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·*Original Article*·

The expression and significance of CATSPER1 in human testis and ejaculated spermatozoa

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Abstract

Aim: To investigate the distribution of cation channel of sperm 1 (CATSPER1) protein and the presence of *CATSPER1* mRNA in human testis and ejaculated spermatozoa. The influence of anti-human CATSPER1 antibody upon human sperm motility was used to evaluate the function of human CATSPER1 and to estimate its possible use as a target for immunocontraception. **Methods:** Human ejaculated sperm from normozoospermic donors (n = 12) and liquid nitrogen frozen human testis were used for the study of mRNA and protein expression of CATSPER1 by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry, respectively. Spermatozoa from normozoospermic donors (n = 12) were individually processed using a swim-up procedure and were then incubated with CATSPER1 antibody at final concentrations of 20, 4 and 0.8 μg/mL. After 1, 2 and 6 h incubation, progressive motility and fast progressive motility were measured by means of computer-assisted semen analysis. **Results:** *CATSPER1* transcript was detected in both human testis and each human ejaculated semen sample. CATSPER1 protein expressed in the membrane of spermatid and was localized in the principal piece of the sperm tail. The application of CATSPER1 antibody at all concentrations significantly inhibited both progressive motility and fast progressive motility after 1, 2 and 6 h incubation, and significant dose-dependent changes were observed. **Conclusion:** CATSPER1 is meiotically and post-meiotically expressed in human testis tissue. *CATSPER1* mRNA in human ejaculated spermatozoa could be a more feasible target for study and infertility screening than testis biopsy. In addition, our results suggest that human CATSPER1 could be a possible target for immunocontraception. *(Asian J Androl 2006 May; 8: 301–306)*

Keywords: cation channel of sperm 1; testis; spermatozoa; motility; immunocontraception; human

1 Introduction

Calcium channel activities are involved in the process of sperm maturation, motility, capacitation and sperm–egg interaction. Several calcium channels have been found to reside in mammalian sperm, including the T-type voltage operated Ca^{2+} channels alpha 1G and 1H, the cyclic nucleotide gated channels and the transient receptor potential (TRP) channel TRP2, the N-type, *R*-type, and *L*-type voltage operated Ca^{2+} channels, and the pkD2 cation channel. Recently, four members of a novel channel-like protein family, cation channel of sperm 1(CATSPER1), CATSPER2, CATSPER3 and CATSPER4, unlike other calcium channels, were identi-

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fied to be exclusively expressed in mice testes and to be linked to mice sperm mobility and male fertility [1–4].

As the first member of the family, mice CATSPER1 was cloned in 2001 [2]. Recently, its functional characterization was further described [5]. In mice, CATSPER1 protein expressed exclusively in the testis and not in other tissues. In mice sperm, CATSPER1 is localized primarily in the tail's principal piece and is directly associated with sperm mobility [2]. Moreover, CATSPER1 is required for sperm hyperactivation and penetrating the zona pellucida [5]. Gene-targeted elimination of the male mouse CATSPER1 results in poor sperm motility and infertility [2].

Based on its restricted expression pattern and important role in sperm mobility and male fertility, CATSPER1 is predicted to be a potential target for male infertility screening and an ideal target for contraception [1, 2, 5]. However, little is known about the expression and function of human CATSPER1. Here, we evaluate the distribution of CATSPER1 protein and the presence of *CATSPER1* mRNA in human testis and human ejaculated spermatozoa, and the influence of CATSPER1 antibody upon human sperm progressive motility.

2 Materials and methods

2.1 Materials

Human testis was obtained from Tongji Hospital (Wuhan, China) after obtaining a signed informing consent and the approval of the Ethical Committee of this University. The tissue was immediately cryostored in liquid nitrogen after surgery. Human semen samples were obtained from 12 consenting donors who met all the World Health Organization (WHO) criteria [6] for normozoospermia by masturbation after 3–5 d of abstinence. Samples with leukocytes and/or immature germ cell concentration greater than 10^6/mL were excluded.

Goat polyclonal antibody against human CATSPER1 (clone: H-20, 0.2 mg/mL, in 0.01 mol/L phosphate-buffered saline [PBS] containing 0.1% sodium azide and 0.2% gelatin) was from Santa Cruz Biotechnology incorporation (California, USA). All reagents for immunohistochemistry were obtained from ZhongShan Golden Bridge Biotechnology Company (Beijing, China). Percoll was purchased from Sigma Chemical Company (St. Louis, USA). TRI Reageant was from the Molecular Research Center (Cincinnati, USA). All reagents for reverse transcription polymerase chain reaction (RT-PCR) were obtained from Toyobo (Osaka, Japan).

2.2 Sperm preparation

1×10^7 spermatozoa of each sample were used for RNA extraction immediately after liquefaction (30–60 min at room temperature). The remaining spermatozoa were processed using the swim-up procedure [6, 7] and used to study of influence of CATSPER1 antibody upon sperm motility. For the swim-up procedure, semen samples were individually fractionated by two-layer (40–80%) Percoll gradient buffered in HEPES-balanced saline (115 mmol/L NaCl, 4 mmol/L KCl, 0.5 mmol/L $MgCl_2$, 14 mmol/L fructose, 25 mmol/L HEPES, pH 8.0). After centrifugation at $800 \times g$ for 20 min, the 80% Percoll fraction was pooled and examined under an optical microscope to verify the efficacy of the regimen.

2.3 RNA extraction

Total RNA from liquid nitrogen frozen human testis and ejaculated spermatozoa were extracted with TRI Reagent according to the manufacturer's instruction. Briefly, approximately 0.1 g human testis tissue and pellets containing approximately 1×10^7 spermatozoa were repetitively individually pipetted in 1 mL TRI Reagent. RNA was separated by chloroform and precipitated with isopropanol, and then washed with 75% ethanol. After briefly air-drying, RNA pellets were dissolved in diethyl pyrocarbonate treated water and then stored at –70ºC. The purity of RNA samples was checked spectrophotometrically at 260 nm and 280 nm.

2.4 RT-PCR

RT-PCR was performed as described in Sambrook and Russel [8]. Briefly, Total RNA (2 μg) was reverse-transcribed to cDNA as follows: 1 h at 42ºC with 100 IU MMLV reverse transcriptase, 500 μmol/L of each deoxynucleotide-triphosphate, 1 μg oligo$(dT)_{18}$ and 50 IU RNasin in a final volume of 25 μL, then 5 min at 95ºC. The cDNA were further amplified by PCR using selected primers. PCR primers for human *CATSPER1* (GenBank accession number AF407333) were designed using Primer 5 software by professional staff of AuGCT Company (Beijing, China). To eliminate possible contamination by genomic DNA, primers were chosen in different exons of *CATSPER1* and were as follows: forward 5'-TTTACCTGCCTCTTCCTCTTCT-3' (1905-1926nt), located in exon 5 and exon 6; reverse, 5'-ACCAGGTTGA GGAAGATGAAGT-3' (2110-2131nt), located in exon 7

and exon 8. PCR primer pairs amplified a 227-bp segment and the PCR amplification was performed on a Perkin Elmer DNA thermal cycler 480 (Wellesley, USA) in a final volume of 25 μL PCR mixture consisting of 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 200 μmol/L of each deoxy-nucleotide-triphosphate, 1.5I U Taq DNA polymerase and 50 pmol/L of each primer. 0.2 μg cDNA reverse-transcribed from human testis or sperm RNA was used as template, and negative control (human sperm RNA reverse-transcribed without MMLV reverse transcriptase) was included. β-actin was used in parallel for each run as internal control. The forward primer (5'-GGGAAATCGTGCGTGACAT-3') and reverse primer (5'-TCAGGAGGAGCAAT-GATCTTG-3') for β-actin were designed to produce a 385 bp amplicon. PCR products were run on a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet transillumination.

2.5 Immunolocalization of CATSPER1 protein in human testis and sperm

Smears of human ejaculated spermatozoa and 10 μm thin frozen sections of human testis were used to study the immunolocalization of CATSPER1. Immunohistochemistry was performed as previously described [9]. Briefly, Goat anti-human CATSPER1 polyclonal antibody at 1: 50 dilution was used as the primary antibody. Fluorescein-isothiocyanate-labeled rabbit anti-goat antibody was used as the secondary antibody at a 1: 100 dilution. 0.01 mol/L phorsphoric acid buffer solution (PBS, pH 7.4) instead of primary antibodiy was used as the negative control. The human testis was stained by Hoechest (Beyotime Biotechnology, China) to discriminate nucleus of the cells in the seminiferous tubules.

2.6 Determination of CATSPER1 antibody influence upon sperm motility

Spermatozoa pooled in 80% Percoll layer described above were diluted to 50×10^6 cells/mL in Biggers–Whitten–Whittingham medium devoid of bicarbonate and BSA and containing 1 mmol/L $CaCl_2$ and 25 mmol/L HEPES (pH 8.0) [10]. Aliquots of 90 μL were incubated with 10 μL CATSPER1 antibodies (200 μg/mL, 40 μg/mL, 8 μg/mL, diluted in 0.01 mol/L PBS, pH 7.4, containing 0. 1% sodium azide and 0.2% gelatin) at final concentrations of 20, 4 and 0.8 μg/mL. 10 μL 0.01 mol/L PBS (pH 7.4, containing 0.1 % sodium azide and 0.2% gelatin) instead of antibody was used as the negative control. After 1, 2 and 6 h at 37ºC with 5% CO_2, progressive motility (WHO motility class a + b) and fast progressive motility (WHO motility class a) was measured using a computer-assisted motion analyzer (Beijing Weili, China) at 37ºC. At least 200 spermatozoa in randomly chosen fields of vision were analyzed.

2.7 Statistical analysis

The data are presented as mean ± SD. Statistical analyses were performed using the SPSS software (version 11.5) by paired sample *t*-tests after checking for normal distribution by means of the Kolmogorov–Smirnov test. All statistical tests were two-tailed and $P < 0.05$ was considered as statistically significant.

3 Results

3.1 CATSPER1 expression

3.1.1 mRNA expression

In RNA isolated from human testis and 12 cases of human spermatozoa, robust PCR products of the expected 227 bp for *CATSPER1* and 385 bp for *β-actin* were identified following analysis on agarose gels (Figure 1). There was no detectable signal in samples without reverse transcriptase. The primers were designed to amplify nucleotides 1905–2131nt. Comparison with the genomic sequence of the human *CATSPER1* indicated that this region encompasses 496 bp of DNA, including exons 5–8 and the intronic sequences. Therefore, it is highly unlike that the *CATSPER1* RT-PCR products in the human testis and in ejaculated spermatozoa were derived from genomic DNA.

3.1.2 Protein expression

In human testis, abundant CATSPER1 protein was detected in the membrane of spermatid (Figure 2). In human ejaculated spermatozoa, CATSPER1 protein was localized in the principal piece of the sperm tail (Figure 3).

3.2 Influence of CATSPER1 antibody upon motility of human sperm

As shown in Table 1, significant and dose-dependent changes in the percentage of progressively motile sperm were observed after 1, 2 and 6 h incubation with CATSPER1 antibody at all concentrations used in the present study. Moreover, under the influence of CATSPER1 antibody, similar changes of the percentage of fast progressively motile

sperm were observed (Table 2). The percentage of slow progressively motile sperm (WHO motility class b) was not affected by this treatment (data not show). Therefore, the decline of progressive motility caused by CATSPER1 antibody was mainly ascribed to the changes of fast progressive motility.

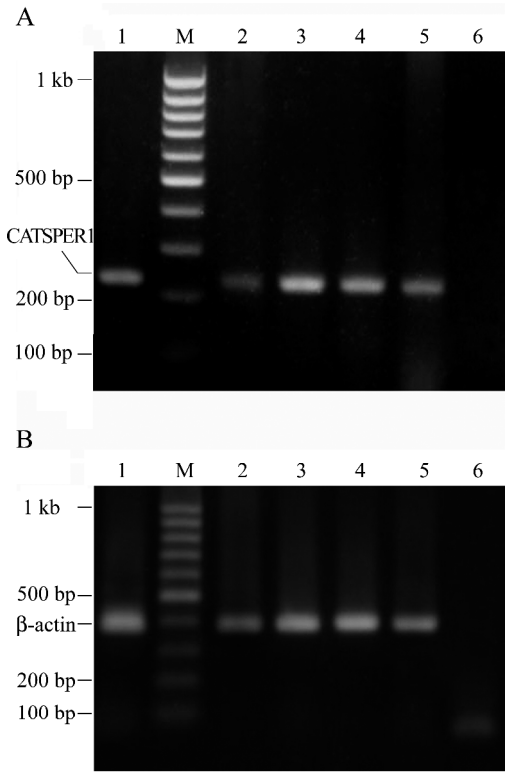

Figure 1. Example of reverse transcription polymerase chain reaction RT-PCR products for *CATSPER1* (A, 227 bp) and β-actin (B, 385 bp) in human testis (lane 1) and human ejaculated spermatozoa (lane 2–5). M, DNA Marker, 100 bp DNA ladder; lane 6: no reverse transcriptase control.

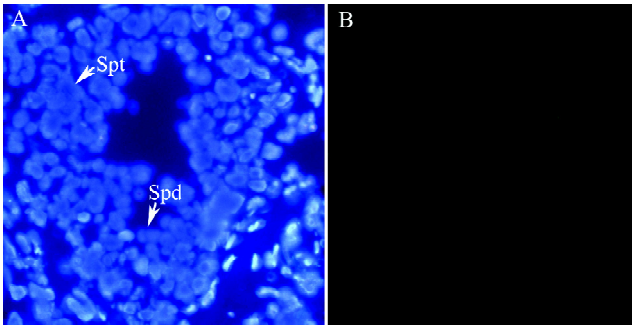

Figure 2. CATSPER1 protein distribution in human testis. Paired photomicrographs are shown. (A): Nucleus of the cells in the seminiferous tubules stained by Hoechest. (B): Expression of CATSPER1 protein in the same field. Abundant CATSPER1 protein was detected in the membrane of spermatid. Spt, Spermatocyte; Spd, Spermatid. Indirect immunofluorescence × 400.

4 Discussion

The recent cloning and characterization of CATSPER1 is a matter of significance to the study of male reproduction and infertility [1, 5]: 1) CATSPER1 is the first calcium ion channel required for sperm mobility and hyperactivation. CATSPER1-deficient mice are infertile as a result of an impairment of sperm mobility and an inability to fertilize intact oocytes; 2) The vital role of

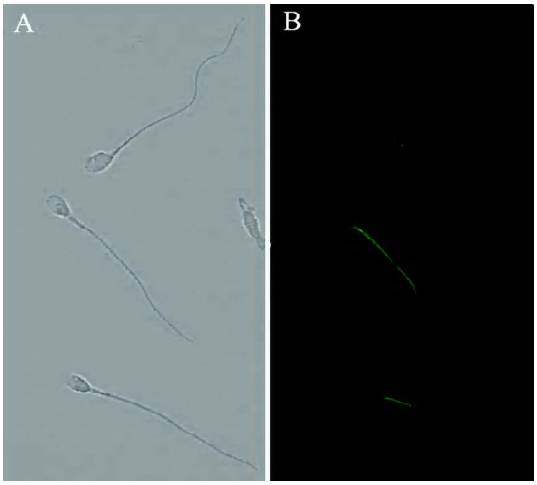

Figure 3. CATSPER1 protein localized in the principal piece of the human sperm tail. Paired photomicrographs are shown. (A): Spermatozoa under light microscope; (B): Fluorescence view of the same field. Indirect immunofluorescence × 400.

Table 1. Percentage of progressively motile sperm (World Health Organization motility class a + b) after incubation with and without (control) CATSPER1 antibody. CATSPER1 antibody decreased significantly ($^{a}P < 0.001$, $^{b}P < 0.005$, $^{c}P < 0.01$, $^{d}P < 0.05$; paired samples *t*-test,) the percentage of spermatozoa with progressive motility (mean ± SD, $n = 12$).

	Progressively motile sperm after incubation (%)		
	1 h	2 h	6 h
Control	61.2 ± 6.5	55.7 ± 7.1	51.3 ± 6.8
20 μg/mL antibody	43.1 ± 7.5^{a}	40.9 ± 12.0^{b}	39.1 ± 7.2^{a}
4 μg/mL antibody	49.2 ± 7.2^{a}	47.5 ± 6.9^{c}	42.7 ± 7.3^{b}
0.8 μg/mL antibody	53.0 ± 7.6^{b}	49.3 ± 6.9^{d}	44.2 ± 4.8^{a}

Table 2. Percentage of fast progressively motile sperm (World Health Organization motility class a) after incubation with and without (control) CATSPER1 antibody. CATSPER1 antibody decreased significantly ($^aP < 0.001$, $^bP < 0.005$, $^cP < 0.01$, $^dP < 0.05$; paired samples *t*-test) the percentage of spermatozoa with fast progressive motility (mean ± SD, $n = 12$).

	Fast progressively motile sperm after incubation (%)		
	1 h	2 h	6 h
Control	39.9 ± 10.5	37.5 ± 8.1	30.8 ± 9.3
20 μg/mL antibody	27.3 ± 7.7[a]	23.5 ± 8.9[a]	17.6 ± 4.5[a]
4 μg/mL antibody	32.0 ± 9.7[b]	28.4 ± 8.7[b]	20.8 ± 6.0[b]
0.8 μg/mL antibody	33.0 ± 11.8[c]	32.1 ± 10.8[d]	22.3 ± 5.6[c]

mice CATSPER1 in male fertility and the restricted localization of CATSPER1 to the mice testis and mature sperm suggest that *CATSPER1* gene could be a potential target for male infertility screening and treatment, and it might be an excellent target for contraception. The study of the evolution of CATSPER1 in primates and rodents also suggests its possibly important physiological role in sperm competition [11, 12]. Frequent incidences of indel substitutions were discovered in the evolution of the first exon of *CATSPER*1 gene. Because exon 1 is supposed to be an important functional part of this gene, it is reasonable to conclude that these indel substitutions, particularly larger indels which are significantly more prevalent in exon 1 than in the neutral genomic regions, are positively selected for the evolution of *CATSPER1*, and positive selection on this gene suggests its important physiological role.

To further explore the role and significance of CATSPER1, the expression and function of human CATSPER1 need to be addressed. In the present study, the expression profiles of CATSPER1 in human testis and ejaculated spermatozoa were investigated for the first time. Consistent with the meiotic and postmeiotic expression in mice testis [2, 13], CATSPER1 protein expressed in the membrane of spermatid. In ejaculated spermatozoa, CATSPER1 protein was highly localized to the principal piece of the sperm tail. The same expression and localization of CATSPER1 in humans as in mice indicates the important role of CATSPER1 in human male fertility.

To further elucidate the function of human CATSPER1 and to estimate its possibility as a target for potential contraception, we studied the influence of CATSPER1 antibody upon human sperm motility *in vitro*. Significant and dose-dependent changes in progressive motility and fast progressive motility after 1, 2 and 6 h incubation with CATSPER1 antibody suggested that, consistent with the function of its mice counterpart, human CATSPER1, which was highly localized to the principal piece of the sperm tail, play an important role in the progressive motility of human spermatozoa. The inhibition effect of CATSPER1 antibody upon human sperm also implies that CATSPER1 could be a possible target for immunocontraception.

CATSPER1 was implicated as a potential target for male infertility screening and treatment. Recently, the profiles of *CATSPER1* mRNA expression in testis biopsy of subfertile patients were investigated [13]. Compared with patients whose infertility cannot be ascribed to a deficiency in motility, a significant reduction in the level of *CATSPER1* gene expression among patients who lack sperm motility was observed. However, testicular puncture is invasive and inconvenient and might not be accepted by patients because of ethical considerations. The presence of *CATSPER1* mRNA in human testis and ejaculated spermatozoa were involved in the present study, and we found that *CATSPER1* mRNA was not only detected in human testis, but also, as a post-meiotically active gene, *CATSPER1* mRNA existed in human ejaculated spermatozoa. In fact, mRNA of some other CatSper family members were detected in human ejaculated spermatozoa by our group (unpublished data). During the last decade, findings from several studies support the conclusion that spermatozoa contain a complex repertoire of mRNA, including mRNA of some ion channels [7, 14–17]. Their putative roles are unknown, but the most common idea is to consider that these transcripts represent remnants of stored mRNA from post-meiotically active genes [15–17]. The study of mRNA in sperm could reflect past events of spermatogenesis and/or spermiogenesis, and could be used as a clinical assay to provide a panoramic view of testis gene expression that can be difficult to achieve from a testicular biopsy [15, 17]. Therefore, if CATSPER1 were to be evaluated as a target for male infertility screening and treatment, it is apparently more feasible to study its mRNA in ejaculated spermatozoa than in testicular biopsy.

Taken together, the work here provides insights into expression and function of CATSPER1 in human testis and ejaculated spermatozoa. Abundant *CATSPER1* mRNA in human ejaculated spermatozoa might be a more fea-

sible target than testis biopsy for male infertility screening and study on human CATSPER1.

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