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Characterization of a Novel Human Testis-Specific Gene: Testis Developmental Related Gene 1 (TDRG1)

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Spermatogenesis is a highly coordinated physiological process that requires the correct expression and functions of thousands of developmentally regulated genes. The regulation of spermatogenesis is not well defined, since majority of the related genes have neither been identified nor fully characterized. Hence, it is meaningful to identify and characterize these genes to reveal the mechanism underlying spermatogenesis. In this study, using digital differential display, we identified a novel human testis-specific gene, testis developmental related gene 1 (TDRG1, GenBank DQ168992), via electronic subtraction of human testis UniGene databases from those of non-reproductive tissues. The transcript of the TDRG1 gene has an open-reading frame that encodes 100 amino acids. We next prepared the anti-TDRG1 monoclonal antibody 10B6 and confirmed that it specifically recognizes an 11-kDa protein in the tissue extracts from an adult human testicular sample (age 31 years) by Western blot analysis. RT-PCR coupled with immunohistochemistry of human tissues demonstrated that TDRG1 is exclusively expressed in the testis but not in any other non-reproductive tissues. TDRG1 is mainly located in spermatogenic cells in seminiferous tubules of adult testis. Furthermore, TDRG1 shows the highest expression level in human post-puberty testis, with the expression levels decreasing afterwards with aging. Importantly, TDRG1 mRNA is undetectable in the fetal testis, as judged by RT-PCR. In conclusion, TDRG1 is a developmentally regulated testicular-specific gene. We suggest that TDRG1, a newly identified testis-specific gene, may play important roles in human spermatogenesis.

Keyword: cloning; expression; gene; spermatogenesis; testis
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Spermatogenesis occurs in successive mitotic, meiotic and postmeiotic phases in the seminiferous epithelium of the testis, and it is a highly developmentally controlled process that relies on the spatiotemporal expression of specific genes directing a number of distinct regulatory pathways (Sassone-Corsi 1997). The expression of a set of germ cell-specific genes in pachytene spermatocytes usually triggers the expression of post-meiotic transcription for many downstream genes that are required for morphological and biochemical reconstructions in spermatids (Sassone-Corsi 2002). These germ cell-specific transcripts mainly fall into three categories: (1) Homologs of genes expressed in somatic cells with different spatiotemporal expression patterns or alternative conformations/functions; (2) Unique genes that are expressed only in male germ cells without homologs in other cells; (3) Alternative transcripts in testicular tissue that are different in size and/or overall sequences of the same gene transcripts in somatic cells (Eddy 2002). Thus, identification and functional characterization of these germ cell-specific genes are crucial for revealing the mechanism underlying spermatogenesis.

A large number of genes, such as many germ-cell unique genes (Hong et al. 2005), general transcriptional factors (Kolthur-Seetharam et al. 2008), kinases/enzymes (Esposito et al. 2004; Miki et al. 2004), hormones (Kendall et al. 1995) and membrane proteins (Griswold 1995), have been proposed to be involved in different stages of spermatogenesis from mitosis to spermiogenesis in various species. Their expression levels are developmentally regulated and stage specific, which determines the unique developmental process of the germline. However, little is known about genes influencing spermatogenesis in humans. Only a limited number of genes have been fully characterized, and thus many genes related to spermatogenesis need further functional determination or remain to be identified.

In this study, we take the advantage of a computerized program, digital differential display (DDD) (Scheurle et al. 2000; Olesen et al. 2001; Yan et al. 2002), to compare the

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differences of gene expression profiles among cDNA libraries from various tissues. We screened and cloned a novel human testis-specific gene: testis developmental related gene 1 (TDRG1). We have demonstrated that TDRG1 is a human testis-specific gene and may play important roles in normal human spermatogenesis.

Materials and Methods

Clinical Biopsy Samples

This study was approved by the National Scientific Council of China and the Institutional Review Board of XiangYa Medical School, Central South University. All human embryonic specimens were collected from spontaneous or medically indicated abortions. Specimens from various tissues of mature healthy human were obtained at autopsies from individuals who died from motor vehicle collisions. Consent has been obtained from all patients or their families. All procedures are in accordance with the China legislation and supervised by the Ethical Committee of Central South University.

DDD

DDD, a bioinformatics tool available at UniGene division at the NCBI server (http://www.ncbi.nlm.nih.gov/UniGene/ddd.cgi?ORG=Hs), allows us to compare the differences in frequencies of cDNA and expressed sequence tags (EST) among libraries from various tissues. The DDD analysis was carried out based on the procedure described in the handbook at the NCBI server. Briefly, in order to identify the genes that may contribute to unique characteristics of testis tissue, electronic subtraction was performed between 9 human testis cDNA libraries (pool A), and 76 cDNA libraries (pool B) from other human tissues and cell lines including heart, spleen, liver, lung, brain, skin, kidney, intestine, ovary, prostate, etc via Unigene database at the NCBI server. The candidate transcripts with abundant expression profiles only in testis but not in any other tissues were selected for further analysis.

Amplification and determination of TDRG1 gene

Based on putative TDRG1 gene transcripts (BC071820.1, BC033995.2 and BC042123.1), two specific primers (P1: 5′-AAG ATCAGGACTGCTG-3′ and P2: 5′-AAAAAAGAATGACATAG-3′) were designed to amplify the hypothetical full-length cDNA of TDRG1. We initially amplified putative full length TDRG1 gene from Marathon-Ready Testis cDNA library (Clontech, CA, USA) using above specific primers. The PCR amplification was performed at 95°C for 1 min, followed by 35 cycles of 95°C for 60 s, 50°C for 60 s, and 72°C for 90 s, with a final extension at 72°C for 5 min. The PCR yielded a product of 1.1-kb size and the fragment was purified from the gel using MinElute Gel Extraction Kit (Qiagen, CA, USA). The PCR fragment was further cloned into pUCm-T vector (BioMatik, DE, USA) and confirmed by DNA sequencing. The TDRG1 gene was identified after comparison of sequence from amplified fragment to the theoretic sequence obtained from DDD analysis.

Real Time Reverse Transcriptase-PCR

Total RNAs from normal human testis, heart, liver, brain, lung, spleen, kidney, epididymis, and different aging stages of testis tissues, were isolated using PURESCRIPT RNA isolation kit (Gentra System, Inc. MN, USA). cDNAs were synthesized from total RNAs obtained from above tissues using SMART PCR cDNA Synthesis Kit (Clontech, CA, USA) according to manufacturer's protocol. Two TDRG1 specific primers (P1: 5′-AAGAGGAGGGAGGCAGTCT-3′; P2: 5′-GCCCAATTCCTCTTGACTGA-3′) were used to yield a 320-bp product for regular RT-PCR and real time RT-PCR analysis. The real time RT-PCR was conducted on Mastercycler ®ep gradient (Eppendorf, Hamburg, Germany). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, a positive control, was amplified simultaneously in the same reaction as the internal control using two specific primers (P3: 5′-CCACCCATGGCAAATTCCATGGCA-3′; P4: 5′-TCTAGACGGCAGGTCAGGTCACC-3′; a 600-bp product) in regular RT-PCR, or was amplified separately from amplification of TDRG1 in real time RT-PCR. Each real time PCR reaction was perform in a 20 μl reaction volume containing 200 ng cDNA template, 10 μl SYBR® Green Realtime PCR Master Mix (BioRad, CA, USA), and 10 pmole of each primer. Amplification was performed at 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 57°C for 15 s, and 72°C for 45 s, with a final extension at 72°C for 5 min. The cycle threshold (Ct) number at which fluorescence crossed a prescribed background level is used for relative quantification (Tan et al. 2003). The relative ratio of target mRNA against internal control is determined by the equation of 2-(target gene Ct - internal control Ct). The data was then normalized to TDRG1 mRNA level in puberty group that was set as 100%. The regular RT-PCR was conducted by using SMART cDNA PCR kit (Clontech, CA, USA), the same primers and amplification program stated above. The PCR products were analyzed on 2% agarose gels.

Northern Blot Analysis

The expression profiles of TDRG1 gene among various tissues were determined by Northern blot using the amplified 320-bp TDRG1 fragment as a probe. The β-actin gene was used as an internal control to assess the equal loading quantity for each sample. The total RNA isolated from each tissue was heated at 65°C for 5 min and separated on a 1.2% agarose gel containing 1.8% formaldehyde. The total RNAs were transferred a Hybond-XL membrane (Amersham Bioscience). Total RNA (20 μg) from each tissue was loaded for each lane of Northern blot. Labeling of the probes, hybridization and signal detection were performed using DIG-High Prime DNA Labeling and Detection Starter Kit II based on manufacturer's manual (Roche, Mannheim, Germany).

Preparation of Monoclonal Antibody against TDRG1 and Western Blot Analysis

TDRG1 open reading frame (ORF) with 6×His tag at 3′ end was cloned into pET21 prokaryotic expression vector (Novagen, CA, USA). The recombinant vector was transformed into BL21 (DE3) and TDRG1 expression was induced by IPTG. Recombinant TDGR1 protein was purified by TALON column (Clontech, CA, USA) based on the protocol described by others (Nie et al. 2006). The purified TDGR1 was used for immunization of BALB/C mouse to produce the monoclonal antibody according to the protocol described by Schook et al. (1987).

Human testis specimens obtained at biopsies were homogenized in lysis buffer [50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM Na3VO4, and Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany)]. The tissue lysates were boiled for 5 min in loading buffer and subject to load on 10% SDS-PAGE. The proteins were transferred to PVDF membrane (BioRad, CA, USA). The membrane was

blocked with 5% milk/PBS containing 0.1% Tween 20 buffer for 1 hour followed by incubation with 1:1,000 dilution of TDRG1 monoclonal antibody for 2 hours. After three time post-primary antibody washes, the secondary HRP-anti-mouse antibody (1:10,000 dilution) (Santa Cruz Biotechnology, CA, USA) was incubated with PVDF membrane for 1 hour. The membrane was rinsed with PBS three times. Immune complexes were visualized using enhanced chemoluminescence kit (Amersham Biosciences, NJ, USA).

Immunohistochemistry

Normal human testis, kidney, heart and spleen specimens were obtained at biopsies. Frozen sections were fixed in 4% buffered paraformaldehyde and embedded in paraffin. Immunohistochemistry was performed on 3-μm-thick sections. The sections were sequentially deparaffinized, rehydrated and washed. The epitope retrieval was performed in citrate buffer (0.01 M, pH 6.0) by high-pressure heating method. After washing, sections were treated by 3% H2O2 for 10 min at room temperature to block endogenous peroxidase. The sections were blocked by normal goat serum for 30 min, and were incubated with anti-TDRG1 monoclonal antibody (1:1,000 dilution) at 4ºC overnight, followed by adding biotin-conjugated rabbit anti-mouse secondary antibody (1:250 dilution) (Vector Laboratories, CA, USA) for 20 min at room temperature and then for streptavidin-peroxidase treatment for 10 min at room temperature (Vector Laboratories, CA, USA). Peroxidase activity was detected using DAB kit (Vector Laboratories, CA, USA). The DAB reaction was stopped by washing in tap water and the sections were counterstained in hematoxylin for 3-5 min. Sections then were dehydrated through series ethanol solutions and xylene, and mounted in Permount.

Statistical Analysis

All data were expressed as the mean ± SEM. Data were analyzed for statistical significance using GraphPad Prism 4 software (GraphPad Software, San Diego, CA). Statistical significance of the difference between the mean values of multiple groups was tested by one-way ANOVA, followed by Tukey-Kramer post-tests. A value of $P < 0.05$ was considered significant.

Results

Molecular cloning of TDRG1 via DDD

A total of 363 ESTs have been shown with abundant expression profiles in testicular tissue with the DDD analysis between the testicular EST library and the cDNA libraries from other non-reproductive tissues. Among them, 168 ESTs are known genes that fall into the following categories: enzymes, ribosome proteins, receptor/membrane proteins, ligands and secreted proteins. The remaining 195 ESTs may represent novel genes with > 10-fold higher expression levels in testis than in non-reproductive tissues. The EST Hs. 180197 shows the highest possibility to be a new testicular specific expression gene. After analyzing three partially overlapped sequences BC071820.1, BC033995.2 and BC042123.1 in this EST cluster, we obtained a 1.1-kb theoretical full-length cDNA sequence and predicted this is a novel gene. We designed two specific primers to amplify the predicted full-length cDNA from the testicular cDNA library. A 1.1-kb PCR product was obtained and subsequently cloned into a T-vector. After sequencing, we confirmed it is a new full-length cDNA, matching the same sequences we predicted. The transcript of new gene has an ORF of 303 bp (504-806 bp) that encodes 100 amino acids. It is located at 6p21.1-p21.2 spanning 1.18 kb with 2 exons and 1 intron. There are five predicted phosphorylation sites in this protein: T16, S58, S60, S91, and S96. This protein is not homologous to any other known protein. We named this new gene as Testis Developmental Related Gene 1 (TDRG1), with a GenBank registration number as DQ168992. The cDNA and protein sequences of TDRG1 are shown in Fig. 1.

TDRG1 is a tissue-specifically expressed gene in testis

In order to examine whether TDRG1 is uniquely expressed in the testicular tissue, we performed semi-quantitative RT-PCR and Northern Blot to analyze the TDRG1 mRNA levels from various tissues. We collected various tissues from human aging from 25 to 32 for semi-quantitative RT-PCR, Northern Blot analysis and immunostaining experiments. We tested 3 samples from different people for each organ. QRT-PCR results showed TDRG1 mRNA was only detected in testicular tissue but not in other tissues such as heart, liver, brain, epididymis, lung, kidney and spleen (Fig. 2A). The full-length transcript of TDRG1 was examined by Northern blot analysis from various tissues using a DIG-labeled TDRG1 specific probe. Consistent with QRT-PCR results, a 1.19-kb transcript was only detected in testicular tissue but not in any other tissues such as brain, heart, liver, lung, spleen, kidney and skeletal muscles (Fig. 2B). Thus, the TDRG1 gene is specifically expressed in testis.

We next examined the TDRG1 protein expression pattern in the testicular tissue. We raised a monoclonal antibody against TDRG1 using recombinant TDRG1 protein expressed and purified from E. coli. After immunization of BALB/C mouse and screening hybridoma cells, we obtained two hybridoma lines 10B6 and 5C5. The subtype for both hybridoma lines of anti-TDRG1 monoclonal antibodies was IgG1. The highest dilution that could recognize the purified TDRG1 protein for both lines antibodies was 1:1.6 × 106 using ELISA method (data not shown). We here used clone 10B6 for the later detection of TDRG1 protein levels in various tissues in this study.

An 11-kDa protein was detected from an adult human biopsy testicular tissue (age 31 years) on Western Blot with the monoclonal antibody 10B6 (Fig. 2C), which was consistent with the theoretical TDRG1 protein size. We repeated the experiments with 3 human biopsy testicular tissues by Western blot analysis. Immunohistochemistry was used to examine cellular locations of TDRG1 protein in human biopsy testicular tissues. The TDRG1 protein was detected in the primary and secondary spermatocytes in seminiferous tubules and localized in cytoplasm, but not in sertoli and germs cells in the adult normal human testis (Fig. 3A). About 27.0% of spermatogenic cells are TDRG1 positive (n = 185 out of 686 cells). TDRG1 expression was not

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1     aagatcaggactgctgaaagaatgaagaagaacttacttggcctaggatg   50
51    tggctagagaacggagcgcactttcacttacctggcaaggtggttttgga  100
101   agtttccacggaagccttcccagggccgctgctgcccgtcctcgcctagg  150
151   tgtggagcttcccgaccggctggggagggaatgtcctgcgggagccgccg  200
201   aggaccctctttagcctcttccctggcttggctgcagctgcagtctggta  250
251   ctcgcccattctgctcttcttcacctccgtattttctctctcacggaaga  300
301   agaattccattctcccatcccaggaaggagagtgccctgcgtgccacgaa  350
351   agagcccaggacccacaggacaaatacgtcactggcagacctgccttcgc  400
401   cagcgccagcccatctctgaaacctccagcatttgtgccccggtccggcc  450
451   cctcccaggcctgactctttccgtgaacggtcactgcgcaggatcaagct  500
501   acaatgaagaggagggaggcagtctgcgcgcaccgccattttctaggaac  550
          M  K  R  R  E  A  V  C  A  H  R  H  F  L  G  T
551   tgggaagccccccccacccctttaggaagatccatccctgtggaaccttgcc 600
        G  K  P  P  H  P  L  G  R  S  I  P  V  E  P  C
601   caggcttaccagcctttgctgaggttgatctattgtccctccttgtcccc  650
      P  G  L  P  A  F  A  E  V  D  L  L  S  L  L  V  P
651   atcaaaatatccagcactccaccttcagggagtagacttgaccctcaaat  700
       I  K  I  S  S  T  P  P  S  G  S  R  L  D  P  Q  I
701   agcaagttcagccttcccaggtctaggttccctgggaggtcaagattcgt  750
        A  S  S  A  F  P  G  L  G  S  L  G  G  Q  D  S
751   ctggttccttagtacagagggctagctgtgagttggaatccccctatgag  800
      S  G  S  L  V  Q  R  A  S  C  E  L  E  S  P  Y  E
801   ctttagaatcagtcaagaggaattgggccccttcccttcatccctcttct  850
       L  *
851   tttccctttttgtcccagagctcagctctgactcaaaagtttttccattt  900
901   accatcaacatggaaacttggctcctcacgtaggtatattatcccccttt  950
951   tgtacatggtcttgttgatccaaactccctttctgtgaaagaggcctgtg 1000
1001  gggctcaagaagcctggtcagccagccaggctagtcccacatacctcaga 1050
1051  accagtttaataaaggctctatgtcattctttttaaaaaaaaaaaaaaa 1100
1101  aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 1150
1151  aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa  1197
```

* Terminator codon

Fig. 1. cDNA and protein sequences of TDRG1.

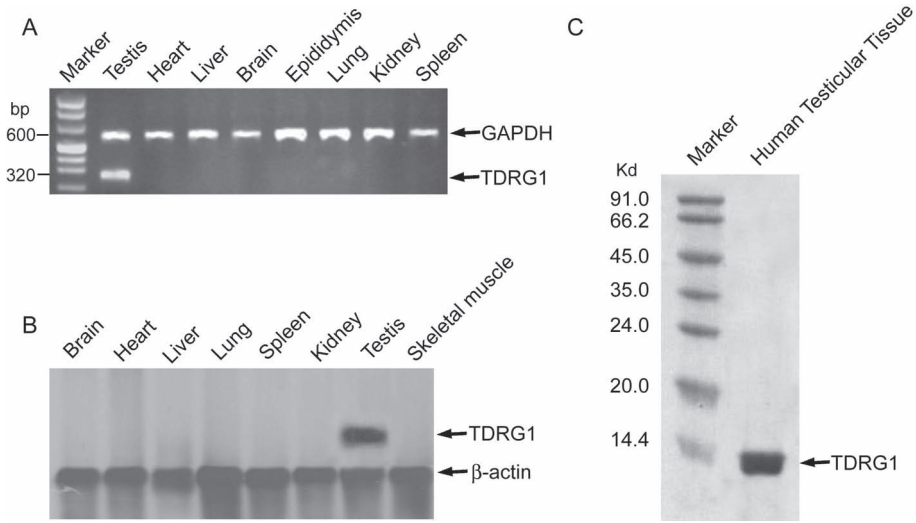

Fig. 2. TDRG1 mRNA and protein are specifically expressed in human testis but not in non-reproductive tissues.
A. TDRG1 mRNA is detected from testis but not any other tissue by RT-PCR analysis. GAPDH mRNA is amplified from each tissue as internal control.
B. Analysis of TDRG1 mRNA levels in various human tissues by Northern blot using a DIG-labeled TDRG1 specific probe. Full length TDRG1 mRNA is detected in testis only but not in any other tissue. β-actin mRNA was detected using DIG-labeled β-actin specific probe as an internal control.
C. TDRG1 protein was detected by the specific monoclonal antibody 10B6 from normal human testicular tissue (31 years old male testis sample).

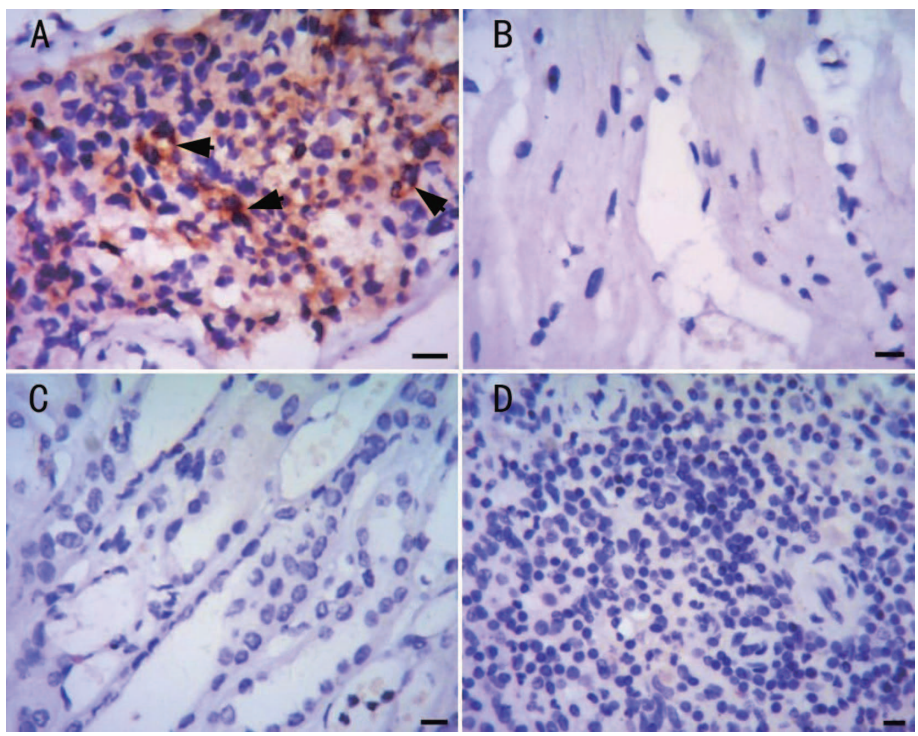

Fig. 3. TDRG1 protein is expressed in human testicular spermatogenic cells, but not in non-reproductive tissues. Immunohistochemistry was performed to detect TDRG1 protein levels in the frozen sections from various human tissues using a specific monoclonal antibody 10B6.
A. TDRG1 is expressed in the cytoplasma of spermatocytes in seminiferous tubules, but not in sertoli and germ cells, in the normal human testis (age 31 years).
B-D. TDRG1 expression is undetectable in human heart (B), kidney (C) and spleen (D). The brown cells are TDRG1-positive cells that are indicated by arrows. Scale bar: 40 μm.

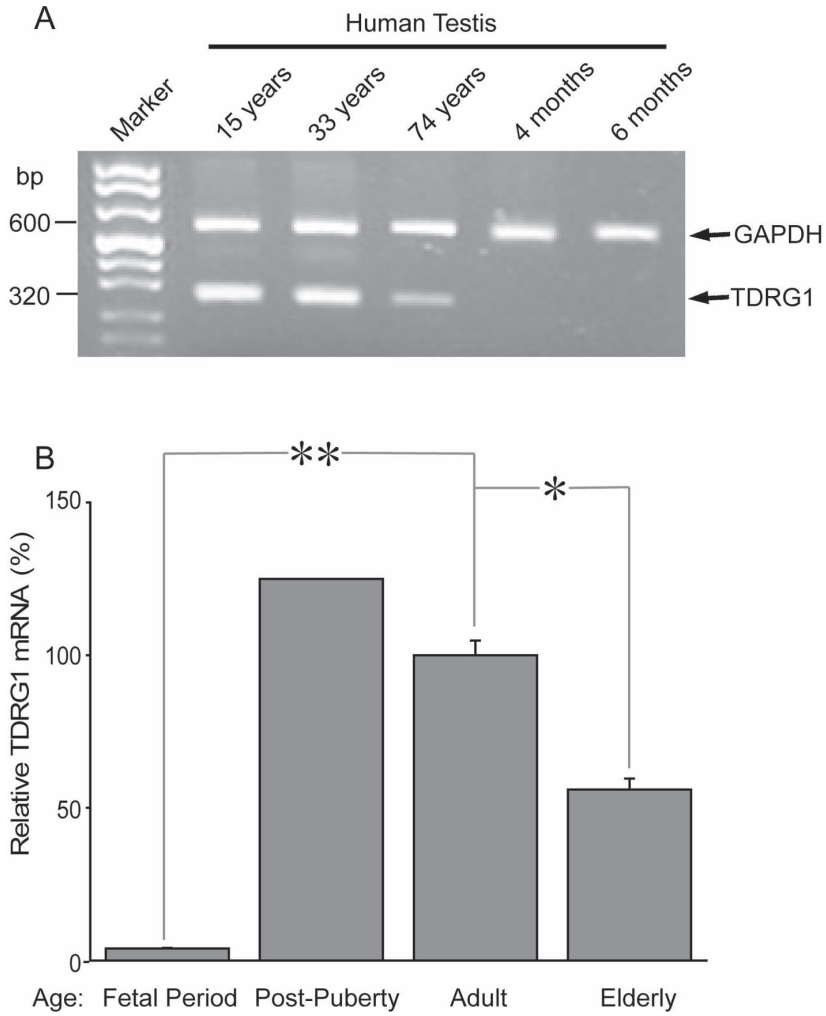

Fig. 4. TDRG1 expression is related to different aging stages of human testis.

A. TDGR1 mRNA levels from human testis of different ages (4, 6 months, 15, 33 and 74 years old) were analyzed using RT-PCR. GAPDH mRNA was used as an internal control.

B. Real time RT-PCR analysis of TDRG1 mRNA levels in human testis for following aging: fetal period (4, 6, 7, 8, 8 and 9 months, $n = 6$), post-puberty stage (15 years old male, $n = 1$), adult (24, 31, 33 and 35 years old males, $n = 4$) and elderly group (74, 79, 65 and 69 years old males, $n = 4$). The data was normalized to relative TDRG1 mRNA level of adult group set as 100%. TDRG1 mRNA shows very low expression level in the fetal period of human testis (4.0 ± 0.1%, $^{**}P < 0.05$) and decrease in elderly group (56.0 ± 3.6%, $^{*}P < 0.05$) comparing with those in adult male testis (100.0 ± 4.8%). Values are presented as mean ± S.D.

detected in heart, kidney, and spleen (Fig. 3B-D). These results confirmed that the TDRG1 protein is exclusively expressed in human testis.

TDRG1 expression pattern is associated with testis aging stages

The TDRG1 expression levels during different testis aging stages were investigated by real time RT-PCR. We extracted total RNAs from human biopsy testicular tissues from 5 different ages: fetal period (4, 6, 7, 8, 8 and 9 months, $n = 6$), post-puberty stage (15 years old male, $n = 1$), adult (24, 31, 33 and 35 years old males, $n = 4$) and elderly group (74, 79, 65 and 69 years old males, $n = 4$). The TDRG1mRNA was barely detected in the testicular tissues during fetal period (4.0 ± 0.1%, $^{**}P < 0.05$ comparing to adult male testis). Its expression in adult men testicular tissues (100.0 ± 4.8%) is higher than elderly men (56.0 ± 3.6%, $^{*}P < 0.05$, Fig. 4). It has the highest expression level in the 15 years old testicular tissue, but we only have one sample from this group and we do not have further information whether he has pubertal or adult spermatogenesis, thus we can not draw a reliable conclusion for this. Nevertheless it indicates the tendency that TDRG1 may reach its peak expression during post-puberty. These results suggest TDRG1 expression is related to certain subtypes of more mature germs cells during development, spermatogenesis and aging.

Discussion

In the present study, we identified human TDRG1, a

testicular specific gene, and characterized its expression pattern in various human tissues and different ages of human testicular biopsy samples. The expression of TDRG1 is found only in testicular tissue, not in other human organs, which confirms it is a unique gene in human germ cells. The germ cell-specific and developmental regulated genes underlying the intrinsic genetic network are the primary regulators of spermatogenesis. Their developmentally regulated and stage-specific expression patterns determine the unique developmental sequence of spermatogenesis, such as from mitotic, meiotic to postmeiotic phase (Eddy 2002; Sassone-Corsi 2002). Testicular unique genes are mostly expressed during the postmeiotic phase, when the spermatozoa are formed with the specialized morphological transformation (Eddy 2002). These genes play significant roles in remodeling of the nucleus, condensation of the chromatin and assembling of some specialized structures during spermatogenesis. For example, unique genes encode SCP1 and COR1 form the major components for synaptonemal complex (Meuwissen et al. 1992; Dobson et al. 1994), one of unique structures present during the meiotic phase. As one of the unique germ cell genes, TDRG1 is expressed in spermatogenic cells, but not in Sertoli cells, with the highest expression level during human puberty stage, and decreasing levels of expression with aging. Our data suggests that TDRG1 expresses in certain types of germs cells during spermatogenesis and may play a role in normal human sperm formation and fertilization.

There are a large number of genes involved in spermatogenesis. The functions of many of these genes still remained to be defined and characterized. It is believed that many genes related to human spermatogenesis remain unknown thus to be discovered. It is of great significance to identify these germ-cell specific genes and characterize their roles in spermatogenesis, which will provide essential information to reveal the causative genes for human infertility. The completion of high throughput genomics projects and construction of many ESTs databases, such as UniGene, has greatly inspired the actions of identification of cell- and tissue-specific transcriptome genes for spermatogenesis. UniGene is featured with tissue types of gene expression profiles and gene-oriented clusters that each one likely represents a single gene. Combined with other powerful bioinformatics tools, such as DDD, UniGene has been successfully screened in discovering many germ cell- and testis-specific novel genes that provide fundamental insights into physiological process of spermatogenesis (Olesen et al. 2001; Fu et al. 2003; Li and Lu 2004; Yang et al. 2005). In the present study, we performed electronic subtraction between 9 human testis cDNA libraries and 76 cDNA libraries from other human tissues and cell lines including heart, spleen, liver, lung, brain, skin, kidney, intestine, ovary, prostate, etc via UniGene databases at NCBI servers, and initially obtained 195 ESTs that may represent novel genes with significant higher expression levels in testis than in any other nonreproductive tissues. After further characterization, we identified TDRG1 as one of the testis-unique genes.

Collectively, we discovered a novel human testicular-specific gene, TDRG1. TDGR1 is exclusively expressed in testicular tissue, in spermatogenic cells. TDRG1 shows the highest expression level in human post-puberty testis biopsy tissue, with expression levels decreasing afterwards with aging, but not expressed in fetal period of human testis. Our study suggests TDRG1 is a developmentally related testicular-specific gene. We predict that TDRG1 is involved in normal human spermatogenesis and fertilization. The characterization of the function of TDRG1 is our next focus.

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Conflict of Interest

The authors declare (1) no conflict of interest that could be perceived as prejudicing the impartiality of the research reported; or (2) no any financial or other potential conflict of interest.

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