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Molecular cloning of a novel nuclear factor, TDRP1, in spermatogenic cells of testis and its relationship with spermatogenesis

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ABSTRACT

We reported the identification of a novel gene termed *TDRP* (encoding testis development-related protein) that might be involved in spermatogenesis. The human *TDRP* gene had two distinct transcripts, *TDRP1* and *TDRP2*, which encoded proteins of 183 aa and 198 aa respectively. *Tdrp* mRNA was predominantly expressed in testis tissue. We generated rabbit polyclonal antibodies specific against human TDRP1. Immunohistochemistry analysis showed TDRP1 was expressed in spermatogenic cells, especially with high expression in spermatocytes. We provided evidence that TDRP1 distributed in both cytoplasm and nuclei of spermatogenic cells. Expression patterns of *Tdrp1* mRNA and its protein were investigated in the rat testis tissues of different developmental stages. Both *Tdrp1* mRNA and its protein were barely detected in the testis of neonatal rats, increased remarkably at 3 weeks postpartum, and peaked at 2 months postpartum. We also investigated TDRP1 expressions in testis tissues of azoospermic men with defective spermatogenesis. Western blot analysis showed that TDRP1 expressions were significantly lower in the testis tissues of azoospermic men compared with normal controls. These current data demonstrated that as a nuclear factor, TDRP1 might play an important role in spermatogenesis.

Introduction

Genes expressed during spermatogenesis are commonly referred to as 'chauvinist genes' [1]. These developmentally regulated genes are either not expressed in somatic cells or produce unique mRNAs in germ cells. Many such chauvinist genes have been cloned and confirmed to be involved in spermatogenesis [1]. However, there are likely to be other genes participating in the process remaining to be identified. Identification of these novel genes and understanding their roles are critical for the biology of spermatogenesis. In previous studies by our group, gene expression profiles of human hypothalamus–pituitary–adrenal axis and insulinoma tissues had been established and several novel full-length cDNAs were cloned from cDNA libraries of those tissues [2–4]. In the present study, a novel full-length cDNA named as *TDRP* (encoding testis development-related protein) was cloned from a cDNA library of human testis tissue. Bioinformatics analysis illustrated the gene structure and predicted the domains of TDRP. Northern blot analysis demonstrated that *Tdrp* mRNA was expressed abundantly in testis. Cellular and subcellular locations of TDRP1 were also investigated. Finally, its possible relationship with spermatogenesis was studied preliminarily.

Materials and methods

Animals. Sprague Dawley (SD) rats obtained from the Experimental Animal Center, Shanghai Medical College of Fudan University, aged from 2 days postpartum to 18 months postpartum, were used for these studies. All experimental procedures were approved by the Shanghai Animal Care and Use Committee on Animals and followed the policies issued by the International Association for the Study of Pain on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the numbers of the animals used.

Cell culture. GC-1 spg, GC-2 spd(ts), TM3, TM4, and HeLa cells from American Type Culture Collection (Manassas, VA) were maintained as subconfluent monolayers in DMEM (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Hyclone, Logan, UT) and 100 units/ml penicillin plus 100 μg/ml streptomycin (Invitrogen) at 37 °C with 5% CO_2. Total RNA and protein were extracted from these cells by using Trizol reagent (Invitrogen) and RIPA buffer (Pierce, Rockford, IL) respectively.

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Bioinformatics analysis and cloning of the full-length cDNA of TDRP. A cDNA library of human testis was screened and a novel EST was selected. *In silico* cloning of the full-length cDNA of human *TDRP* gene was performed as described previously [4]. Nucleic sequences of *TDRP* were amplified by reverse transcription polymerase chain reaction (RT–PCR) with the following primers: forward, 5′-GCCTGACCATGTGGAAGCTG-3′, reverse primer: 5′-GGAGGA ACCCTTCTCTGAAGCAC-3′. The PCR product was recovered and purified using QIAquick gel extraction kits (Qiagen, Chatsworth, CA) and then sequenced using an ABI 3770 sequencer (Applied Biosystems, Foster City, CA).

Semiquantitative RT-PCR. One microgram of total RNA extracted from GC-1 spg, GC-2 spd, TM3, and TM4 cells was reverse-transcribed with oligo(dT) by using Omniscript® Reverse Transcription kit (Qiagen). One microliter of RT product was amplified with primer pairs specific for mouse *Tdrp1* gene by using HotStarTaq® PCR kit (Qiagen). PCR conditions and primer sequences were available on request. Each RT-PCR product was loaded on 1.5% agarose gel containing 0.5 μg/ml ethidium bromide. Gel images were made and saved by Gel Documentation System (UVP, Upland, CA).

Northern blot analysis. Target gene fragments of rat *Tdrp1*, β-actin and human *TDRP1* were cloned into pDrive vectors (Qiagen) and confirmed by restriction enzyme digestion and sequencing analysis. Digoxigenin (DIG)-labeled probes were generated by transcription with SP6/T7 RNA polymerase using the DIG RNA labeling kit (Roche Diagnostics, Indianapolis, IN). Northern blotting was performed using the nonisotopic DIG Northern Starter kit (Roche Diagnostics) as described previously [4].

Generation of rabbit polyclonal antibodies against human TDRP1. Based on the sequence information of TDRP shown in Fig. 1, polypeptides specific to TDRP1 (RQSKGHLTDSPEEAE) were synthesized (GenScript, Piscataway, NJ). 500 μg polypeptides were emulsified in complete Freund's adjuvant and injected subcutaneously into a 6-month-old male rabbit. Additional immunizations with 250 μg were administered on days 14, 35, and 56 in incomplete Freund's adjuvant. Serum was collected 1 month after the last immunization, and then polyclonal antibodies against TDRP1 were purified with antigen affinity. Antibody titers were tested by enzyme-linked immunoabsorbent assay.

Immunohistochemical analysis. Briefly, fresh testis tissues of a 2-month SD rat were fixed in 4% paraformaldehyde solution at 4 °C for 24 h, and then paraffin-embedded sections (5 μm) of the rat testis were incubated with rabbit anti-TDRP1 polyclonal antibody (dilution of anti-TDRP1 is 1:400). TDRP1 staining was visualized using 3,3′-diaminobenzidine, and the sections were counterstained with hematoxylin. The sections were then observed and imaged by using a Leica microscope (Leica Microsystems, Watzlar, Germany).

Immunofluorescence analysis. HeLa cells were seeded onto sterile, acid-treated 12-mm coverslips in 24-well plates. After fixed in freshly prepared 4% paraformaldehyde and blocked with 0.05% Tween 20 in PBS (TPBS) with 1% bovine serum albumin (Sigma–Aldrich, St. Louis), these cells were incubated with rabbit anti-TDRP1 polyclonal antibody in a humidified chamber for 1 h and then washed three times in TPBS. Primary antibodies were visualized using Texas Red-conjugated goat anti-rabbit IgG. DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma–Aldrich).

Confocal analysis. The complete coding sequences of human *TDRP1* gene introducing EcoR I and BamH I sites were obtained by PCR and subsequently subcloned into the pEGFP–C2 vector (Clontech, Mountain View, CA). The pEGFP–*TDRP1* construct was verified by sequencing. HeLa cells were transfected with pEGFP–*TDRP1* and pEGFP–C2 as a control using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Afterwards, DAPI (Sigma–Aldrich) and PKH26 (Sigma–Aldrich) were used to counterstain the nuclei and membrane of the cells respectively. Finally, cells were imaged using a Leica TCS SP2 confocal spectral microscope (Leica Microsystems).

Western blot analysis. Human testis tissues were obtained from patients with prostate cancer by operation and azoospermic men with defective spermatogenesis by testicular biopsy. The uses of those testis tissues were approved by Medical Ethics Committee at Huashan Hospital, Fudan University. Total proteins were extracted from testis tissues and the cell lines by using RIPA buffer (Pierce). Cytoplasmic and nuclear proteins were isolated from HeLa cells and rat testis tissues by using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce). Western blotting was performed as described previously [4].

Results

Cloning and bioinformatics analysis of TDRP gene

The human *TDRP* gene was mapped to chromosome 8p23 and had two distinct transcripts. The mRNA transcript of *TDRP1* was ~3.2 Kb and that of *TDRP2* was ~2.4 Kb. *TDRP1* had three exons and its ORF contained 549 base pairs (bp), which encoded a protein of 183 amino acids (aa). *TDRP2* was produced by splicing a fragment of 788 bp from exon 3 of *TDRP1*. *TDRP2* contained four exons and an ORF of 594 bp encoding a protein of 198 aa. The full-length cDNAs of *TDRP1* and *TDRP2* had been confirmed by sequencing. Fig. 1 demonstrated the nucleotide and amino acid sequences of human *TDRP*. The sketch of genomic structure of *TDRP* gene was shown in Supplementary Fig. S1. RT–PCR and northern blot analysis demonstrated that *TDRP1* was the predominant transcript of *TDRP* gene in human testis tissue (Supplementary Fig. S2). The sequence data of *TDRP1* and *TDRP2* have been submitted to GenBank under accession numbers AY194292 and FJ381685 respectively. When the nucleotide sequence of human *TDRP1* gene was subjected to BLAST searching in the Ensembl database (http://www.ensembl.org/), the homologous mouse (MGI symbol, 2610019F03Rik) and rat (RGD symbol, LOC498662) genes were found. Human TDRP1 shared 77% and 75% aa homologies with the mouse and rat ones respectively.

Tissue expression profile of Tdrp gene in rat tissues

The expression profile of *Tdrp* was investigated in multiple tissues of rat using northern blotting. Of the 13 rat tissues (skeletal muscle, stomach, spleen, brain, bladder, heart, pancreas, adipose tissue, small intestine, testis, kidney, lung and liver), northern blot analysis demonstrated that *Tdrp* mRNA was predominantly expressed in the testis, weakly expressed in adipose tissue and kidney, and essentially not detected in other tissues (Fig. 2A). *Tdrp* mRNA levels were also barely detected in rat ovary, uterus, adrenal gland (data not shown). Northern blotting also detected two transcripts of *Tdrp* mRNA in the rat testis, consistent with the results for human testis.

Expressions of Tdrp1 mRNA and its protein in spermatogenic cells

Immunohistochemistry was utilized to obtain a precise expression pattern of TDRP1 protein within the rat testis tissue. The results demonstrated that TDRP1 proteins were expressed in spermatogenic cells and not detected in Sertoli cells or other somatic interstitial cells, based upon nuclear morphology and position within the seminiferous tubules (Fig. 2B). In detail, spermatocytes expressed high levels of TDRP1 protein, whereas spermatogonia and spermatides expressed lower levels. We also performed experiments to investigate expressions of *Tdrp1* mRNA and its protein in some mouse testis-derived cell lines including

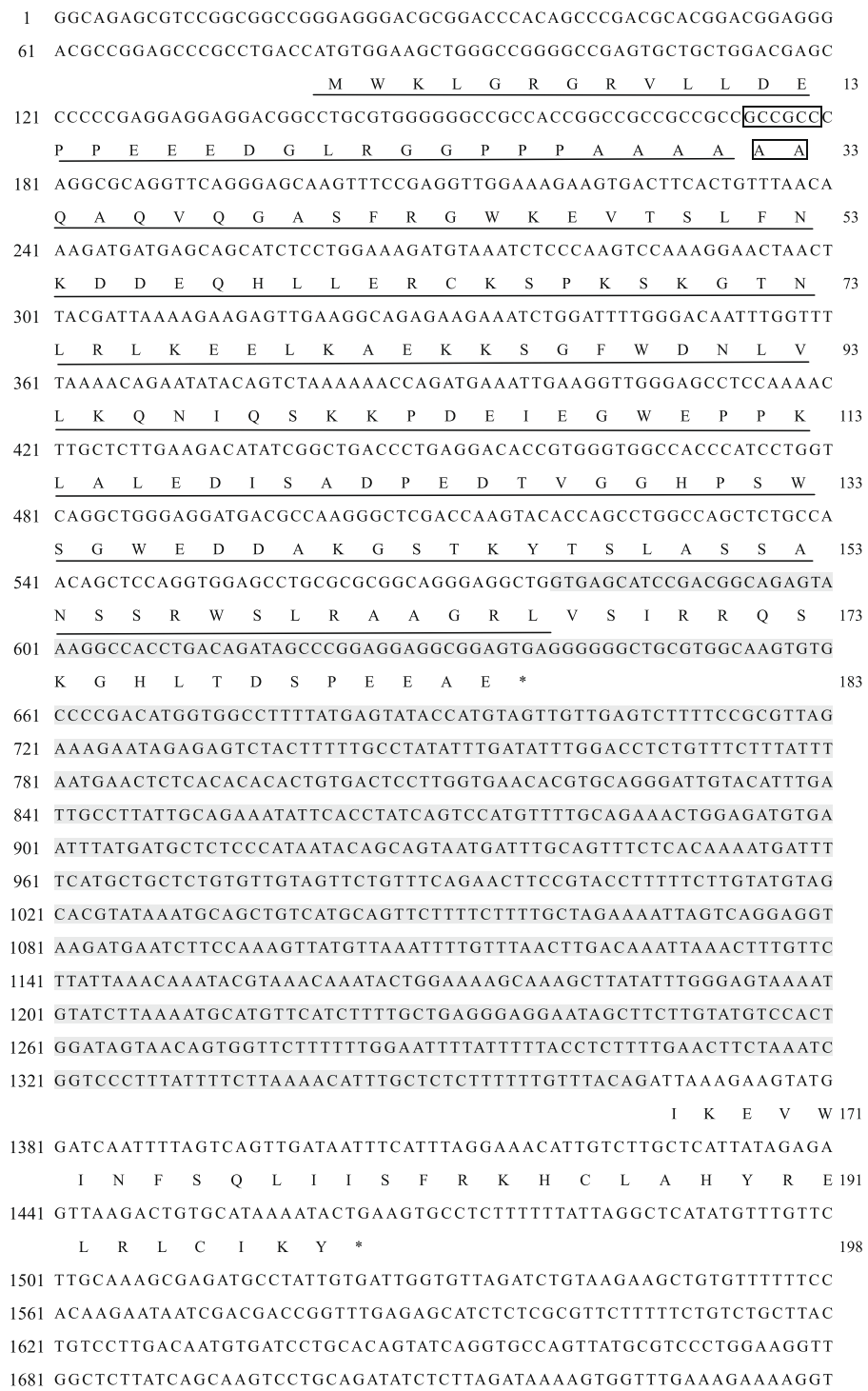

Fig. 1. cDNA sequences of the human *TDRP* gene. It had two distinct transcripts: one was ~3.2 Kb (*TDRP1*), the other ~2.4 Kb (*TDRP2*). The shaded nucleotide sequences were absent in the *TDRP2* transcript. The sequences of nucleotides (GCCGCC) and amino acids (aa) in boxes were absent in the *TDRP1* transcript. The deduced aa sequences of the ORF are numbered starting with the putative initiating methionine. The amino acid sequences (164 aa) shared by TDRP1 and TDRP2 are underlined. Translation of the *TDRP1* transcript stops at aa 183 (glutamic acid, E) and that of *TDRP2* terminates at aa 198 (tyrosine, Y). The polyadenylation signal sequences (AATAAA) are bolded.

GC-1 spg cells derived from type B spermatogonia, GC-2 spd cells derived from spermatocytes, TM3 cells derived from Leydig cells, and TM4 cells derived from Sertoli cells. The data in Fig. 2C and D demonstrated that expressions of *Tdrp1* mRNA and its protein could be detectable in both GC-1 spg and GC-2 spd cells, but undetectable in TM3 or TM4 cells, agreeing with the results shown in Fig. 2B.

TDRP1 acting as a nuclear factor

Online software-based prediction analysis (http://www.cbs.dtu.dk/services/NetNES/) showed that there was a typical leucine-rich nuclear export signal in the aa sequence of the TDRP1 protein (Supplementary Fig. S3). Confocal microscopy showed that the GFP–TDRP1 fusion protein was distributed in both cytoplasm

1741 ATATTTTTCTTTTTTGTTTAAAAGTAAAATAAAACCTCCAGTTACACTGTGCATTCCCCT
1801 CACGTAAAAACAAGACAAAAACCCTTTCCTGAGTTGCTCGGCATGCACTCCTGTGCTGTT
1861 TCATGCATGTGGATGTTCCTTCCGTTTGTTCCTGTGGAATAACTGAGTGTGCCTGATGGC
1921 AGAACACACTGCAGTGTTATCAGTGTCTGCATGTTTTTTAATAGAACAGGTTTACTTGAT
1981 CTGTCATCTGTTATGGAAAAAACAGCAATTACTTTTGCATCCATCTAGCTAAATCTATAA
2041 TCTGTGTCAATCACTTATACCTATCAATCATCCGTATCTATTCACCTGTCCTCTATGTCT
2101 GTCTTCTCTCAGTCTACATCCATCTAGCCTTCTGTCAATCATCTACTTTTTTTTTTAATA
2161 GAACAAGAAGTTTACTTATCAAGTCTTGAAAGGGGGACATGTTTCAATTGGTGTTCAAAC
2221 AAAAATTCCAGCTTGTATTAGAACCTTGAAATGGTGAAGTTGTGGAGGTTTTCTTTGTTC
2281 CATAATACAGAGACAAGTTCATAATTTTTAGTATAACAGCTAAGTTGACAAATTCTAAGT
2341 TTCCTCAGGTAAATAGTCGTAACTGTCCTTTTCCCTAGAGAAGTGCTTGCTGGGATAGTA
2401 AAAACATACACCATTTCATGACTTCTGCAAATACTTTCCAGCGGAAGTCAGTGTAGGTTT
2461 GTTTTTGGCTAACATGGTCTTGCTGCGCAGGAATGTAAACACTGTGTTTGAAACTCTGGA
2521 AATCACGTGTGTGGGGAGATGGGGACGCTTCCCATGTTGTGGGGAGCTCTGTGGCTGTGA
2581 TGGCTGCAGTTGCCGTGCCTCTGTTGGAACGCCAAGTGCCTGCAACTCACGTCAATCATA
2641 GAATTGTGACGCACAGTTGGCAAAATAGTTCTTTATGCTATTTCTCAAAATTTGAGGACA
2701 AACCCAGATTGGGATTGGAATATGCACTGTAAATCAAATTTTTCTTATCTACAAAGACTA
2761 ATGTAAAAATGATTTTTTCTTCTGTGCCTGATTAAATTAACTGTGGTTTTTAATATAAAT
2821 ATTTATTGGTGTGCTTTGGGAGAAAAATTATCTTTTCTTGAAAGAAGTTATCAAAGCAAA
2881 TTTATTATCTTCACAAGTTAATGGGAGAATGTGGTTTTGATTCTGGGTGTTTGAATTGTG
2941 TAAACACACAGCTTCCTTGTGTGAAGAGAGTTGTCCTGTGCTCCTTCTACTGTACTTTTT
3001 TTATTTTTTAATAGGAAAGTGATGTGCTTCAGAGAAGGGTTCCTCCTTAATGTTGAGGTT
3061 TTTTAAAAATAAAAATTAATGTTGAGGTTTTTTAAAAATAAAAATACTGTTTTTAAGCTC
3121 TTTCCTTATTGGTTTGCATGATTATTGGCATCTGCTTACGATGGCTTTAATCACTCAGAG
3181 CTAATGGCACCTTTCCTATTTAGCCTCATTTTAGAATGCAGTCAACACATGTAGACTTTT
3241 CACA**AATAAA**TGGAACAACTTCAAAAAAAAAAA

Fig. 1 (*continued*)

and nuclei of transfected cells (Fig. 2F-a–c). Furthermore, endogenous expression of TDRP1 in HeLa cells was investigated by immunofluorescence analysis. The results (Fig. 2F-d–f) demonstrated that endogenous TDRP1 distributed in both cytoplasm and nuclei of HeLa cells.

Additionally, We isolated cytoplasmic and nuclear proteins from HeLa cells and adult rat testis tissue, and then investigated the expression of TDRP1 in the cytoplasmic and nuclear extracts by western blotting. Although partial GFP could be detected in nuclear extracts of HeLa cells transfected with pEGFP–C2 vector, western blot analysis demonstrated that much more GFP–TDRP1 fusion proteins were expressed in nuclear extracts when compared with GFP expression pattern (Fig. 2E). Importantly, *in vivo* studies also indicated that TDRP1 protein could be detected in nuclei of adult rat testis, although most of TDRP1 expressed in the cytoplasm, comparing with expression patterns of well-known cytoplasmic marker GAPDH and nuclear marker histone H4 (Fig. 2E). Based on these results, it was concluded that TDRP1 distributed in both cytoplasm and nucleus, and might act as a nuclear factor.

Expression patterns of Tdrp1 mRNA and its protein with developmental stages of spermatogenesis

SD rats at ages of 2 days, 7 days, 3 weeks, 2 months, 6 months, and 18 months postpartum respectively were selected, and expression levels of *Tdrp1* mRNA and its protein in their testis tissues were investigated. The expression of *Tdrp1* mRNA or its protein was barely detectable on postnatal days 2 and 7, increased remarkably on week 3, and peaked in month 2 followed by a slight decrease with aging (Fig. 3A and C). Statistical analysis showed there were significant differences in *Tdrp1* mRNA levels between postnatal days 7 and 21 ($P = 0.002$), and between postnatal week 3 and postnatal month 2 ($P < 0.001$) (Fig. 3B). Differential expressions of TDRP1 protein were also detected in testis tissues of the above rats by using western blotting. The statistical results demonstrated that there were also significant differences of TDRP1 protein expression among the rat testis tissues of different developmental stages. Particularly, there were about 10-fold ($P < 0.001$) and 2.5-fold ($P < 0.01$) increases of TDRP1 protein levels in testis tissues of 2-month old rats when compared to those of 7-day and 3-week old rats respectively, and 3.8-fold ($P < 0.001$) increase at 3 weeks postpartum compared to 7 days postpartum (Fig. 3D).

TDRP1 expressions in testis tissues of azoospermic men with defective spermatogenesis

The expression patterns of *Tdrp1* mRNA and its protein in testis tissues of rats with different developmental stages implied that TDRP1 might be related with spermatogenesis. So we investigated TDRP1 expressions in testis tissues of 12 azoospermic men with defective spermatogenesis confirmed by sperm analysis and histology. As normal controls, 12 testis specimens from adult men with normal spermatogenesis verified by testis histology were prepared and then were investigated TDRP1 expressions. Western blot analysis showed that TDRP1 expressions were significantly lower in the testis tissues of azoospermic men compared with normal controls ($P < 0.001$) (Fig. 3E and F). In detail, expressions of TDRP1 were weak in testis specimens of eight azoospermic men, and were absent in those of four azoospermic men.

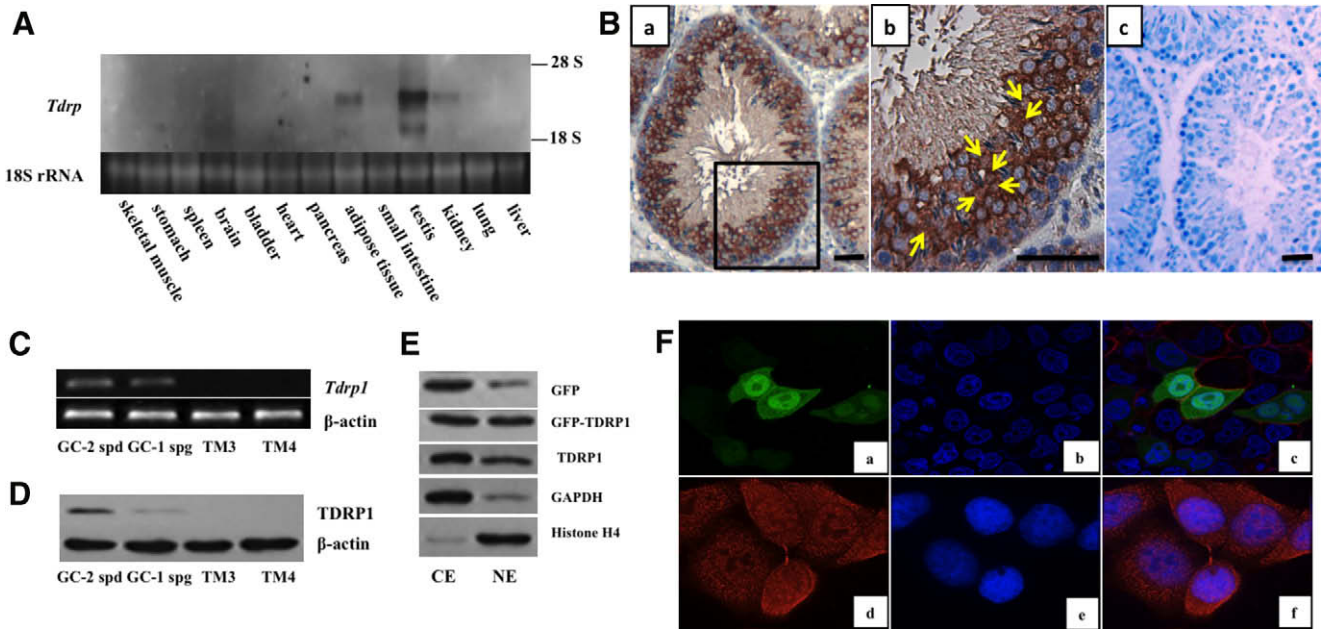

Fig. 2. Expression pattern of *Tdrp1* mRNA and its protein. (A) Northern blot analysis of *Tdrp* gene expression in rat tissues. 18S rRNA was used as control to confirm comparable RNA loading. The locations of 18S and 28S rRNA are indicated on the right. (B) Immunohistochemical analysis of TDRP1 expression in rat testis. *Scale bars*: 50 μm. (a) TDRP1 staining was visualized using DAB (brown), and slides were counterstained with hematoxylin. (b) Magnification of boxed area shown in (a). Yellow arrows indicated TDRP1 was localized in the nuclei of spermatogenic cells. (c) For control, rabbit IgG was used as a primary antibody. No positive signals were detected. (C) *Tdrp1* transcript levels in GC-2 spd, GC-1 spg, TM3, and TM4 cells were determined by RT-PCR. Expression of β-actin was used as loading control. (D) Expressions of TDRP1 in GC-2 spd, GC-1 spg, TM3, and TM4 cells were determined by western blotting. Expression of β-actin was used as loading control. (E) Western blot analysis of expression patterns of green fluorescent protein (GFP), GFP–TDRP1 fusion protein in HeLa cells and TDRP1, GAPDH, histone H4 in testis tissue of a 2-month-old SD rat. Key: CE, cytoplasmic extraction; NE, nuclear extraction; GFP, the expression of GFP in HeLa cells transfected with pEGFP–C2; GFP–TDRP1, expression levels of the GFP–TDRP1 fusion protein in HeLa cells transfected with pEGFP–*TDRP1*; TDRP1, expression levels of TDRP1 in testis tissue of a 2-month-old SD rat; GAPDH, expression levels of GAPDH in the rat testis tissue; Histone H4, expression levels of histone H4 in the rat testis tissue. (F) Expression of TDRP1 in HeLa cells. a, b and c showed confocal images of localization of the GFP–TDRP1 fusion protein in HeLa cells transfected with pEGFP–*TDRP1*. (a) imaging of the GFP–TDRP1 fusion protein, (b) nuclei stained by DAPI and (c) merged image. d, e and f indicated endogenous expression of TDRP1 in HeLa cells by immunofluorescence analysis. (d) staining of TDRP1 by Texas Red, (e) nuclei stained by DAPI and (f) merged image.

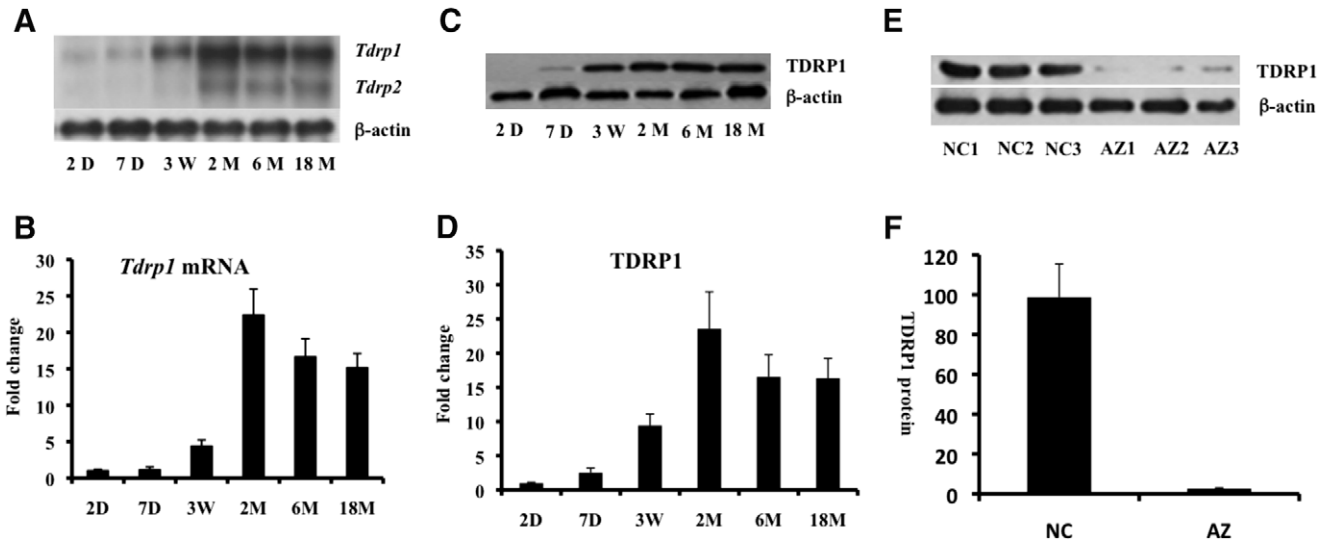

Fig. 3. Expression patterns of TDRP1 in rat and human testis tissues. A, B, C, and D show expression levels of *Tdrp1* mRNA and its protein in testis tissues of rats with different developmental stages. SD rats at ages of 2 days (2 D), 7 days (7 D), 3 weeks (3 W), 2 months (2 M), 6 months (6 M), and 18 months (18 M) postpartum respectively were selected and expression levels of *Tdrp1* mRNA and its protein in their testis tissues were investigated. Three independent experiments were repeated. The expression levels of β-actin were used as controls to confirm comparable RNA or protein loading. The relative expression levels are shown in each graph when the expression level of *Tdrp1* mRNA or its protein in testis of 2-day old rats was set to 1. (A) Expression levels of *Tdrp1* mRNA were investigated in rat testis tissues by northern blotting. (B) The intensity of the RNA signals (as shown in A) were quantified by densitometric scanning. One-Way ANOVA was used to perform statistical analysis. (C) Differential expressions of TDRP1 protein were detected in testis tissues of the above rats by western blotting. (D) Statistical analysis of TDRP1 protein expression shown in C. (E) TDRP1 expressions in testis tissues of azoospermic men with defective spermatogenesis and adult men with normal spermatogenesis by western blotting. Key: NC, normal control (adult men with normal spermatogenesis); AZ, azoospermic men with defective spermatogenesis. (F) Totally, TDRP1 expressions in testis tissues of 12 azoospermic men and 12 normal controls were investigated respectively. Independent sample *t* test was used to perform statistical analysis.

Discussion

Northern blot analysis showed that *Tdrp* mRNA was expressed mostly in the testis, and *Tdrp1* was the predominant transcript of *Tdrp* gene. So experiments in this study were focused on *Tdrp1* transcript. Previous analysis of 49 tissues demonstrated that testis contained more outliers than other tissues, supportive of a conclusion that testis might contain a higher number of cell-specific transcripts than found in most other tissues [5]. In addition to this lineage-specific pattern of expression, most of these genes also exhibit stage-specific expression during spermatogenesis [6]. Immunohistochemistry analysis indicated that TDRP1 protein was mainly expressed in spermatocytes and not detected in Sertoli cells or other somatic interstitial cells. We also investigated expression patterns of *Tdrp1* mRNA and its protein in the rat testis tissues of different developmental stages. Both mRNA and protein levels of TDRP1 were barely detected in rat testis at ages of 2 and 7 days postpartum. Significantly, the levels began to increase at 3 weeks postpartum, continuing to gradually increase afterwards, and peaking at 2 months postpartum. During rat testicular development, after the 2nd postnatal week there are germ cells in all stages of the first meiosis and after the 3rd postnatal week, the early type of haploid cells first appear in the seminiferous tubules. Male rats reach sexual maturity by postnatal days 56–60 [7]. The cell- or stage-specific expression pattern implied a possible role for TDRP1 in meiotic or early postmeiotic development of male germ cells. Meiosis takes place in the spermatocytes. Schultz et al. [8] estimated that ~4% of the mouse genome were dedicated to male germ cell-specific transcripts, >99% of which were first expressed during or after meiosis. The results from studies of Pang et al. [9] further extended the estimation that an even larger proportion of the mouse genome was devoted to male gamete development starting from meiosis. A list of genes involved in meiotic or postmeiotic stages of spermatogenesis have been determined through knockout animal models. Deletions of these genes resulted in arrests of spermatogenesis in various meiotic or postmeiotic stages. The knockout animals were generally infertile and had grossly reduced testicular mass or defective spermatogenesis [10–16]. High levels of TDRP1 expression in spermatocytes implied its role at meiotic and postmeiotic stages of spermatogenesis. Importantly, *in vivo* studies demonstrated that TDRP1 expressions were significantly lower or absent in the testis tissues of azoospermic men with defective spermatogenesis compared with normal controls. The current study provided evidence that TDRP1 might play a role in the process of spermatogenesis, especially at the meiotic or postmeiotic stages.

Bioinformatics analysis demonstrated a significant leucine-rich nuclear export signal (NES) in the aa sequences of TDRP1 protein. NESs are extremely important for the subcellular location of proteins. Most proteins with nuclear functions appear to be actively transported in and out of the nucleus and many of the reported nucleocytoplasmic shuttle proteins are involved in signal transduction events and cell cycle regulation [17]. Both *in vitro* and *in vivo* studies demonstrated that TDRP1 protein could be detected in nuclei, which provided us evidence that TDRP1 might function as a nuclear factor in spermatogenesis. Numerous stage-specific and general transcription factors and components of transcription machinery are expressed specifically or at an unusually high level in both meiotic and early postmeiotic germ cells, suggesting an overall activation of the transcriptional machinery in these cells [8]. On basis of these observations, we speculated that TDRP1 served as a nuclear factor during meiotic or postmeiotic stages of spermatogenesis.

In conclusion, we described a novel gene termed *TDRP*, which was predominantly expressed in spermatogenic cells of testis. We also presented evidence that as a nuclear factor, TDRP1 might play an important role in spermatogenesis, more likely at the meiotic or postmeiotic stages.

Declaration of interest

There were no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.02.061.

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