·Original Article·

Expression and localization of CKLFSF2 in human spermatogenesis

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Abstract

Aim: To investigate the expression and subcellular localization of chemokine-like factor superfamily 2 (CKLFSF2) in human testis and its potential role in spermatogenesis. **Methods:** A specific polyclonal antibody against CKLFSF2 was raised. The expression and cellular localization of CKLFSF2 in the seminiferous tubules was checked by immunohistochemistry method. Also, *in situ* hybridization was applied to localize the mRNA distribution. The EGFP-CKLFSF2 fusion protein was expressed in COS-7 cells to localize its subcellular location *in vitro*. In addition, the abnormal expression of CKLFSF2 in testes of patients with male infertility was assayed by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry methods. **Results:** Having a close correlation with spermatogenesis defects, CKLFSF2 was specifically expressed in meiotic and post-meiotic germ cells, which were localized to the endoplasmic reticulum (ER) near the Golgi apparatus. **Conclusion:** CKLFSF2 could play important roles in the process of meiosis and spermiogenesis, and might be involved in the vesicular transport or membrane apposition events in the endoplasmic reticulum. *(Asian J Androl 2007 Mar; 9: 189–198)*

Keywords: spermatogenesis; testis; chemokine-like factor superfamily; infertility; endoplasmic reticulum

1 Introduction

Mammalian spermatogenesis is a complex phenomenon of cell differentiation, including mitotic stem cell proliferation and meiosis, followed by remodeling of haploid spermatids and progressing to the formation of mature spermatozoa. It takes place within the germinal epithelium of the seminiferous tubules. Abnormality of any single step in spermatogenesis can cause male infertility. Although, spermatogenesis has been extensively studied, molecular details of this complex multistep differentiation remain largely elusive.

Human chemokine-like factor superfamily (CKLFSF) is a novel protein family that provides a structural and functional link between chemokines and members of the transmembrane 4 super family (TM4SF) [1, 2]. In humans, until now, nine genes encoding CKLF and CKLFSF1-8 (CKLF-like MAL-related proteins for vesicle trafficking and membrane link MARVEL transmembrane domain containing1-8, CMTM1-8) have been cloned. Interestingly, most members of the CKLFSF family have

higher expression levels in testis, indicating they might play systemic roles in spermatogenesis. Among members of the CKLFSF family, CKLFSF2 (CMTM2) is highly expressed in testis, mainly in spermatogonia and the seminiferous tubular fluid [3], which has four putative transmembrane regions and a MARVEL domain. In addition, CKLFSF2 is found to be very active during evolution. In mice, CKLFSF2 has two counterparts, mouse Cklfsf2a and Cklfsf2b [4]. Jeong *et al.* [5] reported a novel androgen receptor (AR) corepressor named androgen receptor corepressor-19 kDa (ARR19) was expressed within seminiferous tubules and most abundantly in germ cells, which is in fact the mouse Cklfsf2a. This evidence suggests that human CKLFSF2 might have an important function in spermatogenesis.

The aim of the present study was to analyze the cellular localization of CKLFSF2 within human testes and to characterize the potential role of CKLFSF2 in spermatogenesis.

2 Materials and methods

2.1 Samples

Informed consent was received from the participants and the ethics committee of Peking University (China) granted research approval prior to sample collection. Testis tissue samples with different spermatogenesis defect degrees were acquired via biopsy from azoospermia patients aged 21–35 years. All testes samples were acknowledged by pathologic diagnosis.

2.2 Preparation of the anti-CKLFSF2 polyclonal antibody and specificity verification

2.2.1 Preparation of the anti-CKLFSF2 polyclonal antibody

Polyclonal rabbit anti-CKLFSF2 serum was raised against a synthetic peptide corresponding to the N-terminal 15 amino acids (KPEEDKKDGKEPSDK) of the cytoplamic tail of human CKLFSF2 plus an additional cysteine residue at the C-terminus of the peptide. The peptide was purchased from Biosynthesis Biotechnology (Beijing, China), conjugated to keyhole limpet hemocyanin, and emulsified with an equal volume of Freund complete and incomplete adjuvants. It was then injected subcutaneously into three New Zealand white rabbits at 50 µg in 1 mL every 2 weeks for 4 months. After confirming an increase in the levels of antibody titer to more than × 5 000, the whole blood was collected from each rabbit and the serum was separated. The IgG fraction was purified from the serum using Affi-Gel 10 beads conjugated with CKLFSF2 peptide and concentrated to approximately 100 µg/mL.

2.2.2 Specificity verification of the anti-CKLFSF2 polyclonal antibody

The specificity of the anti-CKLFSF2 polyclonal antibody was verified by transient transfection and Western blot analysis. First, the full-length coding sequences of CKLFSF2 were cloned into the pcDNA3.1-Myc-HisB(-) mammalian expression vector (Invitrogen, Carlsbad, CA, USA) to create pcDNA3.1-CKLFSF2/Myc-His6 (removed the stop codon) and pcDNA3.1-CKLFSF2 (reserved the stop codon) as described in a previous report [3], and COS-7 cells were maintained at 37ºC (5% CO_2) in Dulbecco modified eagle medium (DMEM) with 10% fetal calf serum. Then, cells were transfected with pcDNA3.1-CKLFSF2/Myc-His6 or pcDNA3.1-CKLFSF2 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cells were collected 48 h after transfection and cell lysates were subjected to Western blot analysis with the anti-CKLFSF2 polyclonal antibody (incubation overnight at 4ºC, 1 : 1 000TBST, 1% milk) and the anti-Myc epitope antibody (incubation overnight at 4ºC, 1 : 500TBST, 1% milk; Invitrogen, Carlsbad, CA, USA) as described by Chen *et al.* [6].

2.3 Immunohistochemistry, immunopositive cell count and statistical analysis

2.3.1 Immunohistochemistry

Immunohistochemistry experiments were carried out to detect the cellular localization of CKLFSF2 in the seminiferous tubules and its abnormal expression in infertile patients' testes. After dewaxing and hydrating in descending ethanols (100%, 95%, 80% and 70%), 8-µm paraffin-embedded sections of human testis fixed in Bouin's solution were washed in PBS (154 mmol/L NaCl, 10 mmol/L NaH_2PO4, pH 7.5) and were treated with 0.3% H_2O_2 in methanol for 10 min to inhibit intrinsic peroxidase activity and with 5% normal goat serum for 30 min to prevent nonspecific antibody binding. Subsequently, the sections were then incubated overnight at 4ºC with the anti-CKLFSF2 polyclonal antibody at a 1 : 1 500 dilution in phosphate-buffered saline (PBS), washed three times in PBS, and again incubated with horseradish peroxidase (HRP) conjugated mouse anti-

rabbit IgG antibody for 1 h at room temperature. Sections were washed twice in PBS and the bound antibody was detected using DAB; control sections were stained with pre-immune rabbit serum.

2.3.2 Immunopositive cell count and statistical analysis

The testis samples were divided into four pathologic groups: normal spermatogenesis, spermatogenesis disturbance, spermatogenesis arrest and Sertoli cell-only syndrome (SCOS). With three samples in each group, CKLFSF2 positive cells were identified in 50 seminiferous tubules under a light microscope. Cells were scored as CKLSFSF2-positive when they showed intensely dark brown staining. Data were presented as mean ± SD of CKLFSF2-positive cell number per seminiferous tubule. To compare the significance of the means obtained from pathologic groups, statistical analysis was carried out using Kruskal–Wallis test and SPSS 13.0 for Windows software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

2.4 RNA extraction and RT–PCR

Total RNA of testis tissues was extracted with Trizol reagent (Invitrogen) according to the manufacturer's instructions, and reverse-transcribed to cDNA with Oligo dT and avian myeloblastosis virus (AMV; Promega, Madison, WI, USA).

The CKLFSF2 specific primers sequences were as follows:

Upstream: 5'AGTCATGGCACCTAAGGCGGCAA3'
Downstream: 5'CCTCCAAGTCATTTCTTTCCC3'

The PCR product was 759 bp in size. Beta-actin was used as the positive control. The reagents in 25 μL PCR reaction systems were as follows: H_2O 17.75 μL, 10 × buffer 2.5 μL, Mg^{2+} 1.5 μL, 10 mmol/L dNTP 1 μL, Taq DNA polymerase 0.25 μL, upstream primer 10 pmol 0.5 μL, downstream primer 10 pmol 0.5 μL, and cDNA sample 1 μL. PCR conditions used were as follows: denaturation at 94ºC for 20 s, annealing at 59ºC for 20 s and extension at 72ºC for 30 s. The first cycle had a denaturation period of 3 min and the last cycle had an extension period of 7 min. Thirty-five cycles of PCR were carried out. The PCR products were analyzed by 1% (w/v) agarose gel electrophoresis.

2.5 In situ hybridization on paraffin sections of human testis

In situ hybridization was applied to detect CKLFSF2

mRNA with a commercially available CKLFSF2 mRNA ISH detection kit. Digoxin (DIG)-labeled probe to CKLFSF2 (oligo probe) was obtained from TBD Science Technology (Tianjin, China). The probe sequences were as follows: 5'-TGAAG GGTAT GTATC TATGA ATGGC AAAGC-3'. A testis paraffin embedded section of 5 μm thickness was incubated in 0.3% hydrogen peroxide in methanol for 10 min and rinsed with PBS. After exposed to proteinase K, prehybridization with hybridization solution without the probe was carried out for 1 h at 37ºC. Then hybridization with a labeled probe was carried out at 37ºC for 2 h. After hybridization, the section was washed in 2 × standard saline citrate (SSC) for three changes. Hybridized signal was detected with AP-conjugated anti-DIG antibodies and visualized with nitroblue tetrazolium and 5-bromo-4-chlro-3-indolyl phatase.

2.6 Plasmid construction, cell transfection and confocal microscopy

For analysis of the subcellular localization, the full-length coding sequences of CKLFSF2 were cloned in frame into the pEGFP-N1 and pECFP-N1 expression vectors (BD Biosciences Clontech, Frankin Lakes, NJ, USA) to create pEGFP-N1-CKLFSF2 and pECFP-N1-CKLFSF2 as described previously [3]. The recombinant plasmids were further confirmed by dideoxynucleotide sequencing. COS-7 cells were maintained in Dulbecco modified eagle medium (DMEM) containing 10% fetal bovine serum at 37ºC in 5% CO_2. The cells were transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Then, 24 h after the cells transfected with GFP-CKLFSF2, ER and Golgi apparatus were stained using ER-Tracker™ Red (Molecular Probes; Invitrogern, Carlsbad, CA, USA) and fluorescent BODIPY-TR ceraminde (Molecular Probes) separately according to the manufacturer's protocol. In our previous report [6], Cox7a2 was specifically localized to mitochondria, so in the present study, pEYFP-N1-Cox7a2 was co-transfected with pECFP-N1-CKLFSF2 to label the mitochondria. The treated cells were visualized by laser confocal microscope (Leica, Wetzlar, Germany).

3 Results

3.1 The specificity of the anti-CKLFSF2 antibody

Transient transfection and Western blot experiments

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were carried out to analyze the specificity of the anti-CKLFSF2 polyclonal antibody. The cell lysates of COS-7 cells transfected with pcDNA3.1-CKLFSF2 (lane 1 in Figure 1) were recognized only with the anti-CKLFSF2 antibody, whereas the cell lysates of COS-7 cells transfected with pcDNA3.1-CKLFSF2/Myc-His6 to express recombinant CKLFSF2 containing Myc epitope (lane 2 in Figure 1) were recognized with both the anti-CKLFSF2 and anti-Myc antibody. The molecular masses of detected bands were about 17kDa. No other band was detected. This result confirmed the specificity of the anti-CKLFSF2 antibody.
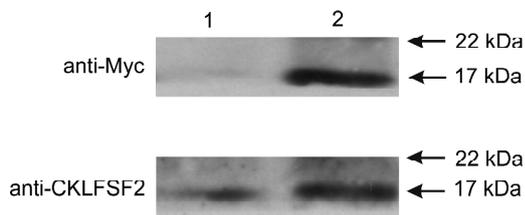

Figure 1. Western blot analysis of the specificity of the anti-CKLFSF2 polyclonal antibody. Cell lysates of COS-7 cells transfected with pcDNA3.1-CKLFSF2 (lane 1) were recognized only with the anti-CKLFSF2 antibody; whereas cell lysates of COS-7 cells transfected with pcDNA3.1-CKLFSF2/Myc-His6 to express recombinant CKLFSF2 containing Myc epitope (lane 2) were recognized with both antibodies.

3.2 The CKLFSF2 protein was localized in a stage-specific manner to the meiotic and post-meiotic germ cells

To confirm the cellular localization of CKLFSF2, we carried out immunohistochemistry in paraffin-embedded human testicular sections using the anti-CKLFSF2 antibody. The immunostaining was located exclusively in the seminiferous tubules and was not detected in cells of the interstitial tissue. Most seminiferous epithelia were intensively immunostained in the cells at the periphery of the lumen, mainly the round and elongating spermatids (Figure 2a). However, in some seminiferous tubules, the strong immunoactivity was detected in the pachytene spermatocytes; the signal was concentrated in juxtanuclear cytoplasm, like the "cap" of the nucleus (Figure 2b). For analysis of the specificity of the reaction, we analyzed the same testis tissue sections with preimmune serum (negative control). As expected, staining with the preimmune serum showed no signal (Figure 2c).

Furthermore, the localization of CKLFSF2 protein in the cycle of the human seminiferous epithelium was detected. As is already known, germ cells in different phases of development are not randomly distributed within the epithelium, but occur in a number of well-defined and easily recognized combinations or associations. Six well defined stages representing six different typical cell associations can be recognized in the cycle of the human seminiferous epithelium [7]. Detecting the localization of CKLFSF2 in each stage could supply the kinetic information during germ cell development. Here, at stage I, CKLFSF2 signals dispersed in the tail cytoplasm of the late elongating spermatids (Figure 2A); At stage II, with the completion of the differentiation of the tail, the spermatozoon was separated from the excess cytoplasm, which remained in the epithelium as a residual body. At this phase, CKLFSF2 immunoactivity appeared mainly in the residual bodies and a little in the flagella of the mature spermatozoa (Figure 2B); at stage III and IV, the CKLFSF2 signal appeared as a "dot" in the caudal part or postnuclear region of round and early elongated spermatids (Figure 2C, 2D). At stage V and VI, the immunoactivity dispersed in the tail cytoplasm of the more advanced generation of elongating spermatids (Figure 2E, F). In brief, the expression of CKLFSF2 protein showed specific characteristics at different stages of the seminiferous epithelium cycle.

In a kinetic view of germ cell development, the immunostaining showed CKLFSF2 protein was first expressed in the cytoplasm of the pachytene spermatocytes; then it appeared in the caudal part or post-nuclear region when the spermatocytes differentiated into round and early elongated spermatids, and further, in the late elongating spermatids and mature spermatozoa, CKLFSF2 protein was localized to the tail cytoplasm and residual bodies; During the whole cell differentiation process, CKLFSF2 was never appeared in the nucleus or the acrosome. In conclusion, this result indicated the protein of CKLFSF2 was localized in a stage-specific manner to the meiotic and post-meiotic germ cells.

3.3 The CKLFSF2 mRNA was detected primarily in the spermatocyte

In situ hybridization with a DIG-labelled antisense riboprobe detected CKLFSF2 mRNA in the normal adult testes. The CKLFSF2 mRNA was detected primarily in the spermatocyte. No signal was detected in the spermatogonia, spermatids or the interstitial tissue (Figure
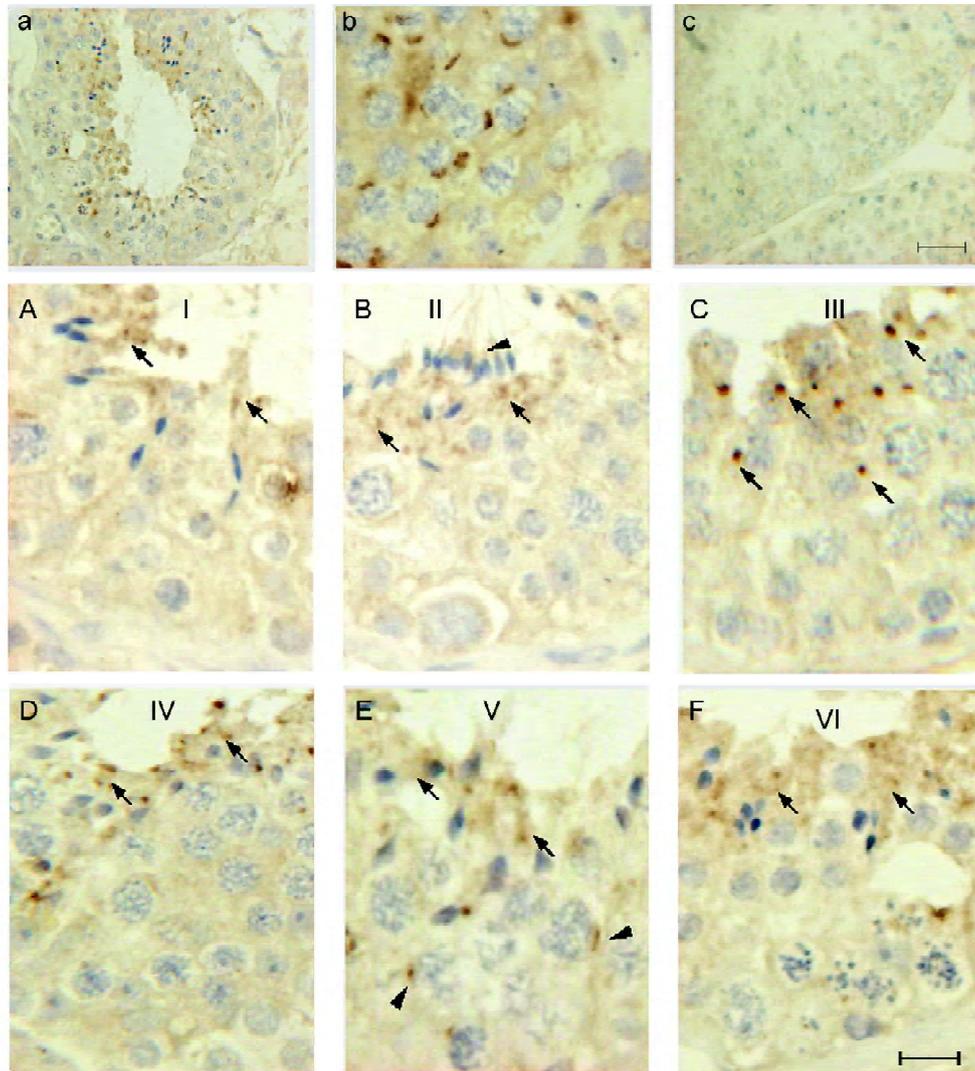
Figure 2. Immunohistochemistry showing the localization of CKLFSF2 in normal adult human testis. The paraffin sections were immunostained with the anti-CKLFSF2 antibody (a, b, A–F) or preimmune serum. (a): Most seminiferous epithelia were intensively immunostained in the cells at the periphery of the lumen, mainly the round and elongating spermatids. (b): In some seminiferous tubules, the strong immunoactivity was localized in the pachytene spermatocytes, mostly in juxtanuclear cytoplasm, like "caps" of the nucleus. (c): No reactivity was present in any portions. (A)–(F): Showed the localization of CKLFSF2 in the cycle of seminiferous epithelium. (A): At stage I, CKLFSF2 immunoactivity appeared in tail cytoplasm of elongating spermatids (arrows). (B): At stage II, the immunostaining appeared in the residual bodies (arrows), with a little in flagella of the mature spermatozoa (arrowhead). (C), (D): At stage III and IV, the CKLFSF2 signal appeared as a "dot" in the caudal part or post-nuclear region of round and early elongated spermatids (arrows). (E), (F) At stage V and VI, the immunoactivity dispersed in the tail cytoplasm of the more advanced generation of elongated spermatids (arrows); (E): Signals in the pachytene spermatocytes were detected (arrow heads). (a), (c): Bar = 50 μm; (b), (A)–(F): Bar = 25 μm.

3A, 3C). Negative control using a sense probe showed no hybridization signal in any cell type under identical experimental conditions, confirming the specific expression of CKLFSF2 mRNA (Figure 3B, 3D).

3.4 Differential expression of the CKLFSF2 protein in the testes of patients with male infertility

Light microscopic immunohistochemistry showed the expression of CKLFSF2 in different spermatogenesis
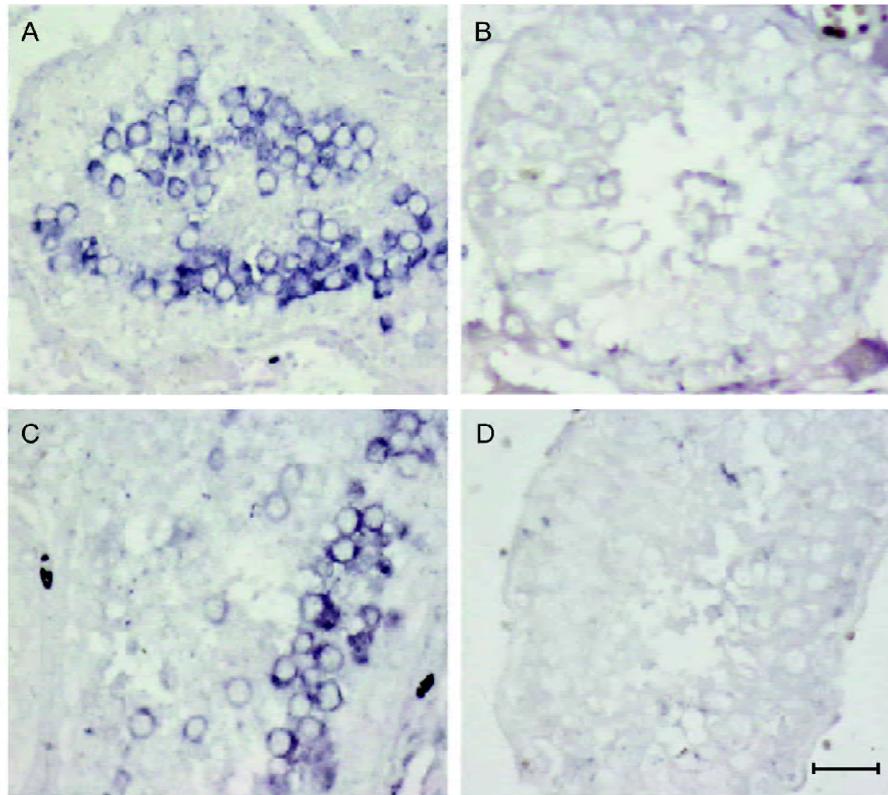
Figure 3. The distribution of CKLFSF2 mRNA in germ cells. Testes were obtained from normal spermatogenesis patients and *in situ* hybridization was carried out using sense and antisense probes, respectively. The signals were detected primarily in the spermatocytes. No signal was seen in spermatogonia, spermatids and Leydig cells (A, C). Hybridization specificity was confirmed by using sense probe (B, D). Bar = 40 μm.

pathologic groups shown in Figure 4 (up panel). With three samples in each group, CKLFSF2-positive cells were identified in 50 seminiferous tubules under a light microscope. As depicted in Figure 4 (graph), the number of CKLFSF2-positive cells per seminiferous tubule in normal testes was the highest with 40.34 ± 12.75 (A), and was lower in spermatogenesis disturbance testes with 22.40 ± 10.06 (B); the number of CKLFSF2-positive cells per seminiferous tubule was the lowest in spermatogenesis arrest testes with 8.44 ± 4.19 (C) and no CKLFSF2-positive cells were found in the Sertoli cell-only syndrome testis tissue (D). After a Kruskal–Wallis test, statistically significant differences in the number of CKLFSF2-positive cells per seminiferous tubule were found within the pathologic groups ($\chi^2 = 108.84$, $P < 0.01$) and between every two spermatogenesis pathologic groups ($P < 0.01$).

3.5 Abnormal expression of the CKLFSF2 mRNA in infertile patients' testes

A total of 14 patients with azoospermia were included in the present study. RT–PCR studies found no detectable CKLFSF2 mRNA in patients with Sertoli cell-only syndrome (1–6 in Figure 5) or in patients with spermatogenesis arrest (7–9 in Figure 5); nevertheless, CKLFSF2 mRNA was detected in five patients diagnosed with spermatogenesis disturbance (10–14 in Figure 5). The result showed that CKLFSF2 is abnormally expressed in infertile patients' testes.

3.6 CKLFSF2 fusion protein was localized to ER apparatus

Laser confocal microscopy localized CKLFSF2 fusion protein to ER apparatus 24 h after transfection. CKLFSF2 chimera appeared to be localized exclusively in the membranous reticular structures in the perinuclear
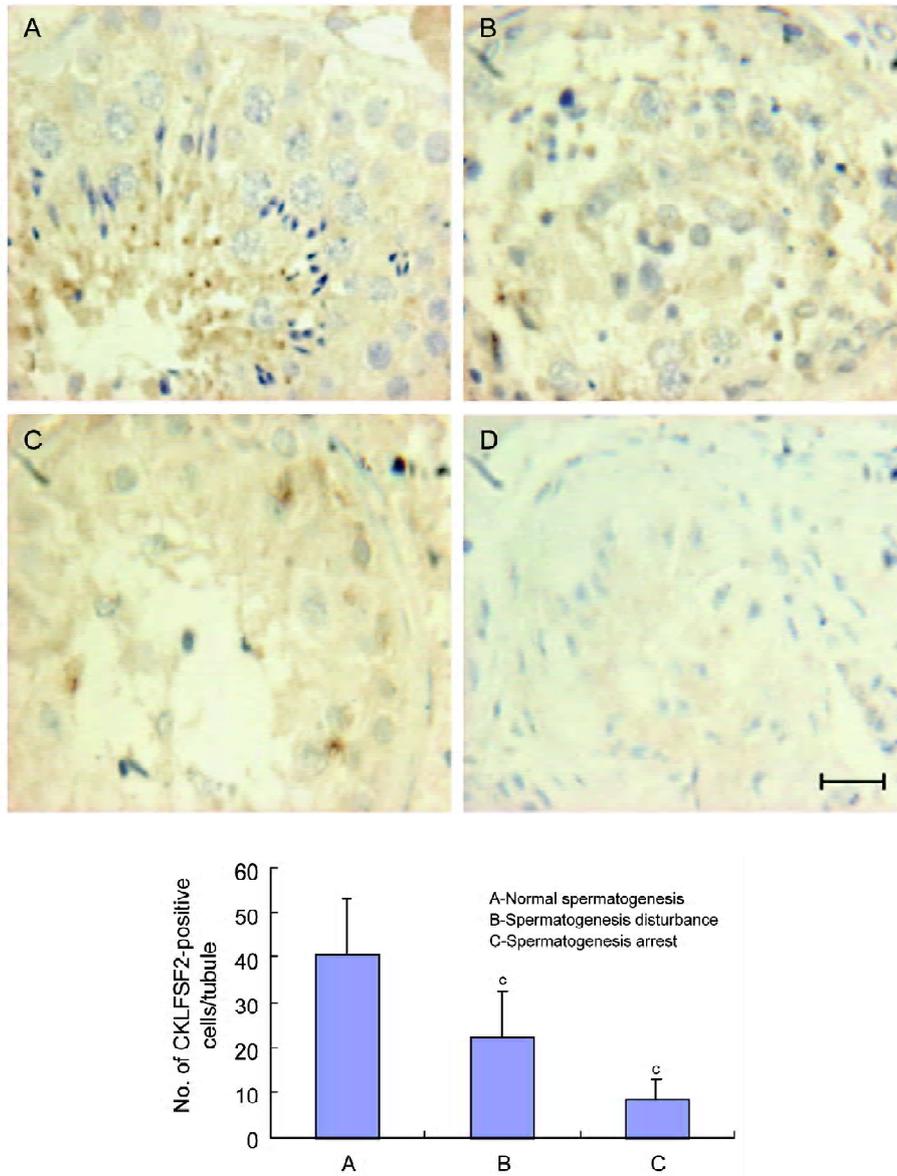
Figure 4. Up panels: Immunohistochemistry showing the expression of CKLFSF2 in different spermatogenesis pathologic groups. Bar = 20 μm; Down graph: data representation of the number of CKLFSF2-positive cells/tubule of three different spermatogenesis pathologic groups. The number of CKLFSF2-positive cells per seminiferous tubule in normal testes was the highest with 40.34 ± 12.75 (A), lower in spermatogenesis disturbance testes with 22.40 ± 10.06 (B), and the lowest in spermatogenesis arrest testes with 8.44 ± 4.19 (C). No CKLFSF2-positive cell was found in the Sertoli cell-only syndrome testis tissue (D). $^cP < 0.01$, compared within groups.

region. In contrast, the GFP control protein as a control was distributed homogeneously in the cytoplasm of the COS-7 cells (data not shown). To further identify the perinuclear compartment where CKLFSF2 fusion protein was localized, the transfected COS-7 cells were stained with a fluorescent ER-specific probe (ER-Traker™ Red) and Golgi-specific probe (BODIPY TR ceramide), or COS-7 cells were co-transfected with CFP-CKLFSF2 and YFP-Cox7a2, a protein that is located in the mitochondria [6]. It demonstrated that CKLFSF2 was co-localized with the ER marker and near the Golgi apparatus, but not co-localized with Cox7a2 (Figure 6).
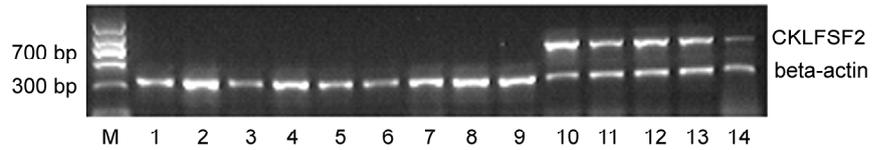
Figure 5. CKLFSF2 mRNA in infertile patients testes. RT–PCR studies examined CKLFSF2 expression in 14 patients with Sertoli cell-only syndrome (1–6), spermatogenesis arrest (7–9) or spermatogenesis disturbance (10–14). CKLFSF2 was not detected in all patients with Sertoli cell-only syndrome or spermatogenesis arrest. As a control, the lower panel displayed the expression level of beta-actin in corresponding patients.
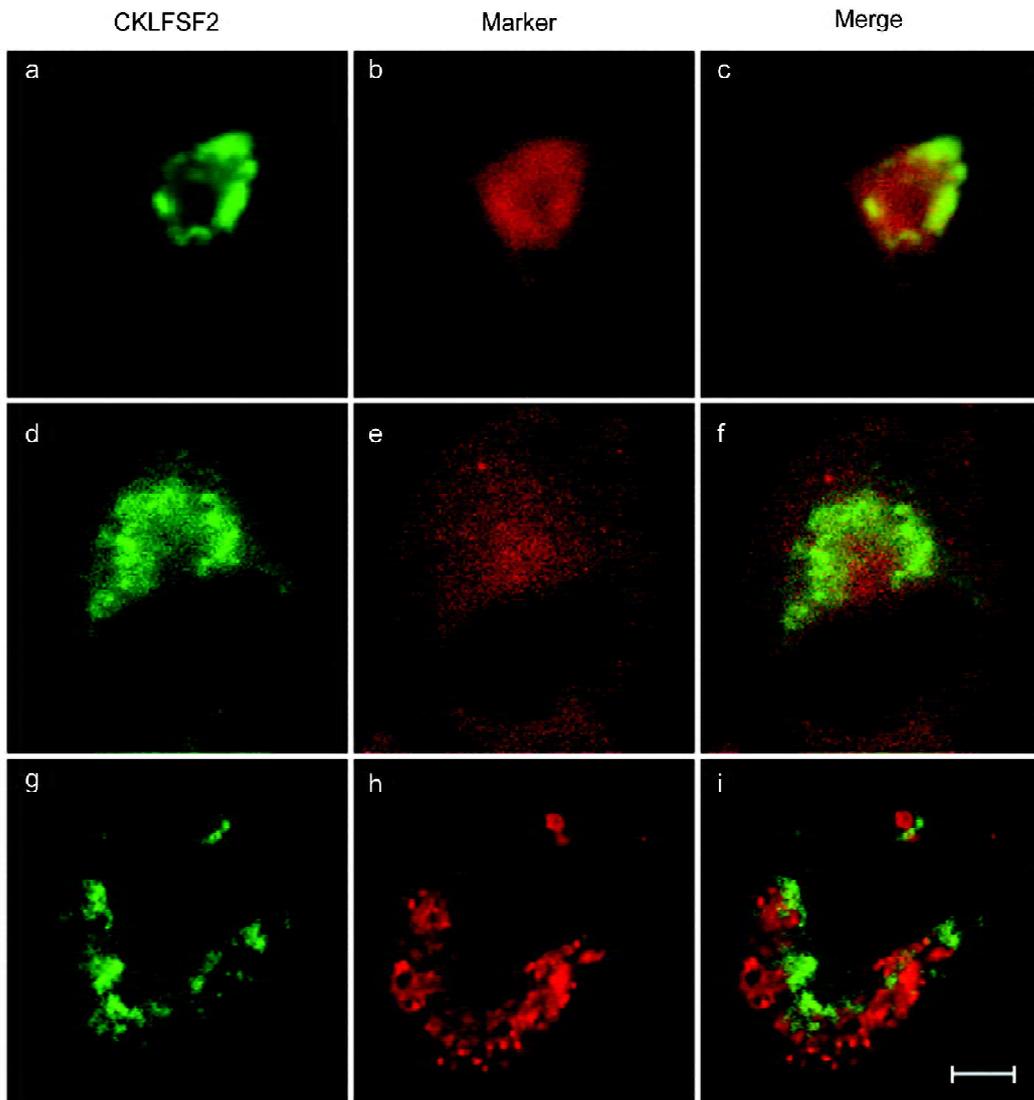

Figure 6. Confocal laser microscope images of CKLFSF2 fusion protein transiently expressed in COS-7 cells. Left panels show green fluorescence corresponding to transiently expressed fusion proteins: GFP-SF2 (a, d) and CFP-SF2 (g). Center panels show fluorescence of the ER (b) and Golgi (e) marker, and YFP-Cox7a2 (h) located in the mitochondria. Right panels show images obtained by merging left and center panels. Bar = 5 µm.

4   Discussion

In present study, we have investigated the cellular localization of CKLFSF2 in the cycle of the seminiferous epithelium, distribution of mRNA, subcellular localization of its fusion protein, and its abnormal expression in patients. These results strongly suggest that CKLFSF2 is localized in a stage-specific manner to the meiotic and post-meiotic germ cells, and might play a crucial role in the process of meiosis and spermiogenesis.

The present study differs from the previous study [3], which only roughly stained CKLFSF2 in human testis, as we have observed the expression of CKLFSF2 in the whole cycle of the human seminiferous epithelium. Immunohistochemistry techniques localized CKLFSF2 in a stage-specific manner to meiotic and post-meiotic germ cells (Figure 2); the staining initially appeared in pachytene spermatocytes and persisted until mature spermatozoa, mainly in the cytoplasm, but never in the acrosome. This expression pattern largely revealed that CKLFSF2 actively participated in the meiotic and post-meiotic process in spermatogenesis, but didn't play a part in the formation of the acrosome.

In contrast, *in situ* hybridization revealed that CKLFSF2 mRNA was only localized in the pachytene primary spermatocytes (Figure 3), which was not wholly consistent with the aforementioned protein localization in meiotic and post-meiotic germ cells. The discrepancy might be due to two reasons: 1) the transcription of CKLFSF2 mRNA terminates in the spermatocytes, whereas the translation of CKLFSF2 persists in the subsequent spermatogenic stages by using the remaining mRNA; 2) the transcription also stops in the spermatocytes, but the protein remains and produces a marked effect in the subsequent steps. Either way, the specific localization of CKLFS2 mRNA in pachytene primary spermatocytes indicated the involvement of CKLFSF2 gene in spermatogenesis originated from the meiosis phase concurrently with the protein. Similar splits between transcription and translation are well recorded for certain testis-specific proteins, such as the transition proteins and the protamines [8], MC31/CE9 [9] and SgIGSF [10].

In addition, the importance of CKLFSF2 in spermatogenesis was suggested by a close correlation between CKLFSF2 abnormal expression and a spermatogenesis defect (Figures 4, 5). With the aggravation of the spermatogenesis defect, the CKLFSF2-positive cell numbers and mRNA level decreased significantly with no expression in the testes of patients with SCOS, which is characterized histologically by a complete loss of the germinal epithelium in testicular tubules [11-13]. This correlation, together with the cell- and stage-specific expression, provided compelling evidence for a crucial role of CKLFSF2 in spermatogenesis.

Although all of the above results indicated a close relationship between CKLFSF2 and spermatogenesis, the exact function remains to be clarified. By confocal microscopy, we observed that CKLFSF2 was localized to the endoplasmic reticulum near the Golgi apparatus, but not in mitochondria (Figure 6). As is known, during the post-meiotic germ cell development, spermatids undergo dramatic morphological transformations and structural modifications of the Golgi apparatus, centriolar-axonemal complex, mitochondria and endoplasmic reticulum [14]. In particular, the endoplasmic reticulum is constituted by a multitude of membrane tubules and vesicles, and undergoes a series of morphological changes and disappears during the late stages of spermiogenesis; this disappearance appears to have an important role in the process of spermatid differentiation [15, 16]. In addition, CKLFSF2 contains MARVEL, a novel domain with a four transmembrane-helix architecture that has been identified in proteins of the myelin and lymphocyte (MAL), physins, gyrins and occluding families. Their function could be related to cholesterol-rich membrane apposition events in a variety of cellular processes, such as biogenesis of vesicular transport carriers [17]. Taking all these together, it is suggested that CKLFSF2 might participate in the vesicular transport carriers or membrane apposition events by sitting on ER.

Compared with a previous study [3], our results were the extension and supplement to the characteristics of CKLFSF2 in spermatogenesis. To the difference between them, it may be due to many reasons, for example, the trait difference of antibodies or the different tissue sections. In summary, data presented in the current study showed that CKLFSF2 was localized in a stage-specific manner to the meiotic and post-meiotic germ cells and had a close correlation with spermatogenesis defects, so it could play important roles in the process of meiosis and spermiogenesis. The function of CKLFSF2 might be involved in the vesicular transport carriers or membrane apposition events by sitting on ER. Further study is needed to address the specific role of CKLFSF2 in ER apparatus-regulated spermatogenesis.

Acknowledgment

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