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Expression and Possible Functions of a Novel Gene *SPATA12* in Human Testis

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ABSTRACT: In our previous study, a novel human testis-specific gene, *SPATA12*, was identified using the digital differential display program. In the current study, both *SPATA12* mRNA and protein levels in the developmental stage of the testis were detected by SYBR real-time reverse transcriptase polymerase chain reaction and Western blot. A high level of *SPATA12* gene expression was observed in normal adult testis but was completely absent in fetal testis. Both in situ hybridization and immunohistochemical analysis showed that *SPATA12* was expressed in seminiferous tubules of adult testis—more precisely in spermatocytes, spermatids, and spermatozoa—but there was no expression in Sertoli and Leydig cells. These results showed that *SPATA12* is a stage-specific and germ cell–specific gene, which suggests that it must be involved in the development of testicular maturation. It was also found that the expression level of *SPATA12* mRNA in the testes of infertile men was associated with the amount and density of germ cells. With a decrease in the number of germ cells, the expression of *SPATA12* mRNA was lower. In addition, the signal in the testes of patients with cryptorchidism or Sertoli cell only syndrome was not detected. Flow cytometry analysis of *SPATA12* in human HeLa cells and mouse GC-1 spg germ cells indicated that the expression of the *SPATA12* gene may delay the progression of G_1 to S in the cell cycle. *SPATA12* was also shown to be lost in testicular germ cell tumors both at the level of transcription and translation. We hypothesized that the putative function of *SPATA12* is to maintain the cell in a differentiated state and/or to suppress cell proliferation.

Key words: Germ cell specific, stage specific, spermatogenesis.
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Spermatogenesis is a complex process of cell development and differentiation that requires the highly regulated expression of multiple genes (Hecht, 1995; Sassone-Corsi, 1997; Vogt, 1998). The unique differentiation mechanisms of spermatogenesis suggest the existence of germ-cell–specific molecules. However, only a few genes involved in spermatogenesis have been identified, such as *Spef1* (Chan et al, 2005), *Sept4* (Kissel et al, 2005), *Rosbin* (Takahashi et al, 2004), *Tektin3* (Roy et al, 2004), *p59^{scr}* (Senoo et al, 2002), *Zfp393* (Yan et al, 2002), *TSGA10* (Modarressi et al, 2001), and *LRTP* (Xue and Goldberg, 2000).

To study the mechanisms of germ cell differentiation, it is vital to isolate and characterize the genes specifically expressed in testicular germ cells. Recently using the digital differential display program (http://www.ncbi.nlm.nih.gov/UniGene) at the National Center for Biotechnology Information, multiple cDNA libraries were screened to identify expressed sequence tags (ESTs) present in libraries derived from testis but absent in libraries derived from other tissues. A novel EST cluster, HS.129794, that is exclusively expressed in testis was identified. Beginning from HS.129794, a full-length cDNA sequence of the new gene named *SPATA12* (Li and Lu, 2004; GenBank accession number AY221117) was identified. The *SPATA12* gene is 2430 bp long, consists of 2 exons, and spans approximately 15 kb on chromosome 3p21.1–3p21.2. The sequence of the open reading frame is 676–1248 bp. The cDNA encodes a novel protein of 190 amino acids with a theoretic molecular mass of 20417.8 daltons and isoelectric point of 5.23. The sequence has no significant homology with any known protein in databases. Northern blot analysis showed that *SPATA12* is specifically expressed in the normal human testis as a major transcript of 2.4 kb but is not detectable in other tissues such as brain, heart, kidney, liver, lung, ovary, spleen, trophoblast, and placenta.

In an attempt to obtain greater insight into the function of *SPATA12*, SYBR real-time reverse transcriptase polymerase chain reaction (RT-PCR), in situ hybridization, Western blot, immunohistochemistry,

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and flow cytometry analysis were used to research the expression pattern and possible function of *SPATA12*. The restricted expression pattern of *SPATA12* in germ cells of adult testis suggested that it maybe involved in the development of testicular maturation. All the results presumed that the putative function of *SPATA12* is to maintain the cell in a differentiated state and/or to suppress cell proliferation.

Materials and Methods

Tissue, Cell Lines, and Ethics

The testis biopsy materials from male infertility patients with spermacrasia, cryptorchidism, or Sertoli cell only syndrome were obtained from Xiangya Hospital of Central South University, Changsha, China. The infertility patients for the expression studies proved to have normal karyotypes; patients with other conditions such as viral parotitis, varicocele, or obstruction of the vas deferens, which may have caused infertility, were excluded. Microdeletion or mutation detection at the AZFa (sY84 and USP9Y), AZFb, AZFc/DAZ, and SRY regions of the Y chromosome was performed as our clinical examination (Fu et al, 2002). No deletions or mutations were found in these patients. The swim-up sperm sample was obtained from a patient with normal spermatogenesis.

Small specimens (0.1–0.2 mg) of human testicular germ cell tumors and surrounding tissues were obtained at the time of surgery from patients undergoing orchiectomy. Histologic evaluations of the testicular germ cell tumors such as seminomas and teratomas were based on routine pathologic reports. Normal testicular tissue was obtained from patients undergoing bilateral orchiectomy for the treatment of prostate carcinoma. The fetal testes were obtained from naturally aborted embryos. These tissues were frozen in liquid nitrogen and then immediately stored at −80°C. All patients signed consent forms approved by the Committee on Human Rights in Research of the Ethics Committee at the Institute of Reproductive and Stem Cell Engineering, Central South University, Changsha, China.

Human tumor cell lines, MCF-7(ATCC HTB22) and HeLa (ATCC CCL2), and mouse GC-1 spg germ cell cell line (ATCC CRL-2053) were purchased from the American Type Culture Collection (ATCC, Manassas, Va). The human MGC cell line (stomach carcinoma) was established by the biology department of Shandong Normal University, China. Cells were cultured in RPMI 1640 (Invitrogen Corp, Carlsbad, Calif) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin.

Primers for PCR

The primers for amplifying the coding region of *SPATA12* cDNA: P1: 5′-CGCGGATCCATGTCCAGTTCTGCTCTGACT-3′ (*Bam*H I site is underlined) and P2: 5′-CCCAAGCTTGCAGGATTATTATTGATTACAG-3′ (*Hin*d III site is underlined); the size of the PCR product is 607 bp. The primers for SYBR real-time PCR: P3: 5′-TCACCTTCCCCTCATCTCCC-3′ and P4: 5′-TTTCACGCTTGTCCACTTTCAC-3′; the size of the PCR product is 170 bp (primers were located in different exons).

Construction of Expression Vectors

The coding region of *SPATA12* cDNA was subcloned into the expression vectors pRevTRE (Clontech Laboratories Inc, Mountain View, Calif) and pQE-30 (QIAGEN GmbH, Hilden, Germany), with a pair of specific primers P1/P2 which had *Bam*H I and *Hin*d III sites. The PCR products, pRevTRE, and pQE-30 were digested with *Bam*H I (Fermentas Inc, Hanover, Md) and *Hin*d III (Fermentas). Digested products were purified with a gel purification kit (TaKaRa Bio Inc, Shiga, Japan). Then ligation, transformation, and screening were carried out using standard protocols (TaKaRa).

RNA Isolation

Total RNA was extracted with the Trizol reagent (Invitrogen) according to the manufacturer's protocol, digested by RNase-free DNase (Sigma-Aldrich, St Louis, Mo), dissolved in diethyl pyrocarbonate–treated water, and stored at −80°C prior to use. The RNA quality and concentration were assessed by agarose gel electrophoresis.

SYBR Real-Time PCR

The primers P3/P4 were designed for SYBR real-time PCR with a human testis cDNA library (Clontech) as the template; the PCR product was subcloned into pUCM-T (Promega, Beijing, China); and the recombinant vector was purified with the QIAquick Purification Kit (QIAGEN), quantified by UV, and diluted into different concentrations to generate a standard curve. The total RNA from different tissues and cell lines was digested by DNase I and quantified by UV. Briefly, cDNA was synthesized from RNA samples with Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. Subsequently cDNA was used as a template for real-time PCR of *SPATA12* mRNA levels with a GeneAmp 5700 thermocycler (Applied Biosystems, Foster City, Calif).

PCR was performed in a 50-μL reaction volume containing 31 μL of nuclease-free water, 5 μL of SYBR 10X reaction buffer, 5 μL of 2.5 mM dNTP mix, 4 μL of 25 mmol/L $MgSO_4$, 1 μL of Taq DNA polymerase, 3 μL of cDNA, and 1 μL each 20 μmol/L gene-specific primers. The PCR profile was 95°C for 3 minutes followed by 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 45 seconds for 35 cycles, with a final extension at 72°C for 10 minutes and storage at 4°C. The level of *SPATA12* mRNA was evaluated in automated analysis by computer.

In Situ Hybridization Analysis

Different types of human testes were fixed in 4% paraformaldehyde, embedded in paraffin, and then cut into sections (5-μm thick) for in situ hybridization. According to the *SPATA12* mRNA sequence, digoxigenin-labeled oligonucleotide antisense probes: 1) 5′-CACCTGGGAAATGAAGGCACTAGACTCTTC-3′, 2) 5′-AAGTTATTCATAACTCTACACCTCAATTTC-3′, and 3) 5′-GGTCCCTCTCTA-

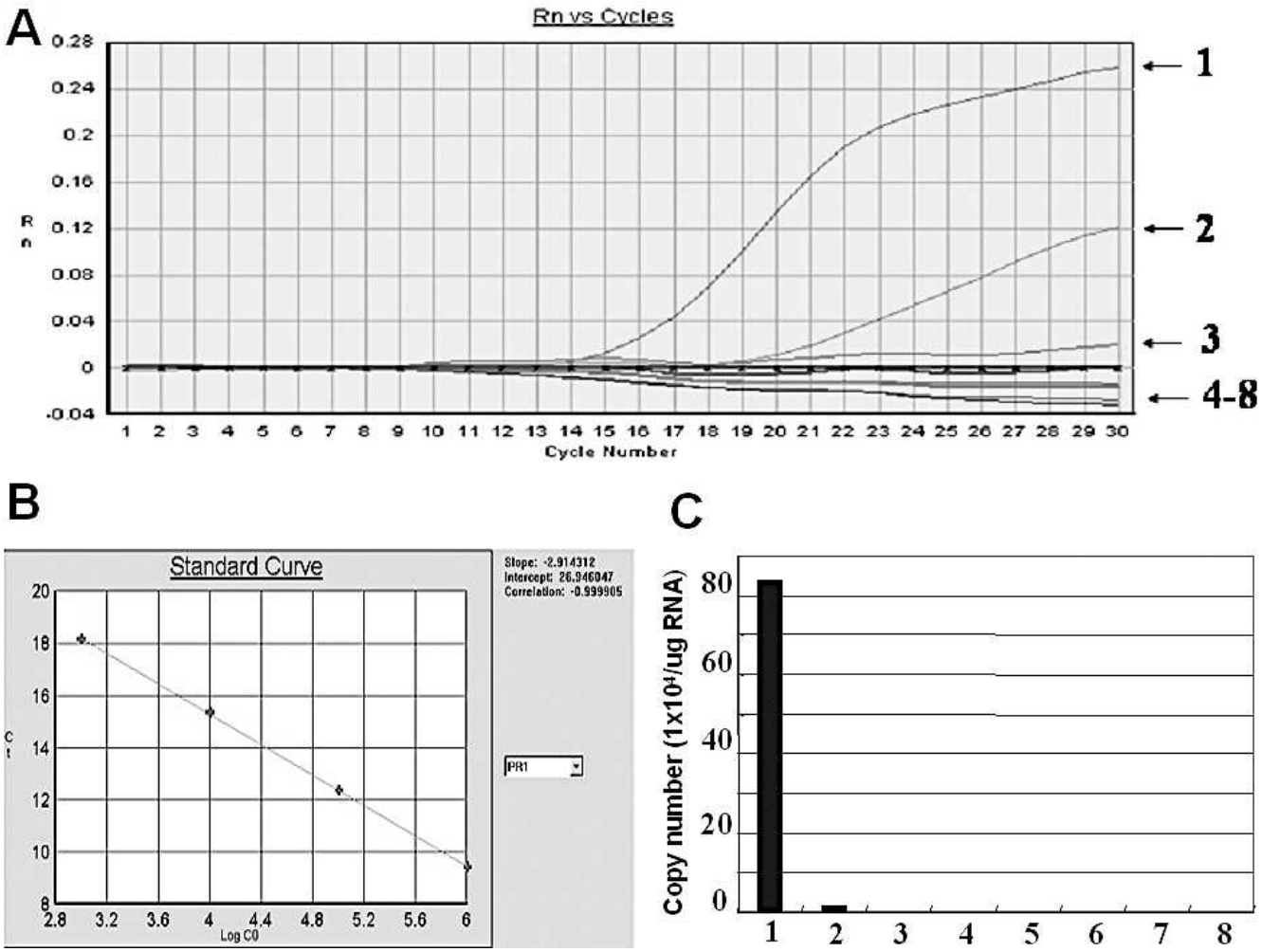

Figure 1. **(A)** SYBR real-time reverse transcriptase polymerase chain reaction (RT-PCR) results of *SPATA12* in the developmental stage of human testis. *SPATA12* mRNA was observed exclusively in adult testis but was completely absent in fetal testis. The level of *SPATA12* was 8.4×10^5/μg RNA in normal mature testis and 1.3×10^4/μg RNA in swim-up sperm. Lane 1: Adult testis. Lane 2: Swim-up sperm. Lane 3: Embryonic testis (3 months). Lane 4: Embryonic testis (4 months). Lane 5: Embryonic testis (5 months). Lane 6: Embryonic testis (6 months). Lane 7: Embryonic testis (8 months). Lane 8: Empty control. **(B)** SYBR real-time RT-PCR standard curve. **(C)** The copy number of *SPATA12* mRNA in human testis.

CAGTATACTCCAACACAC-3′ (which map to position 676–1248 bp) were synthesized commercially by Boster Co Ltd (Wuhan, China). The paraffin-embedded testis tissue slides were dried at 60°C for 30 minutes. These slides were cleared of paraffin with xylene, rehydrated by sequential washings with graded ethanol solution (70% to 100%), treated with proteinase K (100 mg/mL) in 10 mmol/L Tris-HCl (pH 7.5) containing 2 mmol/L $CaCl_2$ for 10 minutes at room temperature, and then fixed in 4% fresh paraformaldehyde for 5 minutes. The deproteinized slides were hybridized with approximately 25 ng of digoxigenin-labeled probes in 40 mL of hybridization buffer (50% formamide, 4X SSC, 2X Denhart solution, 0.1% sodium dodecyl sulfate [SDS], 10% dextran sulfate, and 100 mg/mL salmon sperm DNA). Slides were covered with Parafilm (Montreal, Quebec) and incubated at 37°C for 16 hours in a humid chamber.

Anti-Peptide Immunoglobulin Production

Polyclonal rabbit anti-SPATA12 peptide immunoglobulin (Ig) was synthesized commercially by Boster. The peptide was DTWEMKALDSSRLVP (residues 16–31). The anti-SPATA12 peptide antiserum (200 μg/mL) was concentrated for use in Western blot and immunohistochemistry analyses.

Western Blot Analysis

A single colony of *Escherichia coli* strain M15 (QIAGEN) harboring the expression recombinant pQE-30/SPATA12 was inoculated into 5 mL of LB medium containing ampicillin (final concentration 100 mg/L) and kanamycin (final concentration 25 mg/L) and incubated with shaking at 200 rpm overnight at 37°C. The cells were inoculated into 100 mL of fresh LB medium containing ampicillin and kanamycin in the

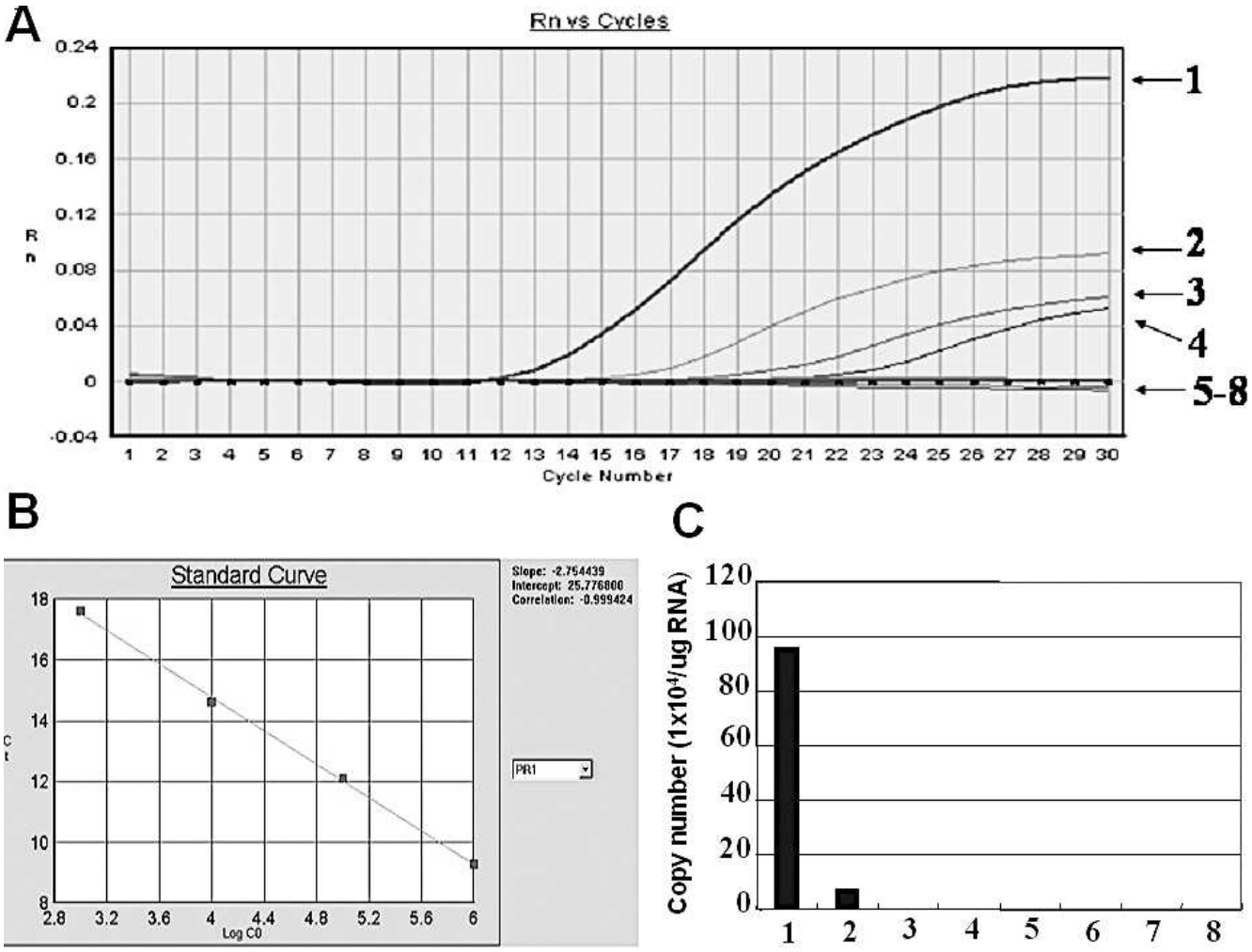

Figure 2. **(A)** SYBR real-time reverse transcriptase polymerase chain reaction (RT-PCR) results of *SPATA12* in testicular germ cell tumors or tumor cell lines. *SPATA12* mRNA expression was absent in testicular germ cell tumors such as seminomas and teratomas and in tumor cell lines such as MCF-7, HeLa, and MCG. The copy number of *SPATA12* mRNA was 9.6×10^5/μg RNA in normal mature testis, 6.3×10^4/μg RNA in testis adjacent to seminomas, 2400/μg RNA in teratomas, and 1900/μg RNA in the MCF-7 cell line. Lane 1: Normal adult testis. Lane 2: Testis adjacent to seminomas. Lane 3: MCF-7 cell line. Lane 4: Teratoma. Lane 5: Seminoma. Lane 6: HeLa cell line. Lane 7. MCG cell line. Lane 8: Empty control. **(B)** SYBR real-time RT-PCR standard curve. **(C)** The copy number of *SPATA12* mRNA in testicular germ cell tumors or tumor cell lines.

ratio of 1:50 and incubated for 3 hours. Then isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 1 mmol/L. The cells were collected by centrifugation after 4 hours. Cells were washed, harvested, and resuspended in phosphate-buffered saline (PBS). The cells were sonicated for 10 minutes with 20% Triton X-100 added to a final concentration of 1%, incubated for 30 minutes, and then centrifuged at 13 000 *g* for 10 minutes. Supernatant was removed to another sterile centrifuge tube. The cells were resuspended in PBS. Total protein from all testicular samples or cell lines were extracted using a mammalian protein extraction reagent (Pierce Biotechnology Inc, Rockford, Ill). The final concentrations of all proteins were determined with the bicinchoninic acid protein assay reagent (Pierce). Both fusion protein and total protein extracts from all testicular samples or cell lines were separated by 12% SDS polyacrylamide gel electrophoresis and transferred electrophoretically to a Hybond-P membrane (Pierce). The membrane was blocked with 5% skim milk in NaCl/Tris (10 mM Tris/HCl, 0.9 M NaCl [pH 7.4]), incubated with affinity-purified anti-SPATA12 peptide Ig (1:500) or β-actin (1:4000; Sigma-Aldrich) at 4°C, shaken overnight, developed with anti-rabbit Ig (1:2000; Pierce), and visualized by using enhanced chemiluminescence (Pierce).

Immunohistochemistry

Sections were dewaxed and rehydrated prior to incubation with 3% H_2O_2 for 8 minutes to remove endogenous peroxidase activity. Nonspecific binding was blocked with normal serum blocking buffer for 20 minutes at room temperature. Sections were incubated with affinity-purified anti-SPATA12 antibody (1:500) in a humidified chamber for 1 hour at 37°C. Following incubation with fluorescein isothiocyanate–labeled goat anti-

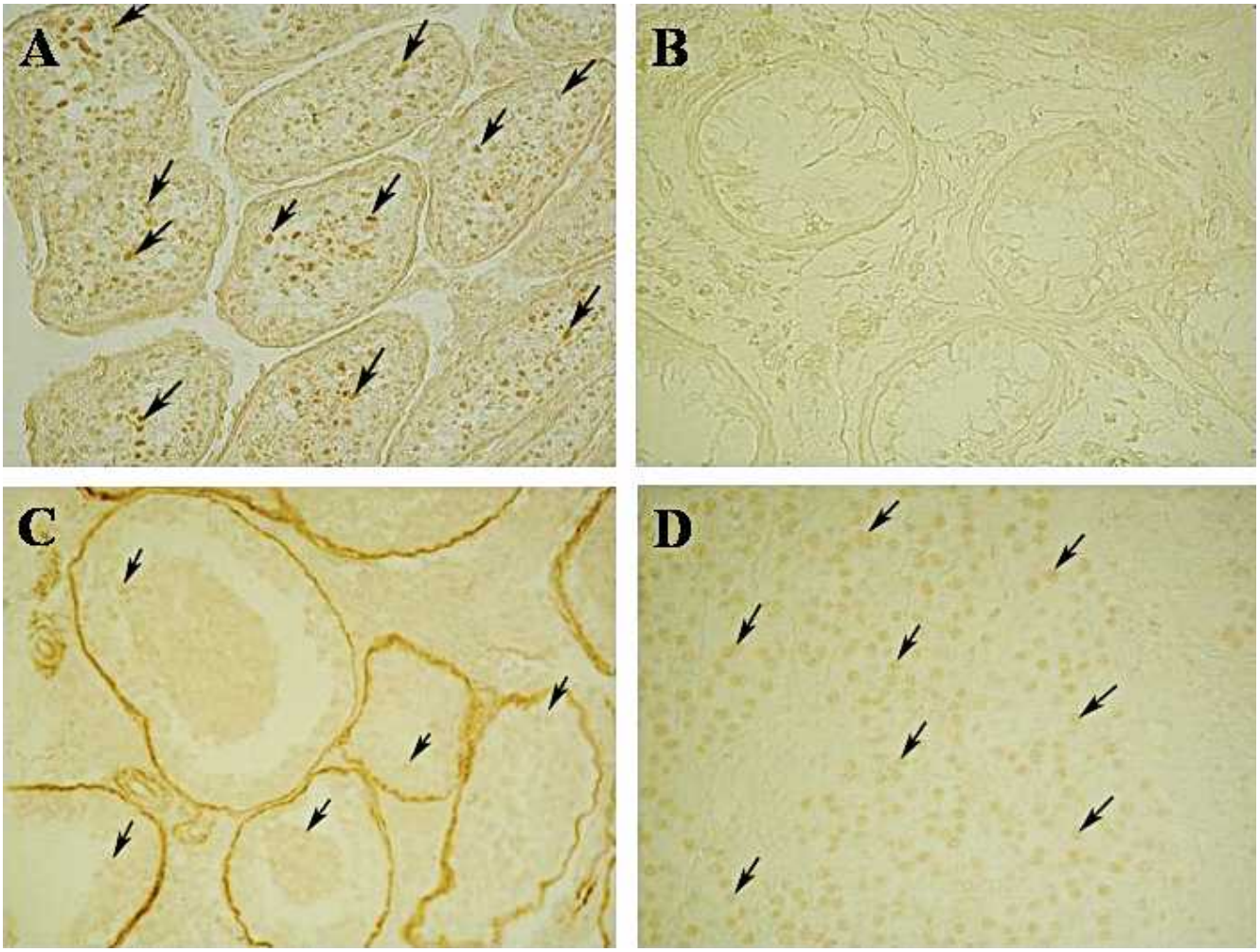

Figure 3. In situ hybridization results of *SPATA12* in human testis. **(A)** Strong hybridization signals were observed in the seminiferous tubules of normal adult testis, more precisely in the spermatocytes, spermatids, and spermatozoa. No expression was detected in Sertoli or Leydig cells. 200 × magnification. **(B)** No positive signals were detected in the testis of a patient with cryptorchidism. 200 × magnification. **(C)** The expression level of *SPATA12* mRNA in the testis of an infertile patient with spermacrasia was associated with the amount and density of germ cells. With decreasing numbers of germ cells, the expression of *SPATA12* mRNA was lower. 200 × magnification. **(D)** The expression of *SPATA12* mRNA was weak in seminomas. 200 × magnification.

rabbit Ig (1:2000; Pierce) for 20 minutes at 37°C, the sections were incubated with streptavidin-biotin complex for 20 minutes at 37°C. Hybridization signals were detected using a 3,3′-diaminobenzidine kit (Boster).

Flow Cytometry Analysis

To investigate the possible roles of the *SPATA12* gene on the cell cycle, pRevTRE/SPATA12 was constructed and flow cytometry (FCM) analysis was used in both the human HeLa cell line and mouse GC-1 spg germ cell cell line. pRevTRE and pRevTRE/SPATA12 transfections were performed with the FuGENE 6 transfection reagent (Roche Ltd, Shanghai, China). Forty-eight hours after the transient transfection, 1×10^6 cells were trypsinized and centrifuged. The cell pellets were washed with PBS and fixed in 70% ethanol overnight. Then cells were centrifuged for 5 minutes and washed with 1X PBS. Following a 30-minute incubation with Hanks buffered saline solution containing propidium iodide (10 μg/mL) and RNase (100 μg/mL) at room temperature, the cells were quantified on a FACScan (BD Biosciences, San Jose, Calif) at an excitation of 488 nm. The data were analyzed by CellQuest software (BD Biosciences).

Results

Expression of SPATA12 mRNA in the Developmental Stage of Human Testis

To examine the developmental changes of *SPATA12* transcription in the human testis, total testicular RNA at 3, 4, 5, 6, and 8 months and from adults was analyzed. *SPATA12* mRNA was not found in fetal testis but was abundantly detected in normal mature testis.

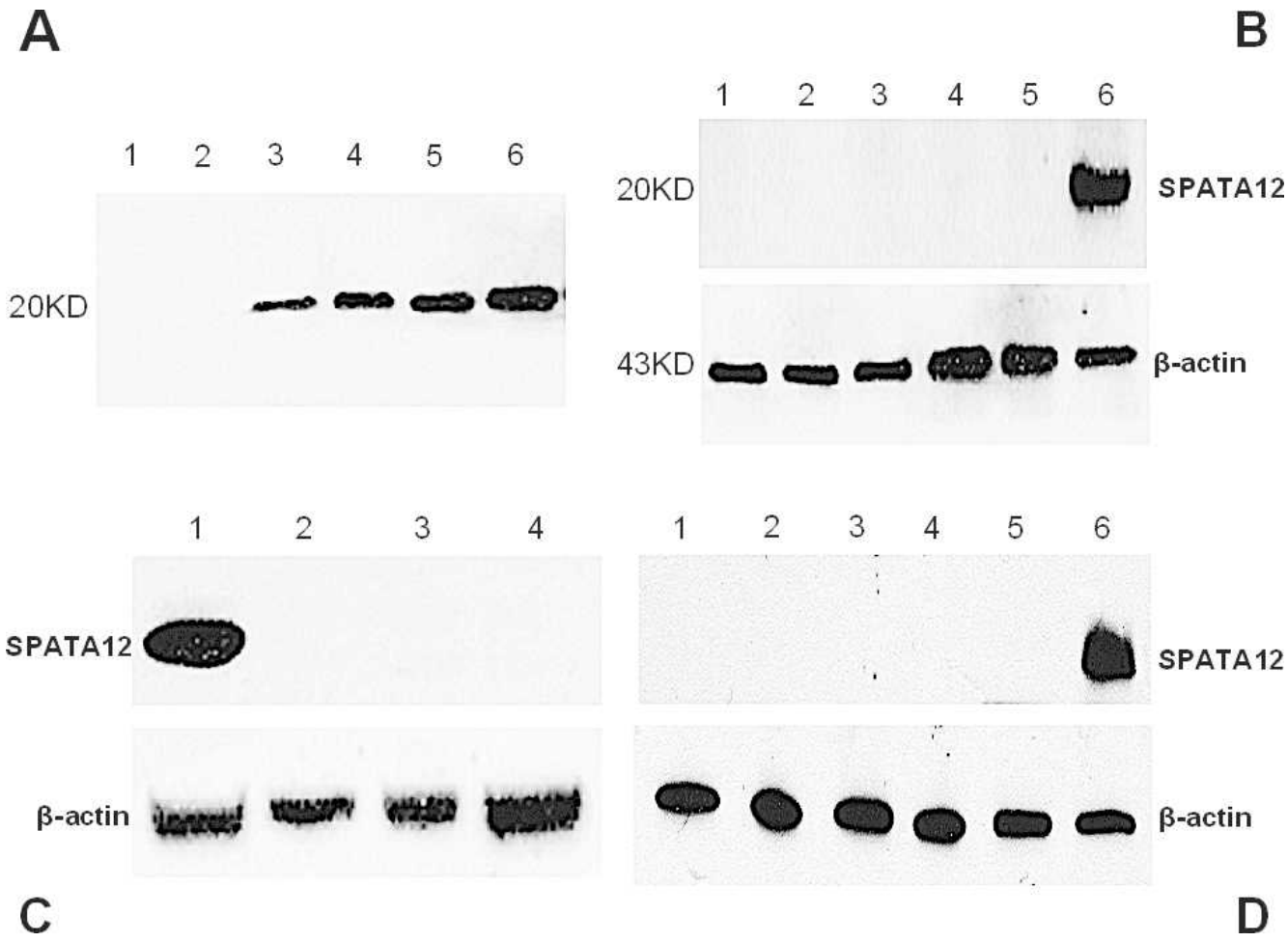

Figure 4. Western blot results of the SPATA12 protein. **(A)** A strong immunoreactive band at approximately 20 kd was shown by Western blot analysis of a recombinant histidine-tagged SPATA12 fusion protein expressed in *Escherichia coli*. Lane 1: pQE-30/SPATA12 without induction by isopropyl β-D-1-thiogalactopyranoside. Lane 2: Supernatant after sonication. Lanes 3–5: pQE-30/SPATA12 after sonication. The amounts of antigen were 50 μg, 100 μg, and 200 μg, respectively. Lane 6: Precipitated protein after sonication. **(B)** Expression of the SPATA12 protein in the developmental stage of the human testis. The SPATA12 protein signal was only detected in normal mature testis, and the size of the SPATA12 protein was approximately 20 kd. Lane 1: Embryonic testis (3 months). Lane 2: Embryonic testis (4 months). Lane 3: Embryonic testis (5 months). Lane 4: Embryonic testis (6 months). Lane 5: Embryonic testis (8 months). Lane 6: Adult testis. **(C)** Expression of the SPATA12 protein in the testis of an infertile patient. The SPATA12 protein was only detected in normal mature testis but was not observed in the testes of patients with cryptorchidism or Sertoli cell only syndrome. Lane 1: Normal adult testis. Lane 2: Testis of patient with cryptorchidism. Lane 3: Testis of patient with Sertoli cell only syndrome. Lane 4: Testis with maturation arrest. **(D)** Expression of the SPATA12 protein in testicular germ cell tumors or tumor cell lines. The SPATA12 protein was absent in testicular germ cell tumors such as seminomas and teratomas and tumor cell lines such as MCF-7, HeLa, and MCG. Lane 1: MCF-7. Lane 2: HeLa. Lane 3: MCG. Lane 4: Seminoma. Lane 5: Teratoma. Lane 6: Normal adult testis.

The copy number of *SPATA12* mRNA was 8.4×10^5/μg RNA in normal mature testis and 1.3×10^4/μg RNA in swim-up sperm (Figure 1). These results suggested that the expression pattern of *SPATA12* is developmental stage specific.

Expression of SPATA12 mRNA in Testicular Germ Cell Tumors and Tumor Cell Lines

The expression of *SPATA12* mRNA in several testicular tumors and tumor cell lines was examined. As shown in Figure 2, the *SPATA12* gene was nearly absent in testicular germ cell tumors such as seminomas and teratomas and in tumor cell lines such as MCF-7, HeLa, and MCG. The copy number of *SPATA12* mRNA was 9.6×10^5/μg RNA in normal mature testis, 6.3×10^4/μg RNA in testis adjacent to seminomas, 2400/μg RNA in teratomas, and 1900/μg RNA in the MCF-7 cell line.

Localization of SPATA12 mRNA in Testicular Germ Cells

In situ hybridization was used to localize *SPATA12* transcripts within the testis. Usually germinal cells or spermatogenic cells are arranged in an orderly manner from the basement membrane up to the lumen.

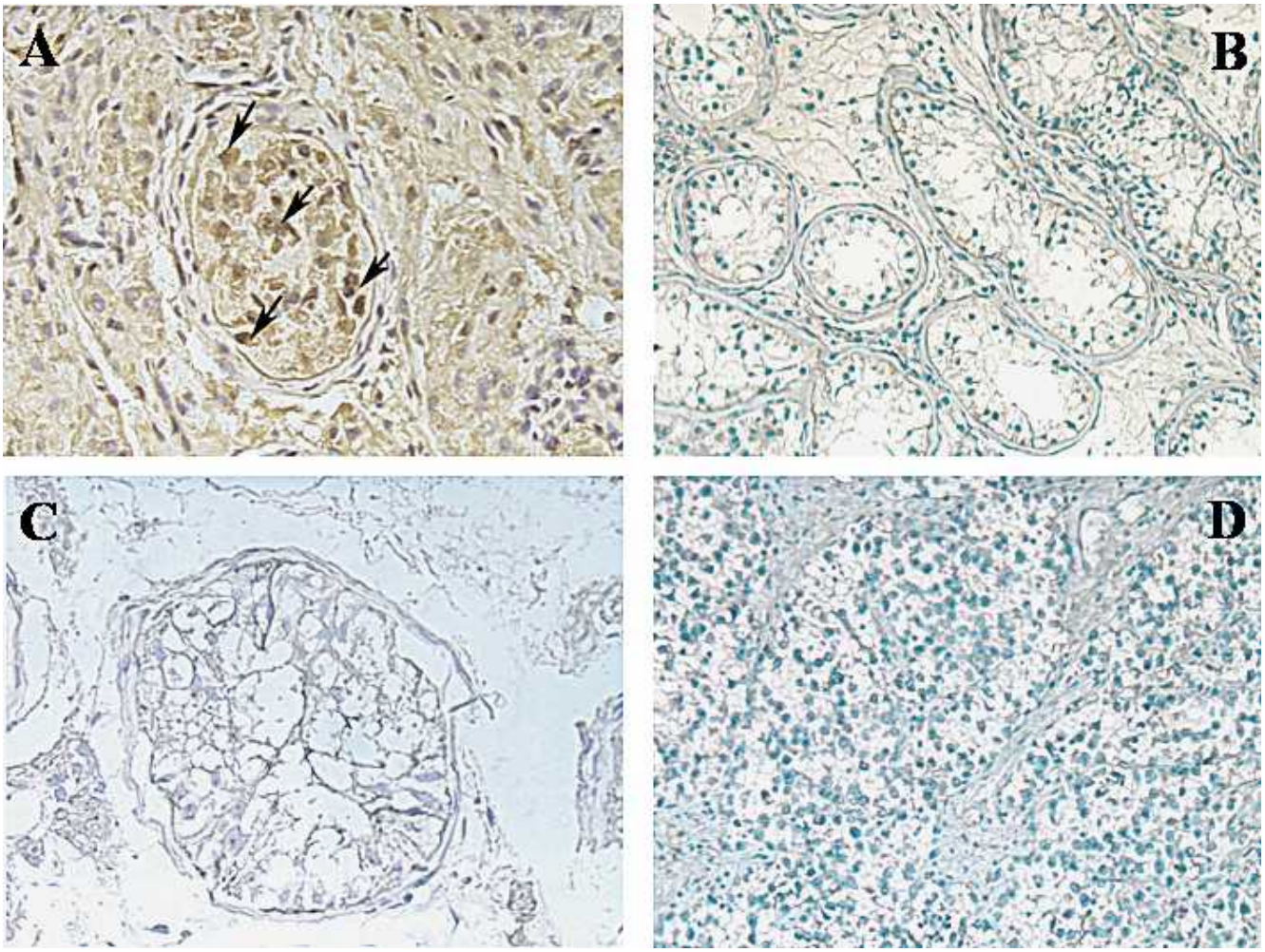

Figure 5. Immunohistochemical staining of the SPATA12 protein in human testis. Immunohistochemical analysis of adult human testis with an anti-SPATA12 antibody revealed a positive signal in germ cells but not in Sertoli or Leydig cells. No staining for SPATA12 was detected in the testes of patients with cryptorchidism or Sertoli cell-only syndrome and in seminomas. **(A)** Normal adult testis. 200 × magnification. **(B)** Testis of patient with cryptorchidism. 200 × magnification. **(C)** Testis of patient with Sertoli cell only syndrome. 400 × magnification. **(D)** Seminoma. 200 × magnification.

Spermatogonia lie directly on the basement membrane, and next, progressing up to the lumen, are the primary spermatocytes, secondary spermatocytes, and spermatids. As shown in Figure 3, the expression of *SPATA12* mRNA varied with the seminiferous cycle during spermatogenesis in the normal mature testis. The brown granules of the hybridization signal were found in spermatocytes, spermatids, and spermatozoa but were absent in spermatogonia and both Sertoli and Leydig cells. And no positive signals were detected in the testes of patients with cryptorchidism. No hybridization signal was observed when a *SPATA12* probe was not used (data was not shown). In addition, the expression level of *SPATA12* mRNA in infertile men with spermacrasia was associated with the amount and density of germ cells. With decreasing numbers of germ cells, the expression of *SPATA12* mRNA was lower. This expression pattern showed that *SPATA12* is a germ cell–specific gene.

Expression and Localization of SPATA12 Protein

Polyclonal antibodies were generated against a unique peptide derived from a nonhomologous, hydrophilic region of the SPATA12 polypeptide sequence to investigate its expression and localization in human testis. A strong immunoreactive band at approximately 20 kd was shown by Western blot analysis of a recombinant histidine-tagged SPATA12 fusion protein expressed in *E coli* (Figure 4A), which demonstrated the specificity of the anti-SPATA12 antibody. The SPATA12 protein signal was only detected in normal mature testis, and the size of the protein in testis was approximately 20 kd (Figure 4B). The signals were not observed in the testes

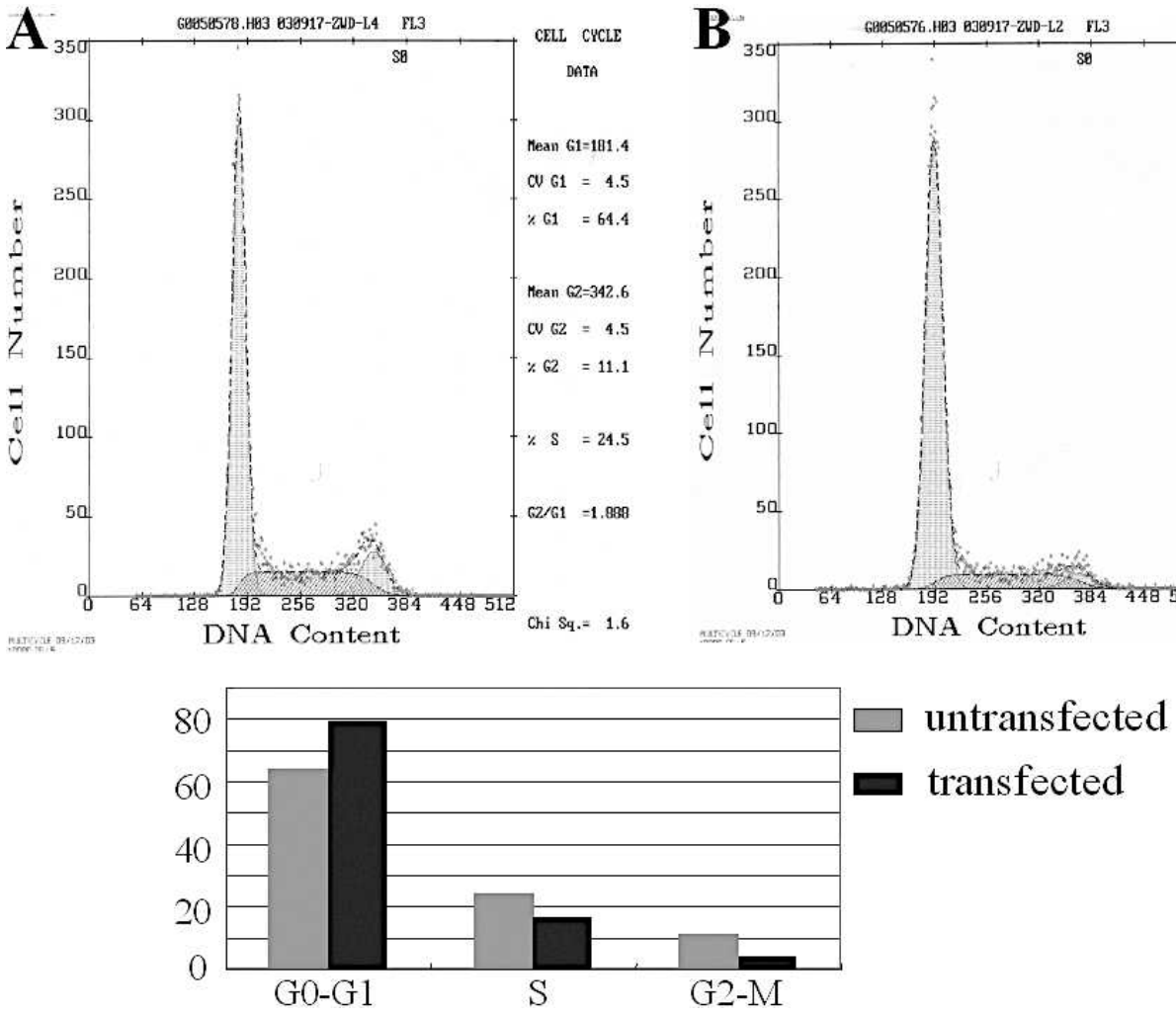

Figure 6. The cell cycle distribution of human HeLa cells by transfection with *SPATA12*. The percentage of cells resident in each cell cycle phase is indicated. **(A)** In control HeLa cells, the cell cycle distribution was 64.4% of cells in G_1 phase, 24.5% of cells in S phase, and 11.1% in G_2 phase. **(B)** In transfected HeLa cells, 79.4% of cells remained in G_1, 16.3% of cells in S, and 4.3% in G_2. The data showed that *SPATA12* could delay the progression of G_1 through S in the cell cycle compared with the nontransfected control.

of patients with cryptorchidism or Sertoli cell only syndrome (Figure 4C). As shown in Figure 4D, the SPATA12 protein was also absent in testicular germ cell tumors such as seminomas and teratomas and in tumor cell lines such as MCF-7, HeLa, and MCG.

Immunohistochemical analysis (Figure 5) of an adult human testis with an anti-SPATA12 antibody resulted in a positive signal in germ cells but not in Sertoli or Leydig cells. Spermatocytes, spermatids, and spermatozoa were stained. No staining for SPATA12 was detected in the testes of patients with either cryptorchidism or Sertoli cell only syndrome. The results indicated that the SPATA12 protein is temporally regulated during human spermatogenesis because the protein was expressed in a stage-specific manner in germ cells correlating with postmeiotic testicular germ cells. These data are consistent with previous results demonstrating the developmental stage–specific and germ cell–specific expression of SPATA12.

Cell Cycle Analysis of the SPATA12 Gene Acting on both HeLa and GC-1 spg Cells

To investigate the possible roles of *SPATA12* on the cell cycle, pRevTRE/SPATA12 was constructed and FCM analysis was used to evaluate the cell cycle distribution in both human HeLa cells and mouse GC-1 spg germ cells. The mRNA expression of *SPATA12* in HeLa and GC-1 spg cells was checked after SPATA12 transfection (data not shown). The percentage of cells resident in each cell cycle phase was determined. In control HeLa cells, the cell cycle distribution was 64.4% of cells in G_1 phase, 24.5% of cells in S phase, and 11.1% in G_2 phase; in transfected HeLa cells, 79.4% of cells remained in G_1

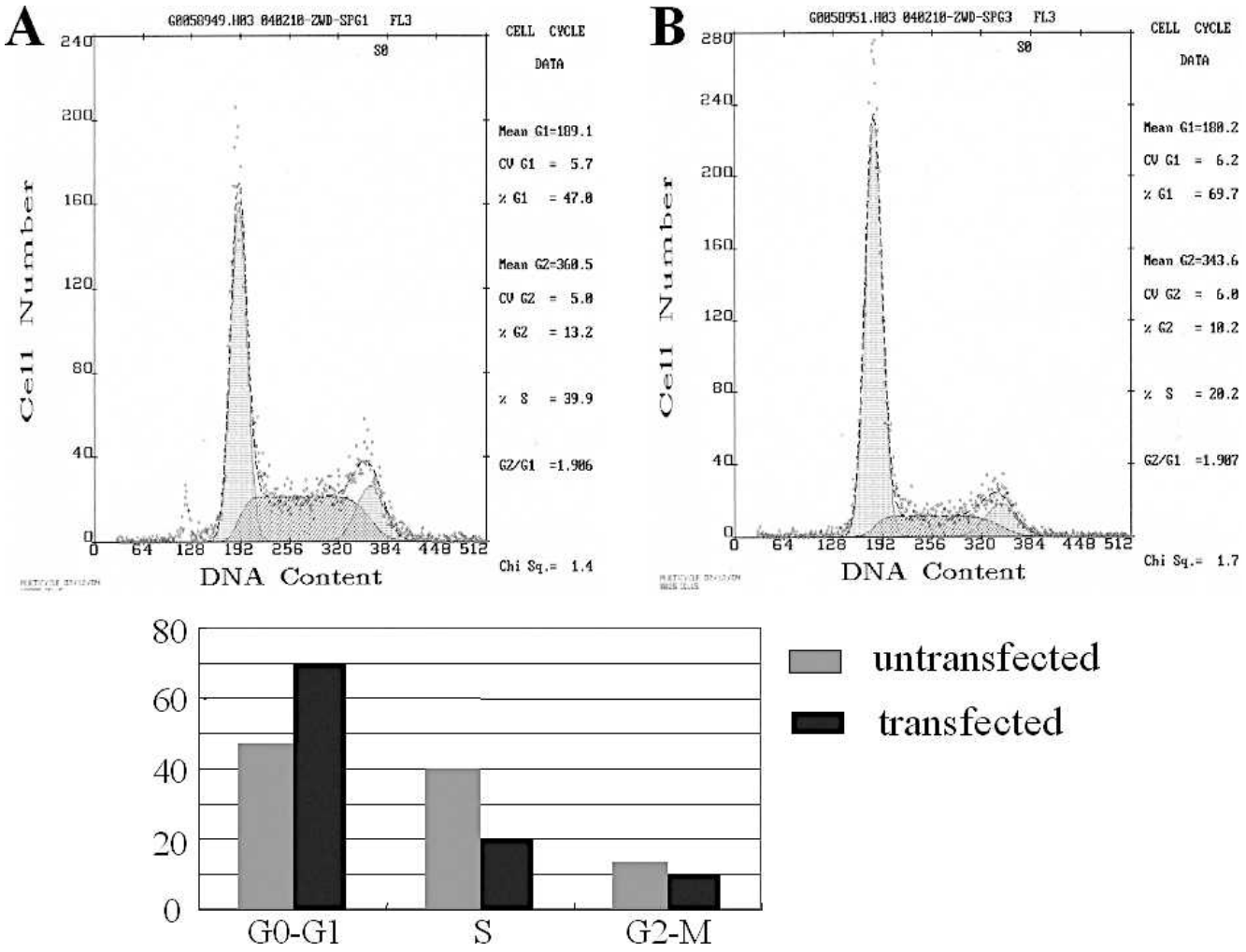

Figure 7. The cell cycle distribution of mouse GC-1 spg cells by transfection with *SPATA12*. The percentage of cells resident in each cell cycle phase is indicated. **(A)** In control GC-1 spg cells, the cell cycle distribution was 47.8% of cells in G_1 phase, 39.9% of cells in S phase, and 13.2% in G_2 phase. **(B)** In transfected GC-1 spg cells, 69.7 % of cells remained in G_1, 20.2% of cells in S, and 18.2% in G_2. The data showed that *SPATA12* could delay the progression of G_1 through S in the cell cycle compared with the nontransfected control.

phase, 16.3% of cells in S phase, and 4.3% in G_2 phase (Figure 6). In control GC-1 spg cells, the cell cycle distribution was 47.8% of cells in G_1 phase, 39.9% of cells in S phase, and 13.2% in G_2 phase; in transfected GC-1 spg cells, 69.7% of cells remained in G_1 phase, 20.2 % of cells in S phase, and 18.2% in G_2 phase (Figure 7). These data showed that *SPATA12* could delay the progression of G_1 through S in the cell cycle compared with the nontransfected controls.

Discussion

In present study, spatial and temporal studies of *SPATA12* were accomplished by SYBR real-time RT-PCR and Western blot. The *SPATA12* gene and protein were only expressed in the adult testicular developmental phase; they were completely absent in fetal testis, which suggests that *SPATA12* is only expressed after puberty. The process of germ cell differentiation begins in the embryo when primordial germ cells differentiate into gonocytes, which subsequently mature into spermatogonia during infancy and early childhood. At puberty, gonadotropic stimulation induces real spermatogenesis. Many genes expressed in the testis are developmental stage specific or cell type specific, which reflects the demands of development in tissues (Guo et al, 2004). It has been demonstrated that *ODF2* (Chan et al, 2005), *AKAP4* (Miki et al, 2002), *CLGN* (Tanaka et al, 1997; Toshimori et al, 2001), *LDHC* (Li et al, 1998), and *PKG2* (Zhang et al, 1999) are differentially displayed between adult testis and fetal testis, which indicates that these are spermatogenesis-specific genes. Temporal studies of *SPATA12* gene and protein

expression demonstrated that it is developmental stage specific and is involved in the development of testicular maturation.

Spermatogenesis is an essential stage in the human reproductive process that consists of 3 principle phases: mitotic proliferation for stem cell renewal, meiosis by which diploid spermatocytes develop into haploid spermatids, and spermiogenesis during which round spermatids differentiate and are released as mature spermatozoa into the lumen of the seminiferous tubule. Both in situ hybridization and immunohistochemical analysis showed that the distribution of the *SPATA12* gene varies with the seminiferous cycle during spermatogenesis in human mature testis. The strong hybridization signal was limited to certain populations of germ cells such as spermatocytes, spermatids, and spermatozoa. These cells belong to the differentiation phase of spermatogenesis. We hypothesized that the putative function of *SPATA12* is control of cell division or maintenance of the cell in a differentiated state. We also found that the expression level of *SPATA12* mRNA in male infertile testis was associated with the amount and density of germ cells. With decreasing numbers of germ cells, the expression of *SPATA12* mRNA was lower. In addition, the signal was not detected in the testes of patients with cryptorchidism or Sertoli cell only syndrome. In these patients, no mutations or deletions were observed in the coding sequence of *SPATA12* (data not shown). As a germ cell–specific gene, *SPATA12* may have some function in male infertility.

In our previous study, chromosome localization showed that *SPATA12* is on chromosome 3p21.1–3p21.2, which has been considered a strong candidate target gene region for tumor suppressor genes (Guo et al, 2000; Wang et al, 2000; Hanke et al, 2001; Imreh et al, 2003). *SPATA12* was lost in testicular germ cell tumors at the level of both transcription and translation. FCM analysis indicated that the expression of SPATA12 could delay the progression of G_1 through S in the cell cycle. According to these results, we presume that SPATA12 may play some role in suppressing tumor cell growth; thus, its absence may permit a proliferative process.

In conclusion, the restricted expression pattern of the *SPATA12* gene in spermatocytes, spermatids, and spermatozoa is consistent with a specialized role during spermatogenesis. Temporal studies of the *SPATA12* gene demonstrated that it is a stage–specific gene that may be involved in development of testicular maturation. A similar expression pattern has been seen in some tumor suppressor genes such as *p53* (Socher et al, 1997; Allemand et al, 1999), *hH-Rev107* (Siegrist et al, 2001), and *Cnot7* (Flanagan et al, 2003; Nakamura et al, 2004), which are also involved in spermatogenesis. We hypothesized that the putative function of *SPATA12* is to maintain the cell in a differentiated state and/or to suppress cell proliferation.

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