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Expression and Identification of a Novel Apoptosis Gene *Spata17* (*MSRG-11*) in Mouse Spermatogenic Cells

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Abstract In this study, anti-spermatogenesis-associated 17 (Spata17) polyclonal antibody was prepared by immunizing New Zealand white rabbits with a synthesized peptide corresponding to the amino acid sequence 7–23 of the mouse Spata17 protein. Immunohistochemical analysis revealed that Spata17 protein was most abundant in the cytoplasm of round spermatids and elongating spermatids within seminiferous tubules of the adult testis. The expression of *Spata17* mRNA in cultured mouse spermatogonia (GC-1) cells was almost undetectable. In an experimental unilateral cryptorchidism model of an adult mouse, the expression of *Spata17* mRNA had no obvious difference with the normal testis until postoperation day 1, but gradually decreased from day 3 and was almost undetectable on day 17. Immunohistochemical analysis revealed that the protein was almost undetectable within seminiferous tubules of an experimental unilateral cryptorchidism model of the adult testis on postoperation day 8. Flow cytometry analysis showed that the expression of Spata17 protein in the GC-1 cell line could accelerate GC-1 cell apoptosis. The effect increases with the increasing of the transfected dose of pcDNA3.1(–)/*Spata17*. By Hoechst 33258 staining, a classical way of identifying apoptotic cells, we further confirmed that the apoptosis was induced by expression of *Spata17* in transfected GC-1 cells.

Key words spermatogenesis-associated 17 (*Spata17*) gene; *MSRG-11*; testis; spermatogenic cell apoptosis

Spermatogenesis is a complex developmental process that consists of three principal phases: mitotic proliferation for stem cell renewal; meiosis, by which diploid spermatocytes develop to haploid spermatids through the stages of leptotene, zygotene, pachytene, diplotene, secondary spermatocyte and round spermatid; and spermiogenesis, during which round spermatids differentiate and are released as mature spermatozoa into the lumen of the seminiferous tubule [1]. The germ cells develop from primordial diploid cells to haploid spermatozoa through a series of dramatic alterations in morphological and biochemical properties, determined by changes of gene expression during spermatogenesis. Loss of germ cells is very common at various stages of mammalian spermatogenesis. It was observed that the amount of mature sperms in mouse testis was 20%–75% less than expected, although testis is one of the tissues with high proliferation ability. The explanation given was that apoptosis in testis resulted in spontaneous degeneration of spermatogenic cells [2–5]. The apoptosis could be induced by many signals, including the Fas and Fas ligand system and/or the Bax and Bcl-2 system in normal adult testis [6–9]. So the interference in spermatogenic cell apoptosis, which involves multiple genes, is an important method to improve male fertility. Although cell death, particularly apoptosis, has been implicated, our understanding of the mechanisms underlying germ cell death is still limited. Cloning and identification of new spermatogenic cell-specific genes related to apoptosis is of physiological and pathological significance, to illustrate both the mechanism of apoptosis and the biological process of spermatogenic cells.

In our previous study, a novel testis-specific apoptosis gene, *MSRG-11*, was cloned from a new contig of the

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expressed sequence tags (Mm.63892) obtained by comparing testis libraries with other tissue and cell line libraries using the digital differential display program [10]. The sequence data have been submitted to the GenBank database under accession number AY747687. The full cDNA length is 1074 bp, and the gene with seven exons and six introns is located in mouse chromosome 1 H5. The protein is recognized as a new member of the calmodulin (CaM)-binding protein family because the sequence contains three short CaM-binding motifs containing conserved Ile and Gln residues (IQ motif) and is considered to play a critical role in interactions of IQ motif-containing proteins with CaM proteins. The putative protein encoded by this gene has 192 amino acid residues with a theoretical molecular mass of 23.7 kDa and a calculated isoelectric point of 9.71. The sequence shares no significant homology with any known protein in databases. Reverse transcription-polymerase chain reaction (RT-PCR) and Northern blot analyses revealed that the 1.3 kb transcript was strongly expressed in adult mouse testis but weakly expressed in the spleen and thymus. The gene was expressed at various levels, faintly at 2 weeks postpartum and strongly from 3 weeks postpartum in adult testes, which suggests that this gene might play an important role in the development of mouse testes [10].

Recently, the gene *MSRG-11* was named *Spata17* (spermatogenesis-associated 17) by the Mouse Genomic Nomenclature Committee. In this study, we further investigate *Spata17* function in spermatogenic cells within seminiferous tubules during mouse spermatogenesis. The results suggested that it might be related to spermatogenic cell apoptosis in the development of normal testis.

Materials and Methods

Animals and cell lines

Male and female Balb/Balc mice were supplied by the Laboratorial Animal Center of Central South University (Changsha, China) and maintained in a temperature- and humidity-controlled room on a light/dark (12 h/12 h) cycle. The mice had free access to food and water. Female mice were naturally mated and observed at 12 h intervals, and the time of parturition was recorded. The day of birth was designated as day 0. All measures taken for the mice referred to "Guidelines for the Care and Use of Laboratory Animals" established by the Chinese Council on Animal Care. Mice spermatogonia cell line GC-1 was purchased from ATCC (Manasses, USA). GC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, USA) supplemented with 10% fetal calf serum (Gibco), 2 mM *L*-glutamine (Sigma, St. Louis, USA), and 1% non-essential amino acids (Sigma). The cultures were incubated at 37 ºC in a 5% CO_2, 95% humidity atmosphere and subcultured every 4 d.

Experimental cryptorchidism

Surgery was performed according to the previous report [11] under light ether anesthesia. Briefly, a midline abdominal incision was made and the right testis was manipulated into the abdomen then sutured to the abdominal wall. The left testis was maintained inside the scrotum to serve as a control. The testes were removed for total RNA extraction on postoperation day 1, 3, 6, 9 or 17. The experimental cryptorchid testes were all confirmed to reside within the abdominal cavity before sample collection. Semi-quantitative RT-PCR analysis and immunohistochemical analysis were performed as below.

Semi-quantitative RT-PCR analysis

Total RNAs of mouse spermatogonia cell line GC-1 and the experimental cryptorchid testes were isolated using an RNA isolation kit (Promega, Madison, USA). Their cDNAs were synthesized according to the manufacturer's instructions and were used as a template in the following PCR reaction with the primer pair LM-1F/LM-1R (forward primer LM-1F: 5′-AGTCGACTCAAGTCTGGTCTCA-3′; reverse primer LM-1R: 5′-GCAGTTTAATAGGAAGGCGAGA-3′) and DNA polymerase. For the glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) gene, the forward primer was 5′-GTCAACGGATTTGGTCGTATT-3′ and the reverse primer was 5′-AGTCTTCTGGGTGGCAGTGAT-3′. Semi-quantitative RT-PCR was performed as follows: initial denaturation at 95 ºC for 90 s; 30 cycles of 94 ºC for 40 s, 57 ºC for 40 s, and 72 ºC for 40 s; and 72 ºC for 5 min; then holding at 4 ºC for *Spata17*, and 20 cycles for *G3PDH* as control in a 10 μl of 1×reaction buffer, containing 200 μM each of dATP, dCTP, dGTP, and dTTP, 2 mM $MgCl_2$, 1 U *Taq* DNA polymerase, approximately 200 ng total RNA reversely transcribed product, 0.4 μM *Spata17*-specific primers and 0.4 μM *G3PDH*-specific primers. The RT-PCR product was separated in 2.0% agarose gel, cloned into pUCm-T vector and sequenced. Equal volumes of the PCR products were analyzed by densitometry with a Gel-Pro analyzer (version 3.1).

Antibody preparation

The peptide corresponding to the amino acid sequence 7–23 of the mouse Spata17 protein was synthesized by

Boster (Wuhan, China). The New Zealand white rabbits were immunized by the synthesized peptide coupled to keyhole limpet hemocyanin to prepare the antiserum. Anti-Spata17 polyclonal antibody was purified from the sera of the rabbits by precipitation of ammonia sulfate and protein A affinity chromatography. The specificity and the titer of the antiserum were tested by Western blot.

Western blot analysis

Cells were lysed as described previously [12]. The protein concentration of each supernatant was determined by the Bradford method [13]. For experiments involving samples from transiently transfected cells, the cotransfected pCMV-*LacZ* plasmid was used to normalize each sample. Western blot was performed as follows. Equal amounts of lysates were separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) then transferred to Hybond membranes (Amersham, Arlington Heights, USA). The membranes were then blocked with phosphate-buffered saline (PBS) buffer containing 5% fat-free milk and 0.05% Tween 20 for 1 h at room temperature or overnight at 4 ºC, then washed three times with PBS with 0.05% Tween 20. They were incubated with primary antibodies for at least 1 h at room temperature, followed by a washing with PBS with 0.05% Tween 20, then incubated with peroxidase-conjugated secondary antibodies and developed with enhanced chemiluminescence (Pierce, Rockford, USA). The Western blot analysis was performed in at least three different experiments, and the representative data are shown.

Immunohistochemical analysis

Adult Balb/Balc mice were euthanized by CO_2 inhalation, and dissected testes were fixed in Bouin's fluid overnight, extensively washed in 70% ethanol, dehydrated in ethanol, embedded in paraffin and sectioned into sections of 5 μm thickness, then rehydrated. Immunohistochemistry was performed according to the procedure described in the manufacturer's instructions (SABC kit; Boster). The peroxidase activity was detected using a DAB kit (Boster). Testicular sections were counterstained with methyl green (Sigma) then observed and photographed under a fluorescence microscope.

Apoptosis analysis of GC-1 cells transfected with pcDNA3.1(−)/*Spata17* by flow cytometry

The effect of Spata17 on cultured GC-1 cells was detected by transient transfection with pcDNA3.1(−)/*Spata17*, a eukaryotic expression plasmid for expression of Spata17 protein constructed by inserting *Spata17* cDNA into pcDNA3.1(−) with *Eco*RI and *Hin*dIII and confirmed by restriction endonuclease digestion and sequencing, and by flow cytometry. GC-1 cells were plated at 50% confluence in 60 mm dishes and 1.5, 3.0, 4.5, 6.0 or 7.5 μg pcDNA3.1(−)/*Spata17* was used for each transfection using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). After transfection for 4 h, the medium was changed to standard DMEM, and the cells were cultured for 32 h. Control cells were transfected with 6 μg of pcDNA3.1(−). The cells were washed with PBS, fixed with ice-cold 70% ethanol, stored at 4 ºC for 2 h then incubated with 50 μl of l mg/ml RNase A at 37 ºC for 60 min. After the addition of 400 μl of 50 μg/ml propidium iodide, the mixture was incubated on ice in the dark for 60 min. The effect of pcDNA3.1(−)/*Spata17* plasmid on cell cycle dynamics was examined using flow cytometry, with at least $l\times10^5$ cells analyzed.

Detection of chromatin condensation of GC-1 cells transfected with pcDNA3.1(−)/*Spata17* or pEGFP-C2/*Spata17*

Chromatin condensation was detected by nucleus staining with Hoechst 33258 (Sigma) as follows. The GC-1 cells were cultured in a six-well plate; transfected with 4 μg pcDNA3.1(−)/*Spata17* or pEGFP-C2/*Spata17*, fixed with 2% paraformaldehyde for 12 h at 32 h post-transfection, washed with PBS, stained with PBS/0.1% Triton X-100/10 μM Hoechst 33258 for 10 min, and washed again with PBS. Stained nuclei were visualized under a Nikon fluorescence microscope at a 400× magnification with an excitation wavelength of 355–366 nm and an emission wavelength of 465–480 nm. In this way, the nuclei of apoptotic GC-1 cells were densely stained bright blue because of their chromatin condensation, whereas those of the normal GC-1 cells were stained evenly with light blue. Enhanced green fluorescent protein (EGFP)-Spata17 fusion protein was also detected at 24 h post-transfection. Observation of fluorescence of the EGFP-Spata17 was performed with the Nikon fluorescence microscope. The transfected cells displayed strong and even green fluorescence excited by blue light. Images were captured digitally and imported into Adobe Photoshop 6.0 for formatting. The GC-1 cells transfected with pcDNA3.1(−) or pEGFP-C2 vector were used as the control.

Results

Expression of Spata17 in cultured GC-1 cells and testis

The RT-PCR was performed using primers LM-1F/LM-

1R. The expression of *Spata17* mRNA in cultured GC-1 cells was almost undetectable [**Fig. 1(A)**], but a single strongly expressed mRNA of 1015 bp was observed in adult testis and the PCR product (1015 bp) was sequenced. The result is identical to the sequence submitted to the GenBank database under accession number AY747687. The control, *G3PDH*, was expressed in both the GC-1 cells and testis.

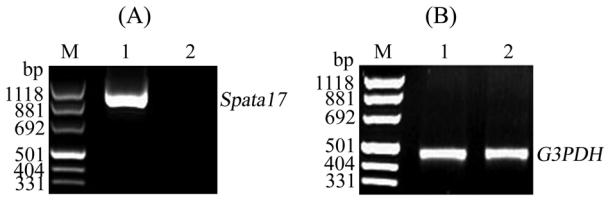

Fig. 1 Expression of *Spata17* mRNA and glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) in cultured mouse spermatogonia (GC-1) cells and testis

(A) The expression of *Spata17* mRNA in GC-1 cells (lane 2) was almost undetectable, but a single strongly expressed mRNA of 1015 bp was observed in adult testis (lane 1). (B) The control, *G3PDH*, was expressed in both GC-1 cells (lane 2) and testis (lane 1). M, DNA marker (pUC Mix8; Fermentas, Vilnius, Lithuania).

Downregulation of *Spata17* mRNA in experimental cryptorchid testes

The changes of *Spata17* mRNA expression in the surgically-induced cryptorchid testes were examined by semi-quantitative RT-PCR. The expression of *Spata17* mRNA in the experimental cryptorchid testes had no obvious difference compared with the normal testis in scrotum on postoperation day 1, but decreased gradually from postoperation day 3. On postoperation day 9, the expression of *Spata17* mRNA in the cryptorchid testis decreased to 10% of that in the scrotal testis and was almost undetectable on postoperation day 17 (**Fig. 2**). As an internal control, *G3PDH* was almost equally expressed on different days. These findings are consistent with previous reports showing that experimental unilateral cryptorchidism begins to disrupt spermatogenesis on postoperation day 6 in mice [21,22].

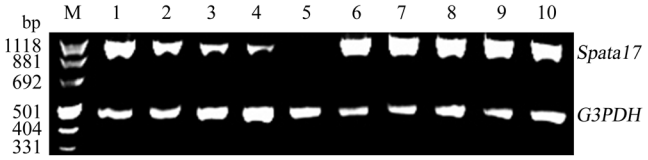

Fig. 2 Downregulation of *Spata17* mRNA in experimental cryptorchidism *in vivo*

M, DNA marker; 1–5, experimental cryptorchid testes on day 1, 3, 6, 9 and 17 postoperation, respectively; 6–10, scrotal testes on day 1, 3, 6, 9 and 17, respectively. Glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) was used as an internal control. The data were from a representative experiment repeated three times with similar results.

Western blot analysis

The GC-1 cells transiently transfected with pcDNA3.1(–)/*Spata17* or pcDNA3.1(–) were lysed and blotted with anti-Spata17 serum, antibody of β-actin and preimmune serum. The result showed that the final preparation of GC-1 cells transiently transfected with pcDNA3.1(–)/*Spata17* gave a single stained band of 24 kDa on SDS-PAGE (**Fig. 3**) by immunoblotting using anti-Spata17 serum. No immunoblotting signal was detected in GC-1 cells transiently transfected with pcDNA3.1(–), whereas the internal control, *β-actin*, was expressed in both GC-1 cells transfected with pcDNA3.1(–)/*Spata17* and GC-1 cells transfected with pcDNA3.1(–). No immunoblotting signal was detected using preimmune serum.

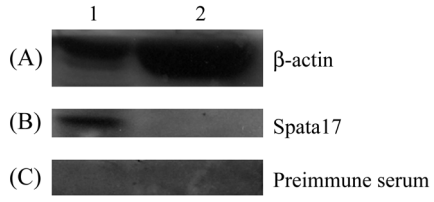

Fig. 3 Western blot result of Spata17 protein

(A) Blotting with antibody of β-actin. (B) Blotting with anti-Spata17 serum. (C) Blotting with preimmune serum. 1, cultured mouse spermatogonia (GC-1) cells transfected with pcDNA3.1(–)/*Spata17*; 2, GC-1 cells transfected with pcDNA3.1(–). As shown in the figure, the final preparation of GC-1 cells transiently transfected with pcDNA3.1(–)/*Spata17* gave a single stained band of 24 kDa on sodium dodecyl sulphate-polyacrylamide gel electrophoresis by immunoblotting using anti-Spata17 serum. No immunoblotting signal was detected in GC-1 cells transiently transfected with pcDNA3.1(–), and no immunoblotting signal was detected using preimmune serum.

Immunohistochemical analysis

Immunohistochemical analysis was performed according to the procedure described in "Materials and Methods". The results (**Fig. 4**) showed that Spata17 protein was

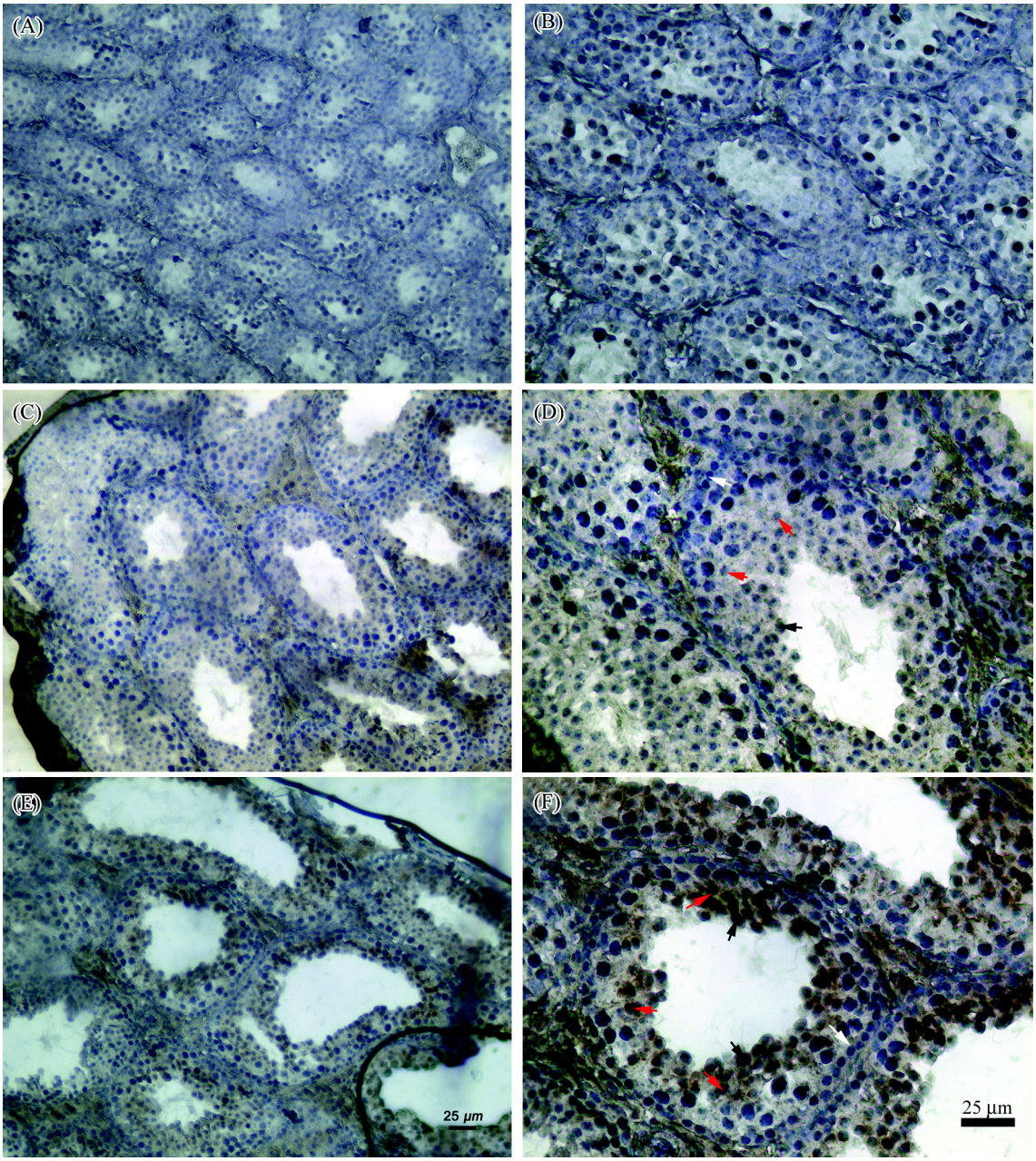

Fig. 4 Immunohistochemical analysis of spermatogenesis-associated 17 (*Spata17*) in mouse testis

Polyclonal rabbit anti-Spata17 and biotin peroxidase-conjugated goat antirabbit antibodies were used and the presence of Spata17 was revealed by brown staining. Spata17 was detected in the cytoplasm of round spermatids (marked by red arrows) and elongating spermatids (marked by black arrows). No significant signals were detected in spermatogonia and spermatozoa (marked by white arrows) in the seminiferous tubules of controls. (A,B), 10-day old mouse testis stained with polyclonal rabbit anti-Spata17 and biotin peroxidase-conjugated goat antirabbit antibodies. (C,D) 5-week old mouse testis stained with preimmune serum and biotin peroxidase-conjugated goat antirabbit antibody. (E,F) 5-week old mouse testis stained with polyclonal rabbit anti-Spata17 and biotin peroxidase-conjugated goat antirabbit antibodies. Bar=25 μm.

strongly expressed in round spermatids and elongating spermatids, and weakly or not expressed in spermatogonia and spermatozoa, suggesting that *Spata17* might be involved in the process of meiosis or posttranscriptional regulation of elongated spermatids. In the experimental cryptorchid testes, spermatocyte, spermatozoa and most spermatids were lost from the testis tubules, and Spata17 protein was not detected (**Fig. 5**).

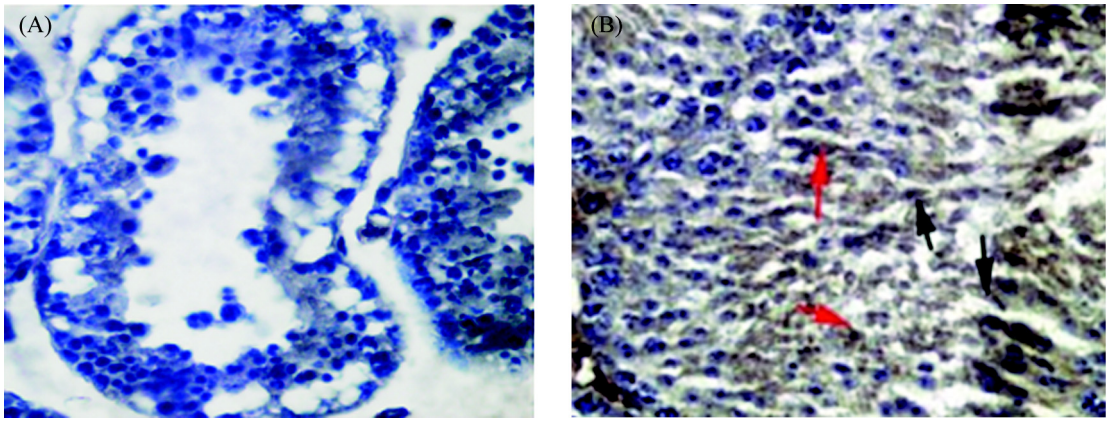

Fig. 5 Immunohistochemical analysis of *Spata17* in mouse experimental cryptorchid testis

Experimental cryptorchid testes (A) and scrotal testes (B) on postoperation day 8 stained with polyclonal rabbit anti-Spata17 and biotin peroxidase-conjugated goat antirabbit antibodies, respectively. Spata17 was detected in the cytoplasm of round spermatids (marked by red arrows) and elongating spermatids (marked by black arrows) in the seminiferous tubules of scrotal testes (B). No significant signals were detected in experimental cryptorchid testes on postoperation day 8 (A).

Effects of Spata17 on cultured GC-1 cell apoptosis detected by flow cytometry

To determine whether the *Spata17* gene results in changes in cultured cell proliferation or apoptosis, we examined transient expression of the Spata17 protein in GC-1 cells (**Fig. 6**) by transfection with pcDNA3.1(–)/*Spata17* expression plasmid using Lipofectamine 2000. The effects of such treatment on cultured cell growth were examined *in vitro* using flow cytometry. The percentage of the cells

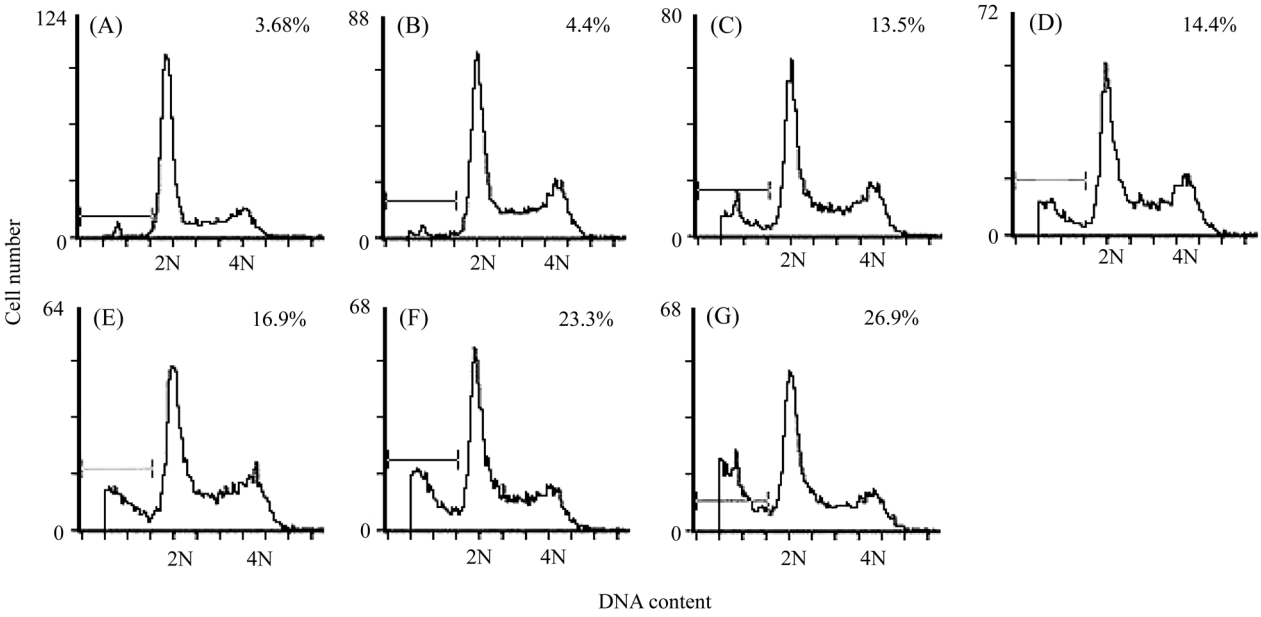

Fig. 6 Effect of the transfection of *Spata17* on cultured mouse spermatogonia (GC-1) cell apoptosis detected by flow cytometry

(A) Normal GC-1 cells, the percentage of the apoptosis cells was 3.68%. (B) GC-1 cells transfected with pcDNA3.1(–), the apoptosis rate was 4.4%. (C–G) GC-1 cells transfected with pcDNA3.1(–)/*Spata17* of 1.5, 3.0, 4.5, 6.0 and 7.5 μg, respectively. The percentage of the apoptosis cells is 13.5%, 14.4%, 16.9%, 23.3% and 26.9%, respectively. The results indicated that transfection of *Spata17* can accelerate GC-1 cell apoptosis and the effect increases along with the increasing of the transfected dose of pcDNA3.1(–)/*Spata17* DNA.

resident in each cell cycle phase is indicated in **Fig. 7**, showing that the percentage of the apoptosis cells is 3. 68% in the normal GC-1 cells, 4.4% in the GC-1 cells transfected with pcDNA3.1(−), and 13.5%, 14.4%, 16. 9%, 23.3%, and 26.9% in the GC-1 cells transfected with pcDNA3.1(−)/*Spata17* of 1.5, 3.0, 4.5, 6.0 and 7.5 μg, respectively. The results indicated that transfection of *Spata17* can accelerate GC-1 cell apoptosis and the effect increases along with the increasing of the transfected dose of pcDNA3.1(−)/*Spata17* DNA.

Effects of Spata17 on cultured GC-1 cell apoptosis detected by Hoechst 33258 staining

To confirm effects of Spata17 on GC-1 cell apoptosis, we further investigated the action of Spata17 on cultured GC-1 cells transfected with pcDNA3.1(−)/*Spata17* by Hoechst 33258 staining, a classical way of identifying apoptotic cells. At 24 h post-transfection, the GC-1 cells transfected with pcDNA3.1(−)/*Spata17* were stained by Hoechst 33258 to test their nuclei morphology. Approximately 23% of GC-1 cells transfected with pcDNA3.1(−)/*Spata17* and 1.5 % of the control GC-1 cells transfected with pcDNA3.1(−) showed the typical apoptotic nuclear morphology and their highly condensed nuclei were stained bright blue. The nuclei of the rest of the cells showed the normal morphology and were stained an even light blue (**Fig. 7**). To confirm that the apoptosis was induced by expression of *Spata17*, we further investigated the effect of Spata17 on cultured GC-1 cells transfected with pEGFP-C2/*Spata17*. By staining with Hoechst 33258 at 32 h post-transfection, the results showed that the nuclei of the control GC-1 cells transfected with pEGFP-C2 and expressing EGFP showed an even light blue stain [**Fig. 8(A,B)**], whereas 75% of the nuclei of GC-1 cells transfected with pEGFP-C2/*Spata17* and expressing EGFP-Spata17 were stained highly condensed bright blue [**Fig. 8(C,D)**].

Discussion

Apoptosis, which happens selectively to certain spermatogenic cells, maintains normal spermatogenesis. However, unbalanced apoptosis could lead to spermatogenic dysfunction and infertility. Thus, a thorough understanding of the apoptosis mechanism might uncover the causes for many testicular failures and help to find efficient strategies against these defects. Many testis spermatogenic cell apoptosis-related genes have been reported to be involved in this process, including *Mcl-1*, *p53*, *CREM*, *Fas*, *Hsp*, *TRAIL*, *C-myc* and *TR2* [23−30]. In our previous research, we cloned a novel testis-specific high expression gene *Spata17* (*MSRG-11*) [10].

In the present research, we further investigated the expression and function of *Spata17*. Our results showed that *Spata17* was immunolocalized in the cytoplasm of round spermatids and elongating spermatids, but undetectable in spermatogonia, spermatocytes or spermatozoa in the seminiferous tubules of adult normal testis. No significant signals were detected in spermatogenic cells of 10-day old mouse, suggesting that it was a new spermatogenic

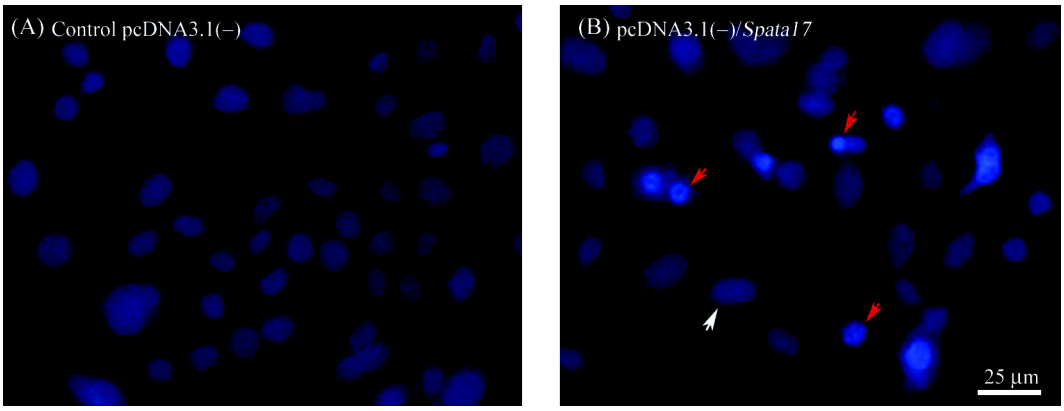

Fig. 7 Apoptosis of cultured mouse spermatogonia (GC-1) cells induced by transfection of pcDNA3.1(−)/*Spata17*

The highly condensed nuclei of apoptotic cells were stained bright blue by Hoechst 33258 (marked by red arrows); those of normal cells were stained an even light blue (marked by white arrows). Approximately 23% of GC-1 cells transfected with pcDNA3.1(−)/*Spata17* (B) and 1.5% of the control GC-1 cells transfected with pcDNA3. 1(−) (A) showed typical apoptotic nuclear morphology and their highly condensed nuclei were stained bright blue. The nuclei of the rest of the cells showed normal morphology and were evenly stained light blue. The data were from a representative experiment repeated three times with similar results. Bar=25 μm.

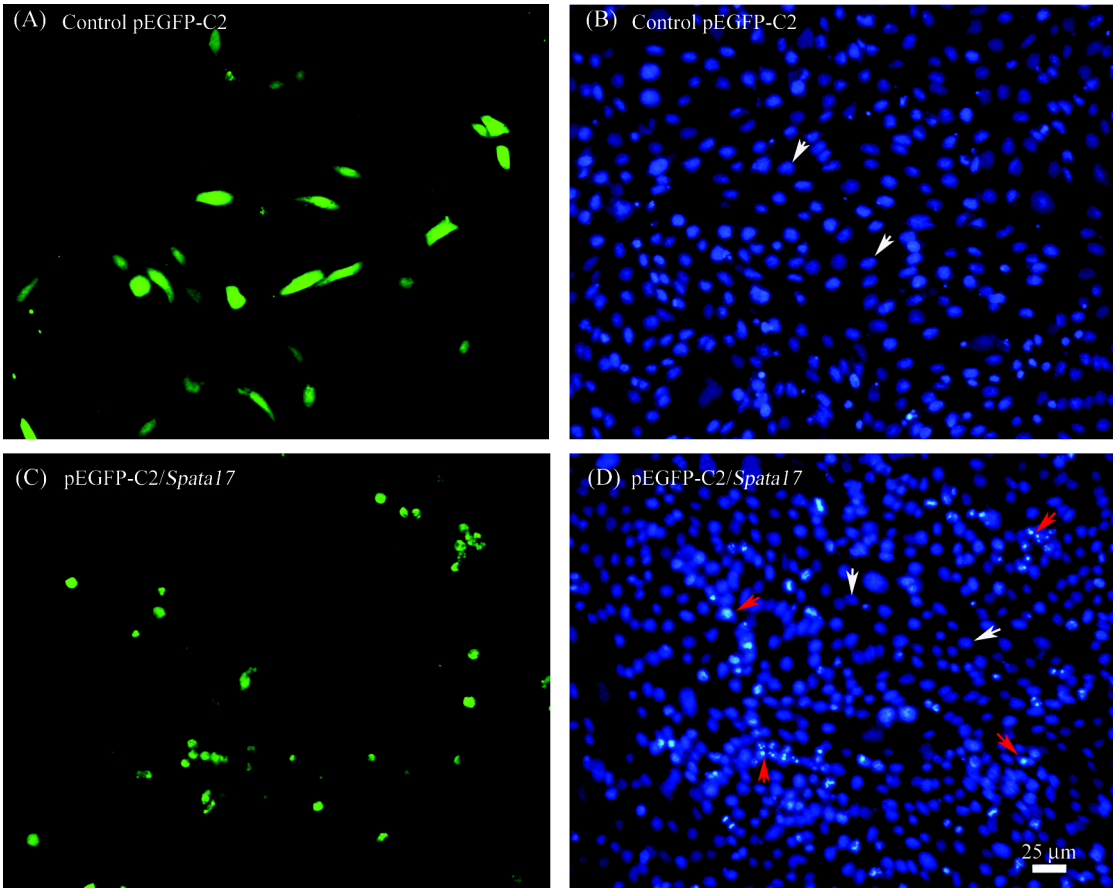

Fig. 8 Apoptosis of cultured mouse spermatogonia (GC-1) cells induced by transfection of pEGFP-C2/ *Spata17*

The highly condensed nuclei of apoptotic cells were stained bright blue by Hoechst 33258 (marked by red arrows); those of normal cells were stained an even light blue (marked by white arrows). Approximately 75% of the nuclei of GC-1 cells transfected with pEGFP-C2/*Spata17* and expressing enhanced green fluorescent protein (EGFP)-Spata17 showed typical apoptotic nuclear morphology, and the highly condensed nuclei were stained bright blue by Hoechst 33258 (C,D). The nuclei of the control GC-1 cells transfected with pEGFP-C2 and expressing EGFP were stained an even light blue (A,B). The result further confirmed the apoptosis was induced by expression of *Spata17*. The data were from a representative experiment repeated three times with similar results. Bar=25 μm. (A,B) GC-1 cells transfected with pEGFP-C2. (C,D) GC-1 cells transfected with pEGFP-C2/*Spata17*.

cell-specific gene. RT-PCR results also showed that *Spata17* was not detected in GC-1 cells. So we considered that the involvement of *Spata17* in spermatogenesis could be further limited to meiosis or postmeiosis stages. In mouse testis, it is known that late pachytene spermatocytes first appear on postnatal days 22–23, haploid round spermatids on days 24–25, and elongating spermatids on days 30–31 [31]. The present research results are also in accordance with *Spata17* expression levels in testis at different developmental stages, faintly before 2 weeks postnatal and strongly from 3 weeks postnatal [10], earlier than the beginning of spermatogenesis. This suggested that *Spata17* expression is strongly associated with the development of germ cells.

Apoptosis analysis of GC-1 cells transfected with pcDNA3.1(–)/*Spata17*, a eukaryotic expression plasmid for expression of Spata17 protein, was performed by flow cytometry and revealed that Spata17 protein can accelerate GC-1 and COS7 [10] cell apoptosis. The effects of Spata17 on cultured GC-1 cell apoptosis was also confirmed by Hoechst 33258 staining, suggesting that this gene could play an important role in the development of testis and might be involved in maintaining an equilibrium between normal spermatogenesis and normal apoptosis. Therefore, it might be a testis apoptosis-candidate gene.

In the experimental cryptorchid testes, spermatocytes, spermatozoa and most spermatids were lost from the testis tubules and Spata17 protein was not detected by immunohistochemical analysis. The downregulation of *Spata17* in the experimental cryptorchid testis with time

might be caused by the loss of spermatids, suggesting that *Spata17* might be involved in programming apoptosis of spermatogenic cells, but not involved in apoptosis due to heat stress in the adult testis.

Based on these observations, it is considered that *Spata17* is a new gene that could cause apoptosis of mouse testis spermatogenetic cells, but its apoptosis mechanism remains to be elucidated.

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