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SHORT COMMUNICATION

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Isolation and Expression Analysis of the Human Testis-Specific Gene, *SPERGEN-1*, a Spermatogenic Cell-Specific Gene-1

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INTRODUCTION

Spermatogenesis in testis is an excellent model system to study regulation of gene expression during cell differentiation. Previously more than 100 cDNA fragments, which include several novel as well as already identified genes with developmentally upregulated expression in rat testis, were cloned by using differential display method. One of them is the gene encoding iba1, an ionized calcium-binding adapter molecule-1, which was expressed in haploid spermatids, but not in other germ cells, in rat testis (1). Recently another gene, *spergen-1*, a spermatogenic cell-specific gene-1, was isolated by the same procedure (2). The rat *spergen-1* is highly expressed in testis; however, it is undetectable in other organs examined. It was first detectable at 4 weeks of postnatal development and its expression increased thereafter. In situ hybridization analysis demonstrated that *spergen-1* mRNA is expressed specifically in spermatids of steps 5–11 in the seminiferous epithelium of the rat testis. Genes specifically expressed in haploid spermatids are very interesting, because such genes might be involved in spermatid differentiation or spermiogenesis. For example, haploid-specific genes, such as *hapsin*, *hsp70t*, and *oaz-t*, have provided valuable clues to understand the mechanism regulating spermiogenesis (3–5). In addition, spergen-1 protein has a mitochondria-targeting signal at the N terminus in rat (2). Previously we have reported that isolation of the human *RNH2* and *STRA8* cDNA and their expressions, which are limited to the human testis (6,7). In this study, we have isolated the human *SPERGEN-1* cDNA and analyzed its expression patterns in human tissues.

MATERIALS AND METHODS

Isolation of Human *SPERGEN-1* cDNA

The mouse and rat *spergen-1* cDNAs were isolated previously (2). Using mouse amino acid sequences (AK005610in GenBank), we found the region including homology in amino acid level in the human genome sequences (AP002834 in GenBank). The primers encompassing introns, SPER1F, SPER2F, SPER1R, and SPER2R were made using homology, and nested RT-PCR was performed with human testis cDNA library (Clontech) as a template. The resultant PCR product was sequenced with both directions. 3′RACE was carried out with the primers, 3RACE1SPER and 3RACE3SPER. The used oligonucleotides are as follows: SPER1F; 5′-TTCAATGG AAGGAGGGAGCC-3′, SPER2F; 5′-AAGAAGG GGGCCTGGTATAC-3′, SPER1R; 5′-TCGTCTCA CCTGCTCTATTC-3′, SPER2R; 5′-GATCTCGCAT GATGTCCTCTG-3′, 3RACE1SPER; 5′-GTGAAG CACCACCTCTCTAAGTCTG-3′, and 3RACE3SP ER; 5′-CCTTTTGCAGCCACACTCGTATCTTCC-3′. The RACE product was sequenced with both directions. The isolated cDNA sequences were compared to human genome sequences.

Expression Analysis by RT-PCR

For the expression analysis of the human *SPERGEN-1*, RT-PCR was done with the primers, SPER1F, SPER4F, SPER1R, SPER2R, and SPER3R. The analyzed human cDNAs were as follows: spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocyte, brain, heart, kidney, liver, lung, and pancreas (Clontech).

RESULTS

We found partial nucleotide sequences representing a putative human *SPERGEN-1* gene in the human genome sequences (Homo sapiens chromosome 11 clone RP11-709J12 map 11q). To isolate human *SPERGEN-1* cDNA, nested RT-PCR was performed with primers, SPER1F, SPER2F, SPER1R, and SPER2R. The resultant nested-PCR product was sequenced by both directions. Based on the

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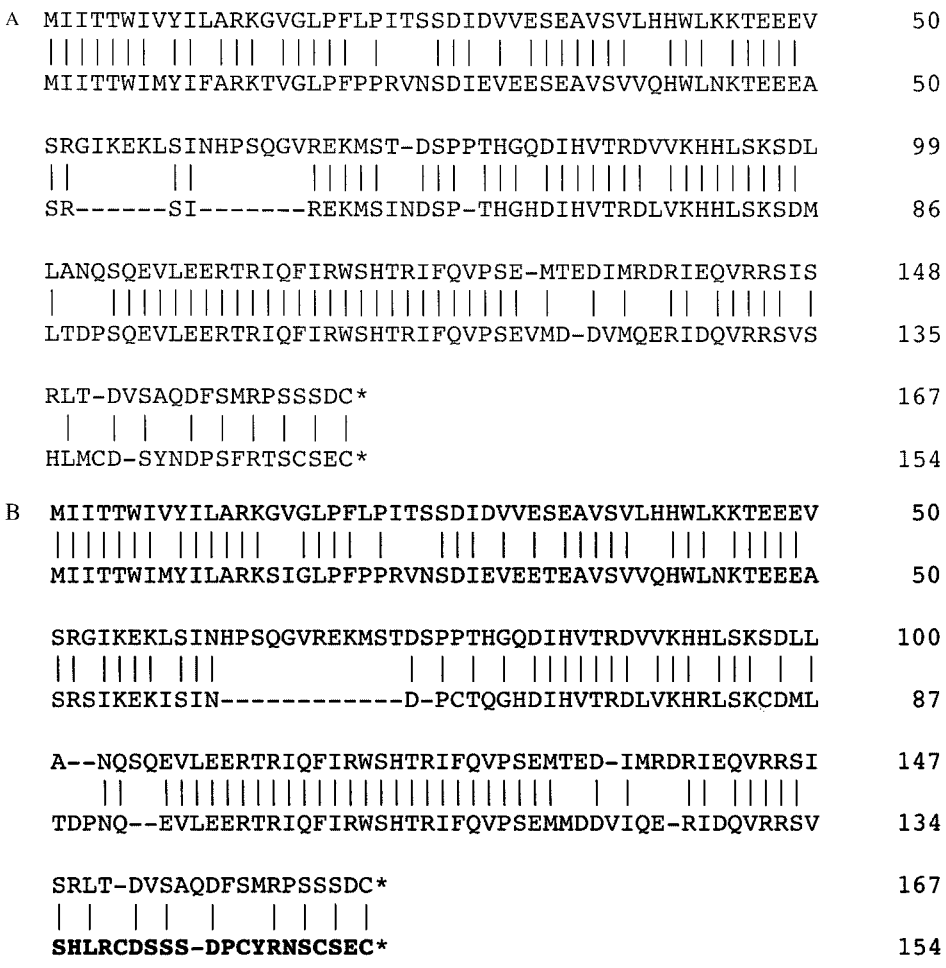

Fig. 1. The comparison of amino acid sequences among human, mouse, and rat SPERGEN-1. Vertical lines indicate identical sequences. (A) Upper sequences are human and the other is mouse. There is 67% homology between them. (B) Upper sequences are human and the other is rat. There is 63% homology between them, and bold letters indicate the position for transport of the protein to mitochondria.

sequences, the primers were made and 3′RACE was carried out. The PCR products were sequenced. The *SPERGEN-1* cDNA containing whole open reading frame (ORF) is 832 bp (GenBank accession no. AF521713). It has 63% homology to both of the mouse and rat cDNA sequences in nucleotide levels. The genomic structure of *SPERGEN-1* was determined by comparison of the cDNA sequence with genomic sequence found in the human genome sequences and HTGS database. A BLAST search with the *SPERGEN-1* cDNA sequence showed identical regions in chromosome 11 sequences. The ORF region of the human *SPERGEN-1* has six exons and five introns. The putative protein of the human *SPERGEN-1* consists of 167 amino acid residues. As shown in Fig. 1(A), human SPERGEN-1 protein has some homology to mouse SPERGEN-1 (67% identity overall). On the other hand, there is 63% homology between human and rat (Fig. 1(B)). However, there is little homology between them at 20 N-terminal amino acid residues (Fig. 1(B)).

To determine the expression patterns of the human *SPERGEN-1* in normal tissues, RT-PCR was done with various tissues as templates. RT-PCR was performed with primers, SPER1F and SPER1R. The 428-bp-sized band was clearly detected specifically in the testis on the *SPERGEN-1* (Fig. 2(A)). No bands could be detected in the other 15 tissues. To make sure if the human *SPERGEN-1* is expressed specific to testis, we made two more primers, SPER4F, SPER2R and SPER3R using homology to mouse and rat cDNA sequences. Only one band was detected

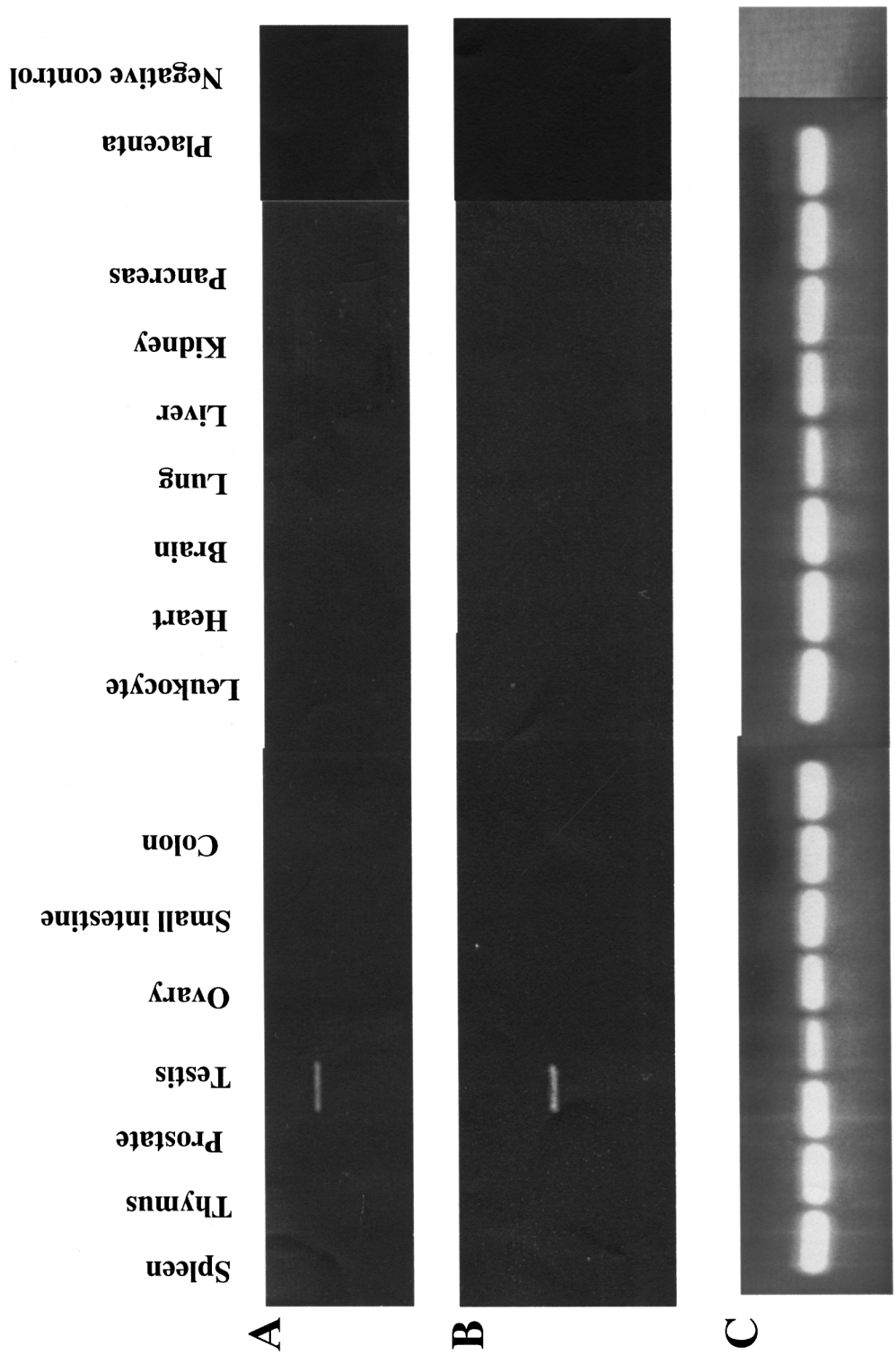

Fig. 2. RT-PCR analysis of human *SPERGEN-1* cDNA with the primers, SPER1F and SPER1R (A) and SPER4F and SPER2R (B). Distribution patterns of the human *SPERGEN-1* in 14 adult human tissues were examined by RT-PCR. *β*-Actin was used as a positive control (C).

with the primers, SPER4F and SPER2R (Fig. 2(B)). The same result was shown with the primers, SPER4F and SPER3R (data not shown). Then, the human *SPERGEN-1* is certainly expressed specific to testis like the rat *spergen-1*.

DISCUSSION

In this study, we report the isolation and characterization of human *SPERGEN-1*, a spermatogenic cell-specific gene-1. Rat spergen-1 protein has an N-terminal hydrophobic region interrupted by several basic amino acid residues, which is also conserved in mouse spergen-1 (70% identity). It was shown that rat spergen-1 is transported to mitochondria in COS-7 cells by transfection experiments. In addition, it was proved that 20 N-terminal amino acid residues in spergen-1 protein are essential for transport of the protein to mitochondria (2). However, there is little homology between human and rat at 20 N-terminal amino acid residues (45% identity). Then it is uncertain whether human SPERGEN-1 has a function in transport of the protein to mitochondria or not.

In rat, the *spergen-1* is highly expressed only in the testis. In addition, the rat *spergen-1* mRNA is expressed specifically in spermatid of steps 5–11 in the seminiferous epithelium of the testis. It has been known that haploid spermatid-specific genes play critical roles to understand the mechanisms regulating spermiogenesis. Then, it was suggested that the rat *spergen-1* plays important roles in spermiogenesis. However, it has not been reported that a study of knockout mouse of *Spergen-1*. Then it is unclear how the *Spergen-1* plays a role in spermiogenesis.

In this study, the expression analysis on histological level was not performed on the human *SPERGEN-1*. However, its expression is also specific to the human testis. Then, we suggest that the human *SPERGEN-1* may play a critical role in human spermiogenesis. The study with the patients of azoospermia will be needed in the future.

In summary, the present study suggests that the human *SPERGEN-1* be also expressed only in the testis. It has not known that its expression is specific to spermatid or not. However, from the expression patterns and homology of amino acid sequences among rat, mouse, and human, it is suggested that the human *SPERGEN-1* may play some roles in human spermatogenesis.

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