-NY were as following: P1 5' GACCATCTATCTAAGGAGTTG 3 ' and P2 5' TTGTCTACCCGGATGCAC 3'. The PCR products (255 bp) of sixteen tissues were resolved by electrophoresis on 1 % agarose gel and transferred to Hybond-N+ nylon membrane (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK).

The Ap2β-NY cDNA probe was generated with the same primers used for the PCR. The template was Ap2β-NY clone plasmid, and digoxigenin (Dig)-labeled dNTPs were used (Dig DNA Labeling Mix; Roche Diagnostics, Indianapolis, USA). The reaction protocol was as following: 35 cycles of 94 °C for 30 sec, 44 °C for 30 sec and 72 °C for 60 sec. Hybridization was performed according to the Instruction Manual of Roche (DIG High Prime DNA Labeling and Detection Starter Kit II, Cat. No.1585614). After hybridization, the membrane was incubated with alkaline phosphatase (Ap)-conjugated anti-DIG antibodies and visualized with immunological detection through chemiluminescent substrate for alkaline phosphatase (CSPD; Roche). Human β-actin was used as the positive control and the primers used were: P3 5'CGGTTGGCCTTGGGGTTCAGGGGG 3' and P4 5' ATCGTGGGGGCGCCCCAGGCACCA3'.

2.4 Expressional state in a different development stage and in patients of abnormal spermatogenesis

Human adult (37 years) and aged (73 years) testes were obtained from the Body Donor Center (Nanjing Medical University). Embryo testes were from accidentally aborted 6-month fetuses. Human ejaculates were obtained from healthy volunteers of proven fertility and of normal semen quality as assessed by WHO criteria (1999). Samples of patient testes were obtained via biopsy from 12 infertile men at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China). The clinical diagnoses based on testicular biopsy were Sertoli-cell-only Syndrome (SCOS), spermatogenesis-arrest at different stages and spermatogenesis-disturbance, each

in four patients. All the samples were obtained after ethics approval and consent from all participants or their family. The total mRNA was isolated using the TRIzol Reagent (Gibcol, Grand Island, USA.) according to the manufacturer's instructions. Reverse transcription was performed using random primers (1 μL, 0.2 mg/mL; Sangon company, Shanghai, China) and 1 μL Moloney murine leukemin virus (MMLV) Reverse Transcriptase (10 U /μL, Promega, Madison, USA) in a 15 μL reaction mixture. Then RT-PCR was carried out to determine the expression of Ap2β-NY and Ap2β in subjects mentioned above. The primer-pairs used respectively were p1, p2 and p5, p6, which are also specific to Ap2β and its sequence is: P5 5' ATGTGGGAGGTGGATAGGTC 3' and p6 5' ATGGGTTGGCTCAGTCAGG 3'. The desired fragment for Ap2β is 283 bp. The resulting PCR products were identified by 1 % agarose gel electrophoresis and the expression level was analyzed.

3 Results

3.1 cDNA microarray hybridization

After hybridization and data analysis, genes differentially expressed in human adult and embryo testes were considered as testicular development and spermatogenesis-related. Among those, a novel alternative splicing form of Ap2β was identified. The hybridization intensity of Ap2β-NY in the adult testis and embryo testis was 140.22 and 38.73, respectively (Figure 1). Obviously, Ap2β-NY was expressed both in the adult and embryo testis, but the expression level in the adult testis was approximately 3-fold more than that in the embryo testis.

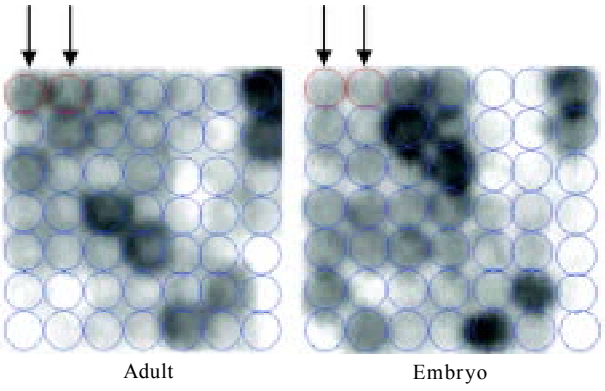

Figure 1. Partial cDNA hybridization images showing differential expression of Ap2β-NY in adult and embryo testes. Arrows indicate Ap2β-NY cDNA. The signal intensity in the adult testis (left) and embryo testis (right) was 140.22 and 38.73, respectively.

3.2 Sequence identification and analysis of the Ap2β-NY gene

The full nucleotide and putative amino acid sequences of Ap2β-NY are displayed in Figure 2. The 3457 bp Ap2β-NY cDNA contains a complete open reading frame (ORF) of 2642 bp with a methionine start codon at the position 396 and a TGA stop codon at the position 3038. The first start codon is preceded by an in-frame stop codon TAA at position 354, suggesting that ATG at position 396 is the start codon for the Ap2β-NY protein. Ap2β-NY encoded an 880-amino acids protein with predicted molecular weight of 98.12 kDa and isoelectric point, 5.01. In addition, two different promoters, located at –2300 and –1200 respectively, were found upstream in the sequence by use of Promoter 2.0 (Promoter 2.0 [Internet]. S. Knudsen. Bioinformatics: 15, 356-361; [cited 22 February 2005]. Available from: http://www.cbs.dtu.dk/services/Promoter/).

3.3 Homologous analysis of Ap2β-NY and Ap2β

A Basic local alignment search tool (BLAST) search in the human genome database localized the Ap2β-NY gene to 17q11.2-q12. BLAST-nr showed Ap2β-NY had a marked similarity to Ap2β (Figure 3). The former has a distinct 5-UTR and it is 57 amino acids shorter than the latter at the amino terminus. The Simple Modular Architecture Research Tool (SMART) (SMART [Internet]. Schultz J, Proc Natl Acad Sci, 95(11):5857-64, USA; [cited 22 February 2005] Available from: http://smart.embl-heidelberg.de/) results showed that both genes have an adaptin N, a low complexity and an adaptin C motif, but the adaptin N motif of Ap2β-NY is shorter than that of Ap2β. Protein composition analysis, using Gene Runner software (http://www.generunner.com), indicates that the deleted region is hydrophilic and is most likely exposed on the protein surface (Figure 4).

3.4 Tissue distribution of Ap2β-NY

The results of multiple tissue PCR and hybridization indicate that Ap2β-NY is expressed in several human tissues (Figure 5). It is highly expressed in the spleen, thymus, prostate, testis, ovary, blood leukocyte and brain, but not in the heart, placenta, lung, liver, skeletal muscle, kidney and pancreas.

3.5 Development-dependent expression

```
   1 AGTGGCTTAGACCTAGAAAAGAATCGTGACGGGCAGGAAACCATTACACCACCACCTGG
  60 GCTGTGCTCTCCGGCTCCCGCCGCCACCCCCGCCCTCGCCTTCGCCTCCGCCTCCGGTGC
 120 ACATTAAAGATCCAAAGTCATGACTGACTCCAAGTATTTCACAACCAATAAAAAAGGAGA
 180 AATATTTGAACTAAAAGCTGAACTCAACAATGAAAAGAAAGAAAAGAGAAAGGAGGCTGT
 240 GAAGAAAGTGATTGCTGCTATGACCGTGGGGAAGGATGTTAGCACATAAAGCACATTTGG
 300 TTTTTAAATTGCTGTCCTGTGACCATCTATCTAAGGAGTTGGCAAACGTTTTTGTAAAGG
360 ACAAAATATTCTCTCTTTCCAGACGTAGTGAACTGTATGCAGACTGACAATCTGGAACTA
                                        M  Q  T  D  N  L  E  L
420 AAGAAGCTTGTGTATCTCTACTTGATGAACTACGCCAAGAGTCAGCCAGACATGGCCATC
     K  K  L  V  Y  L  Y  L  M  N  Y  A  K  S  Q  P  D  M  A  I
480 ATGGCTGTAAACAGCTTTGTGAAGGACTGTGAAGATCCTAATCCTTTGATTCGAGCCTTG
     M  A  V  N  S  F  V  K  D  C  E  D  P  N  P  L  I  R  A  L
540 GCAGTCAGAACCATGGGGTGCATCCGGGTAGACAAAATTACAGAATATCTCTGTGAGCCG
     A  V  R  T  M  G  C  I  R  V  D  K  I  T  E  Y  L  C  E  P
600 CTCCGCAAGTGCTTGAAGGATGAGGATCCCTATGTTCGGAAAACAGCAGCAGTCTGCGTG
     L  R  K  C  L  K  D  E  D  P  Y  V  R  K  T  A  A  V  C  V
660 GCAAAACTCCATGATATCAATGCCCAAATGGTGGAAGATCAGGGATTTCTGGATTCTCTA
     A  K  L  H  D  I  N  A  Q  M  V  E  D  Q  G  F  L  D  S  L
720 CGGGATCTCATAGCAGATTCAAATCCAATGGTGGTGGCTAATGCCGTAGCGGCATTATCT
     R  D  L  I  A  D  S  N  P  M  V  V  A  N  A  V  A  A  L  S
780 GAAATCAGTGAGTCTCACCCAAACAGCAACTTACTTGATCTGAACCCACAGAACATTAAT
     E  I  S  E  S  H  P  N  S  N  L  L  D  L  N  P  Q  N  I  N
840 AAGCTGCTGACAGCCCTGAATGAATGCACTGAATGGGGCCAGATTTTCATCCTGGACTGC
     K  L  L  T  A  L  N  E  C  T  E  W  G  Q  I  F  I  L  D  C
900 CTGTCTAATTACAACCCTAAAGATGATCGGGAGGCTCAGAGCATCTGTGAGCGGGTAACT
     L  S  N  Y  N  P  K  D  D  R  E  A  Q  S  I  C  E  R  V  T
960 CCCCGGCTATCCCATGCCAACTCAGCAGTGGTGCTTTCAGCGGTAAAAGTCCTAATGAAG
     P  R  L  S  H  A  N  S  A  V  V  L  S  A  V  K  V  L  M  K
1020 TTTCTAGAATTGTTACCTAAGGATTCTGACTACTACAATATGCTGCTGAAGAAGTTAGCC
     F  L  E  L  L  P  K  D  S  D  Y  Y  N  M  L  L  K  K  L  A
1080 CCTCCACTTGTCACTTTGCTGTCTGGGGAGCCAGAAGTGCAGTATGTCGCCCTGAGGAAC
     P  P  L  V  T  L  L  S  G  E  P  E  V  Q  Y  V  A  L  R  N
1140 ATCAACTTAATTGTCCAGAAAAGGCCTGAAATCTTGAAGCAGGAAATCAAAGTCTTCTTT
     I  N  L  I  V  Q  K  R  P  E  I  L  K  Q  E  I  K  V  F  F
1200 GTGAAGTACAATGATCCCATCTATGTTAAACTAGAGAAGTTGGACATCATGATTCGTTTG
     V  K  Y  N  D  P  I  Y  V  K  L  E  K  L  D  I  M  I  R  L
1260 GCATCTCAAGCCAACATTGCTCAGGTTCTGGCAGAACTGAAAGAATATGCTACAGAGGTG
     A  S  Q  A  N  I  A  Q  V  L  A  E  L  K  E  Y  A  T  E  V
1320 GATGTTGACTTTGTTCGAAAAGCTGTGCGGGCCATTGGACGGTGTGCCATCAAGGTGGAG
     D  V  D  F  V  R  K  A  V  R  A  I  G  R  C  A  I  K  V  E
1380 CAATCTGCAGAGCGCTGTGTAAGCACATTGCTTGATCTAATCCAGACCAAAGTGAATTAT
     Q  S  A  E  R  C  V  S  T  L  L  D  L  I  Q  T  K  V  N  Y
1440 GTGGTCCAAGAAGCAATTGTTGTCATCAGGGACATCTTCCGCAAATACCCCAACAAGTAT
     V  V  Q  E  A  I  V  V  I  R  D  I  F  R  K  Y  P  N  K  Y
1500 GAAAGTATCATCGCCACTCTGTGTGAGAACTTAGACTCGCTGGATGAGCCAGATGCTCGA
     E  S  I  I  A  T  L  C  E  N  L  D  S  L  D  E  P  D  A  R
1560 GCAGCTATGATTTGGATTGTGGGAGAATATGCTGAAAGAATTGACAATGCAGATGAGTTA
     A  A  M  I  W  I  V  G  E  Y  A  E  R  I  D  N  A  D  E  L
1620 CTAGAAAGCTTCCTGGAGGGTTTTCACGATGAAAGCACCCAGGTGCAGCTCACTCTGCTT
     L  E  S  F  L  E  G  F  H  D  E  S  T  Q  V  Q  L  T  L  L
1680 ACTGCCATAGTGAAGCTGTTTCTCAAGAAACCATCAGAAACACAGGAGCTAGTCCAGCAG
     T  A  I  V  K  L  F  L  K  K  P  S  E  T  Q  E  L  V  Q  Q
1740 GTCTTGAGTTTGGCAACACAGGATTCTGATAATCCTGACCTTCGAGACCGGGGCTATATT
```

(to be continued)

```
      V  L  S  L  A  T  Q  D  S  D  N  P  D  L  R  D  R  G  Y  I
1800 TATTGGCGCCTTCTCTCAACTGACCCTGTTACAGCTAAAGAAGTAGTCTTGTCTGAGAAG
      Y  W  R  L  L  S  T  D  P  V  T  A  K  E  V  V  L  S  E  K
1860 CCACTGATCTCTGAGGAGACGGACCTTATTGAGCCAACTCTGCTGGATGAGCTAATCTGC
      P  L  I  S  E  E  T  D  L  I  E  P  T  L  L  D  E  L  I  C
1920 CACATTGGTTCTTTGGCCTCTGTGTATCATAAGCCTCCCAATGCTTTTGTGGAAGGAAGT
      H  I  G  S  L  A  S  V  Y  H  K  P  P  N  A  F  V  E  G  S
1980 CATGGAATTCATCGTAAACACTTGCCAATTCATCATGGGAGCACTGATGCAGGTGACAGC
      H  G  I  H  R  K  H  L  P  I  H  H  G  S  T  D  A  G  D  S
2040 CCTGTTGGCACTACCACTGCAACGAACCTGGAACAGCCTCAGGTTATCCCCTCTCAAGGT
      P  V  G  T  T  T  A  T  N  L  E  Q  P  Q  V  I  P  S  Q  G
2100 GATCTTCTAGGGGATCTTTTAAACCTTGACCTCGGTCCCCCAGTCAATGTGCCACAGGTG
      D  L  L  G  D  L  L  N  L  D  L  G  P  P  V  N  V  P  Q  V
2160 TCCTCCATGCAGATGGGAGCAGTGGATCTCCTAGGAGGAGGACTAGATAGTCTGGTGGGA
      S  S  M  Q  M  G  A  V  D  L  L  G  G  G  L  D  S  L  V  G
2220 CAATCCTTCATCCCATCATCGGTCCCTGCAACCTTTGCTCCTTCACCTACACCTGCTGTG
      Q  S  F  I  P  S  S  V  P  A  T  F  A  P  S  P  T  P  A  V
2280 GTCAGCAGTGGACTGAATGACCTGTTTGAACTCTCCACAGGGATAGGCATGGCACCTGGT
      V  S  S  G  L  N  D  L  F  E  L  S  T  G  I  G  M  A  P  G
2340 GGATATGTGGCTCCTAAGGCTGTCTGGCTACCTGCAGTAAAGGCTAAAGGCTTGGAGATT
      G  Y  V  A  P  K  A  V  W  L  P  A  V  K  A  K  G  L  E  I
2400 TCCGGAACATTTACTCACCGCCAAGGGCACATCTATATGGAAATGAACTTCACCAATAAA
      S  G  T  F  T  H  R  Q  G  H  I  Y  M  E  M  N  F  T  N  K
2460 GCTCTGCAGCACATGACAGATTTTGCAATCCAGTTTAACAAAAATAGCTTTGGTGTCATC
      A  L  Q  H  M  T  D  F  A  I  Q  F  N  K  N  S  F  G  V  I
2520 CCCAGCACTCCTCTGGCCATCCATACACCACTGATGCCAAACCAGAGCATTGATGTCTCC
      P  S  T  P  L  A  I  H  T  P  L  M  P  N  Q  S  I  D  V  S
2580 CTGCCTCTCAATACCTTGGGCCCAGTCATGAAGATGGAACCTCTGAATAACCTCCAGGTG
      L  P  L  N  T  L  G  P  V  M  K  M  E  P  L  N  N  L  Q  V
2640 GCTGTGAAAAACAATATCGATGTCTTCTACTTCAGCTGCCTCATCCCACTCAATGTGCTT
      A  V  K  N  N  I  D  V  F  Y  F  S  C  L  I  P  L  N  V  L
2700 TTTGTAGAAGATGGCAAAATGGAGCGCCAGGTCTTCCTTGCAACATGGAAGGATATTCCC
      F  V  E  D  G  K  M  E  R  Q  V  F  L  A  T  W  K  D  I  P
2760 AATGAAAATGAACTTCAGTTTCAGATTAAGGAATGTCATTTAAATGCTGACACTGTTTCC
      N  E  N  E  L  Q  F  Q  I  K  E  C  H  L  N  A  D  T  V  S
2820 AGCAAGTTGCAAAACAACAATGTTTATACTATTGCCAAGAGGAATGTGGAAGGGCAGGAC
      S  K  L  Q  N  N  N  V  Y  T  I  A  K  R  N  V  E  G  Q  D
2880 ATGCTGTACCAATCCCTGAAGCTCACTAATGGCATTTGGATTTTGGCCGAACTACGTATC
      M  L  Y  Q  S  L  K  L  T  N  G  I  W  I  L  A  E  L  R  I
2940 CAGCCAGGAAACCCCAATTACACGCTGTCACTGAAGTGTAGAGCTCCTGAAGTCTCTCAA
      Q  P  G  N  P  N  Y  T  L  S  L  K  C  R  A  P  E  V  S  Q
3000 TACATCTATCAGGTCTACGACAGCATTTTGAAAAACTAACAAGACTGGTCCAGTACCCTT
      Y  I  Y  Q  V  Y  D  S  I  L  K  N  #
3060 CAACCATGCTGTGATCGGTGCAAGTCAAGAACTCTTAACTGGAAGAAATTGTATTGCTGC
3120 GTAGAATCTGAACACACTGAGGCCACCTAGCAAGGTAGTAACTAGTCTAACCTGTGCTAA
3180 CATTAGGGCACAACCTGTTGGATAGTTTTAGCTTCCTGTGAACATTTGTAACCACTGCTT
3240 CAGTCACCTCCCACCTCTTGCCACCTGCTGCTGCTATCTGTCCTTACTTGTGGGCTTCTC
3300 CATGCTGTGCCAATGGCTGGCTTTTTCTACACCCTCTTTTGAGTGTAGTTTGGTATTTTG
3360 TAATTGAGAGCTCATTTCAAAAGCAGAAAAAGACAACAAATATTAAAGCAAGGAAAAGTG
3420 TAACTGAAATAAAAAAAAAAAAAAAAAAAAAAAAAA
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Figure 2. Nucleic acid and deduced amino acid sequences of the cDNA for Ap2β-NY. Underlining shows PCR primers for the determination of expression profile. Upstream primer is located in the specific region of Ap2β-NY. Downstream primer is homologous with that of other genes of the Ap2β family. Initiation and stop codons are in italic type.

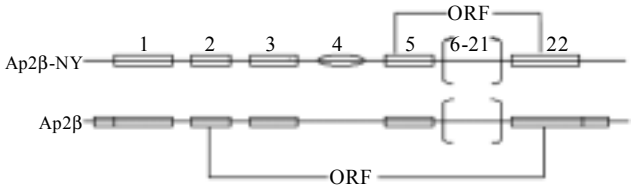

Figure 3. Transcript and splicing comparison of Ap2β-NY (top) and Ap2β (bottom). Middle line represents genome DNA and rectangles represent exon, which was numbered by Arabic numerals. Exon 4 only existed in Ap2β-NY. Exon 1 and Exon 22 were shorter (40 bp and 2270 bp, respectively) in Ap2β-NY than in Ap2β. Exon 2, exon 3 and exons 6–21 (in brackets) were largely identical except for a 2 base mutation. Open reading frame (ORF) was marked by "ORF".

RT-PCR results show that Ap2β is strongly expressed in embryo, adult and aged testes (throughout the development stage of testis) while its expression level in spermatozoa is relatively lower (Figure 6). On the contrary, in spermatozoa and adult testes, Ap2β-NY has higher expression level, while it is poorly expressed in embryos and old testes. This expressional profile validates the microarray result at the same time. Together all these evidences support the concept that different splicing isoform of Ap2β-NY are predominant in various testis developmental stages and Ap2β-NY may be involved in testis development and spermatogenesis.

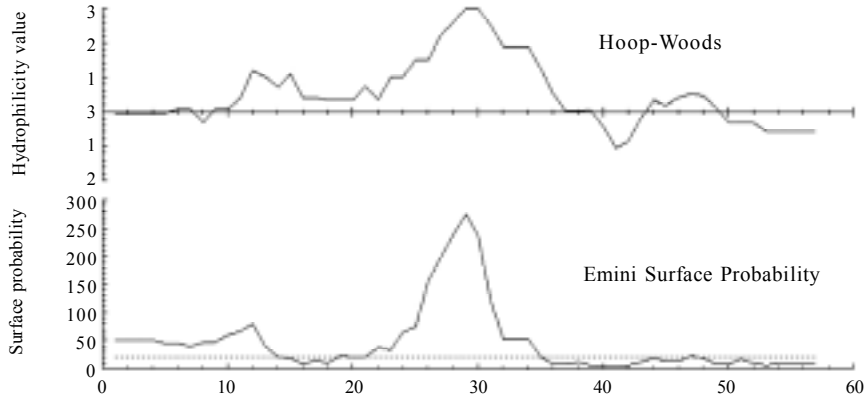

Figure 4. Hydrophilicity profile (top) and surface probability (bottom) of deleted 57Aa predicted by Gene Runner.

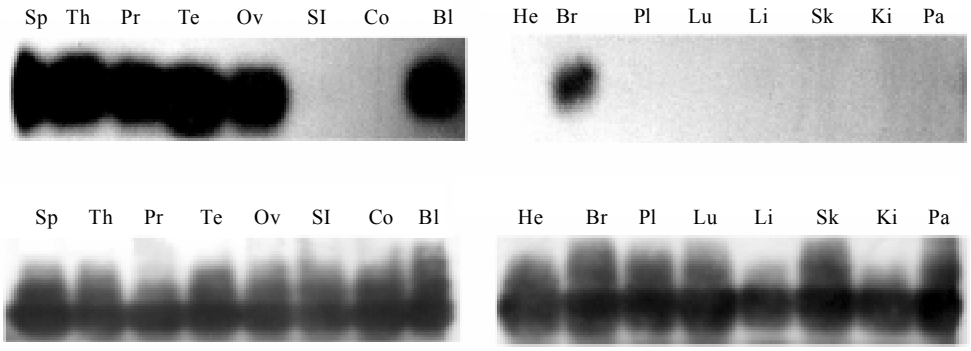

Figure 5. Tissue distribution of Ap2β-NY. Top: Ap2β-NY was expressed in several human tissues. Bottom: β-actin was expressed in all tissues. Blood leukocyte (Bl); colon (Co); small intestine (SI); ovary (Ov); testis (Te); prostate (Pr); thymus (Th); spleen (Sp); pancreas (Pa); kidney (Ki); skeletal muscle (Sk); liver (Li); lung (Lu); placenta (Pl); brain (Br); heart (He).

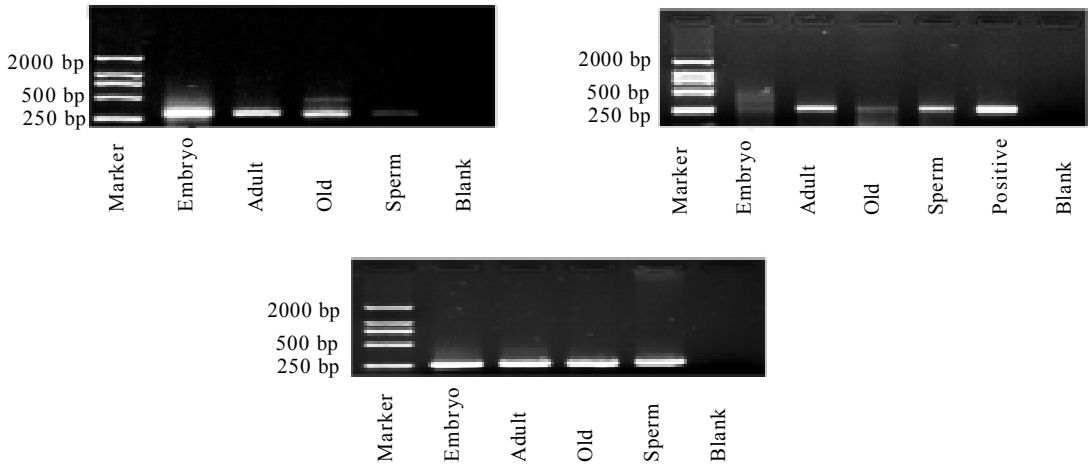

Figure 6. Top: age-dependent expressional status of Ap2β(left) and Ap2β-NY (right). Bottom: β-actin as positive control.

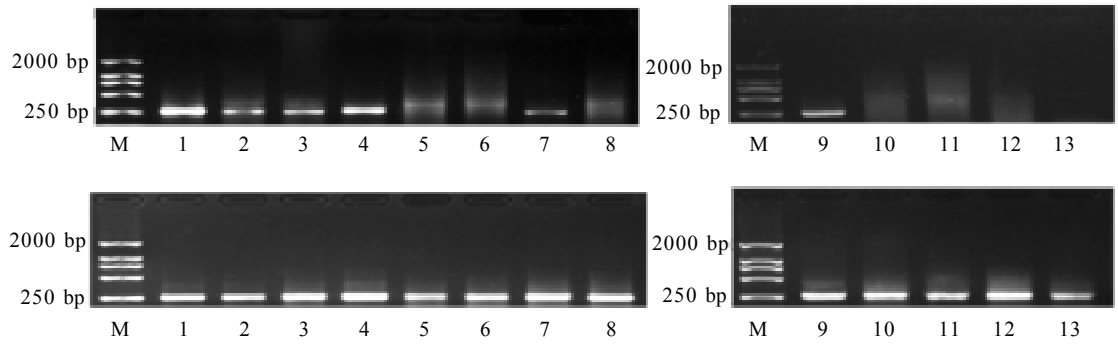

Figure 7. Abnormal expression of Ap2β-NY in infertile men. Top: expression of Ap2β-NY in patients with spermatogenic disturbance (lanes 1–4), with spermatogenesis arrest (lanes 5–8) or SCOS (lanes 10–13; lane 9 is normal adult testis as control). Bottom: Expression of human β-actin mRNA as a positive control.

3.6 Ap2β-NY expression in infertile patient testes

The results of RT-PCR reveal that Ap2β-NY was not expressed in patients with Sertoli-cell-only syndrome (SCOS) and was variably expressed in those with spermatogenic disturbance and spermatogenesis-arrest (Figure 7). It is well known that in SCOS, there is no germ cell but only Sertoli cells in the seminiferous tubules. Therefore, Ap2β-NY was not expressed in Sertoli cells and Leydig cells, but in spermatogenic cells.

4 Discussion

In the present study, a novel isoform of the Ap2β gene, named Ap2β-NY, was identified. Previously the Ap2β subunit was found in human fibroblast, rat lymphocyte, bovine lymphocyte, rat brain and bovine brain [4]. In the rat brain there was a 42 bp insert in the coding region, which was thought to be a result of alternative splicing of the Ap2β subunit [16]. This evidence reminds us that there may be a different splicing isoform

of Ap2β in human tissue. Indeed, two promoters, located at –2300 and –1200 bp were found in the upstream of Ap2β using Promoter 2.0 (Position Score Likelihood was 1.101 and 1.112, respectively, highly likely prediction). In the present study, we further validated that Ap2β-NY was expressed in several human tissues. In testes, Ap2β-NY showed a developmental stage-dependent expression pattern. It was highly expressed in adult, spermatogenesis-active testes, but weakly in embryo and senile testes, suggesting its localization in spermatogenic cells and its relationship with spermatogenesis. Our further PCR analysis of infertile patients confirmed this. Ap2β-NY was not expressed in SCOS, but disordered in those of spermatogenic disturbances and spermatogenesis-arrest. Ap2β-NY was indicated to be a newly found regulator of spermatogenesis in male germ cells.

4.1 Hypothetic traffic model mediated by Ap2β-NY in testes

Bioinformatical analysis provides some information regarding the function of Ap2β-NY in spermatogenesis. SMART analysis showed that Ap2β-NY contained all the three motifs of Ap2β and is 57 amino acids shorter in the N-terminal region than Ap2β. The deleted region is hydrophilic and likely exposed on the surface of the protein, which, in general, was the position of protein interacting with others. Deletion of this region may have an influence on the capability of Ap2β binding with these cargo molecules, leading to an even greater default of binding. In embryo testes, Ap2β was predominant. It can transduce signals into cells by linking cargo proteins and clathrin. While in adult testes, Ap2β-NY was increasingly expressed. It was shortened in trunk domain, which may result in decreasing affinity with cargo protein in adaptin. Consequently, the endocytosis of cargo proteins was curtailed. These cargo proteins, such as TGFβ receptor (TGFβR) and BubR1, are capable of regulating cell proliferation and differentiation, and thus affecting testicular development and spermatogenesis (Figure 8).

Spermatogenesis begins at puberty so there may be some growth inhibitory mechanism to prevent prepubertal spermatogenic cell proliferation. TGFβ is generally considered as a potent growth inhibitor and differentiation factor [17–19]. It has recently been revealed by Ingman that members of the transforming growth factor beta (TGFβ1, TGFβ2 and TGFβ3) play a key role in testicular development and spermatogenesis. It is responsible for the appropriate development and prevention of premature onset of spermatogenesis [20]. TGFβ reduced the number of gonocytes cultured *in vitro* by increasing apoptosis [21]. Yao *et al.* have proved that an N-terminal trunk domain of Ap2β was the only direct binding site of TGFβR. Shortened regions in the trunk domain may reduce the affinity of Ap2β with TGFβR.

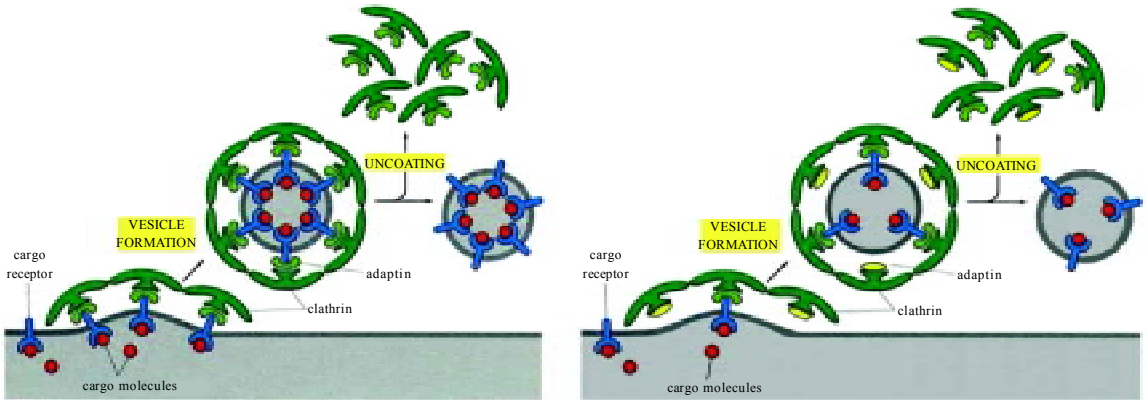

Figure 8. A hypothetical model for membrane traffic mediated by AP2β-NY in the different developmental stages of testes. Left [23]: in embryo testes, AP2β links cargo protein and clathrin, transducing signals into cells. Right (edited from left): in adult testes, AP2β-NY was increasingly expressed. Compared with AP2β, AP2β-NY is shortened in the trunk domain, which may result in a decreasing affinity with cargo protein in adaptin. Consequently, the endocytosis of cargo proteins was curtailed. These cargo proteins, such as TGFβ receptor and BubR1, are capable of regulating cell proliferation and differentiation, and thus affecting testicular development and spermatogenesis.

Fewer quantity of TGFβR could be transported into the cytoplasm. Accordingly, the inhibitory effect of TGFβ on gametogenesis was weakened, and at the same time spermatogenesis started. Although much attention was paid to the signal transduction of TGF, the mechanism is still unclear. Here, the existence of Ap2β splicing variant may, to some extent, shed light on how the signal of TGF is down-regulated not only in testes but also in other tissues containing Ap2β-NY. Of course more evidence is needed to support the hypothesis. In addition, the trunk domain of Ap2β was also the only binding site of mitotic checkpoint kinase BubR1 and Bub, both of which participate in the regulation of cell division [22]. It is suggested that Ap2β and its new isoform, Ap2β-NY, regulate the transportation of some other signal proteins in male gametogenesis. The disordered expression of Ap2β-NY in infertile patients also indicated its function in gametogenesis. Further investigation on the isoform of Ap2β should be valuable to elucidating the mechanism of spermatogenesis and infertile disease.

In summary, there is a novel splicing isoform of Ap2β, Ap2β-NY, in human testes. With regard to Ap2β and its cargos such as TGFβ and BubR1, the splicing isoform Ap2β-NY may, cooperating with Ap2β, regulate the transportation of signal proteins and thus play an important role in spermatogenesis and testicular development.

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