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SHORT COMMUNICATION

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Isolation and Expression Analysis of the Testis-Specific Gene, Human *OPPO1*

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***Purpose*:** To investigate human spermatogenesis, we isolated human testis-specific genes.
***Methods*:** Using mouse amino acid sequences, we found the region including homology in amino acid level in the human genome sequences. The primers encompassing introns were made and RT-PCR and RACE were carried out. The resultant PCR products were sequenced.
***Results*:** The full-length cDNA of human *OPPO1* was isolated. It encodes 257 amino acid residues. The expression of the human *OPPO1* was predominantly in the testis. On the other hand, partial cDNAs of *ZNF8*, *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200* were expressed in the various tissues.
***Conclusions*:** Our data suggests that the human *OPPO1* may play important roles in human spermatogenesis.

KEY WORDS: OPPO1; spermatogenesis; testis; ZNF8.

INTRODUCTION

During mammalian spermatogenesis, spermatogonial stem cells become mature spermatozoa through a highly specialized and complex process. During this process, spermatogonia proliferate, and some undergo meiosis to give rise to haploid spermatids, which transform into morphologically and functionally differentiated spermatozoa. The formation of the tail is one of the primary events in male haploid germ cell differentiation, which is known as spermiogenesis.

Recently, a lot of genes specifically expressed in haploid germ cells from subtracted cDNA library that was generated by subtracting the mRNA from 17-day-old mouse testes from the cDNA of 35-day-old mouse testes (1,2). Last year another gene that is exclusively expressed in the testis, *Oppo1*, was isolated in mouse (3). The mRNA was detected from late pachytene stage-spermatocytes to elongated spermatids in the seminiferous tubules by in situ hybridization. Its protein product is localized in the sperm tail, thus, it was speculated that it is involved in sperm tail structure and/or sperm motility. On the other hand, the *Znf8* gene encoding a novel Kruppel-type zinc finger protein was identified in mouse (4). This transcription is specifically observed in adult mouse testes. It was suggested that the mouse *Znf8* played critical roles in mediating BMP signalling during spermatogenesis. In 2000, high-throughput gene expression analysis was carried out to identify genes specifically expressed in mouse gonad (5). Some of the isolated genes are *GR194*, *GR219*, *GR093*, *GR046*, GR163, and *GR200*. Their expression patterns are specific to testis and ovary in mouse. Previously we have reported the isolation of the human *RNH2*, *STRA8*, and *SPERGEN-1* cDNA and their expressions, which are limited to the human testis (6–8). In the present study, we have isolated full-length cDNA of *OPPO1* and partial cDNA of *ZNF8*, *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200* and analyzed their expression patterns in human tissues.

MATERIALS AND METHODS

Isolation of Human *OPPO1* cDNA

The mouse *Oppo1* cDNA was isolated previously (3). Using mouse amino acid sequences (NM_145746 in GenBank), we found the region with homology at the amino acid level in human genome sequences (AC106019 in GenBank). The primers encompassing introns, HOPPO1F1, HOPPO1F2, HOPPO1R1, and HOPPO1R2 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. 5′RACE and 3′RACE were carried out with the primers, 5RACEOPPO1-1, 5RACEOPPO1-2, 3RACEOPPO1-1, and 3RACEOPPO1-2. The oligonucleotides used are: HOPPO1F1; 5′-TAGGAGCCTCTTCTACCAGC-3′, HOPPO1F2; 5′-ACAGAATCCACACATCAGCC-3′, HOPPO1R1; 5′-TTCTGCTCAGGATCCAGGAC-3′, HOPPO1R2; 5′-GGTGTCTGTGATCGTCTGTG-3′, 5RACEOPPO1-1; 5′-TGCTGCTGCAGGACACGGCACCACTGG-3′, 5RACEOPPO1-2; 5′-GTGGCTCAGAATCAGACTCCAGAAAC-3′, 3RACEOPPO1-1; 5′-GGAAAGTCACCTTCATCTTCTCCACC-3′, and 3RACEOPPO1-2; 5′-ACTTCACCTGCGCGATCCTTTGCTAC-3′. Both

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RACE products were sequenced with both directions. The isolated full-length cDNA sequences were compared to human genome sequences.

Expression Analysis by RT-PCR

For expression analyses of the human *OPPO1*, *ZNF8*, *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200*, RT-PCR and nested RT-PCR were done with the primers, HOPPO1F1, HOPPO1F2, HOPPO1R1, and HOPPO1R2 (*OPPO1*), ZNF8F1 and ZNF8R2 (*ZNF8*), GR194F, and GR194R (*GR194*), GR219F and GR219R (*GR219*), GR093F and GR093R (*GR093*), GR046F and GR046R (*GR046*), GR163F and GR163R (*GR163*), GR200F and GR200R (*GR200*). The oligonucleotides used are: ZNF8F1; 5′-CAGGAACCAGTGACCTTCCG-3′, ZNF8R1; 5′-GGCTTGAACTCAGGACGCAG-3′, GR194F; 5′-GGTCAGAGGCGACATGAGTG-3′, GR194R; 5′-TTGCCATCTTCTATCCGAGC-3′, GR219F; 5′-GCTGAATTCTCTGCCATCTG-3′, GR219R; 5′-TAAGCAGCTATTGACCCGTG′-3′, GR093F; 5′-TCAGATCGTCGTGGCTCAGG-3′, GR093R; 5′-ACTGGTTCTGAGGAGGAGCC-3′, GR046F; 5′-CCAGGCTGTTGTGTCCTACC-3′, GR046R; 5′-GGAAAGTCTTCTACAGGTGG-3′, GR163F; 5′-TATCATGAACCCCATCGGGC-3′, GR163R; 5′-GGCAGGAAGGGTCCATACAG-3′, GR200F; 5′-AACCGACTCGTGTACTCTGG-3′ and GR200R; 5′-CCCACCTTGTTGCTGAAGAG-3′. The analyzed human cDNAs were: 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 *OPPO1* gene in the human genome sequences (Homosapiens chromosome 17 clone CTD_2540E3 map 17). To isolate human *OPPO1* cDNA, nested RT-PCR was performed with primers, HOPPO1F1, HOPPO1F2, HOPPO1R1, and HOPPO1R2. The resultant PCR product was sequenced in both directions. Based on the sequences, primers were made for 5′RACE and 3′RACE. The *OPPO1* cDNA containing the whole open reading frame (ORF) is 1149 bp (Fig. 1) (GenBank accession no. AY237799). This ORF is from nucleotide 183 to 956, encoding a putative 257-amino acid protein. The coding region of human *OPPO1* cDNA has some homology to the ORF region of the mouse *Oppo1* at nucleotide level (57% identity). The genomic structure of the *OPPO1* was determined by comparison of the

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GGGTTGGGGCCTTCTCTTGGATCAAGAGTCTCTTATTTGATCGAGGTCTGTTATTAGACTTCCTCATTCTTTCTCAGACC      80
TCAGCTGCATGGTGGCAGCTGATGAAAATATCCAAAAGATGAGAAGAGACAATTGAGTCTGGTGAGGGAAAGGGGAGACA     160
GAGGGTGAAGTGGTGCTCAAGATGGATGCAGAGTACTCTGGGAATGAGTTCCCCAGGTCAGAAGGAGAAAGAGACCAACA     240
                    M  D  A  E  Y  S  G  N  E  F  P  R  S  E  G  E  R  D  Q  H
TCAGAGACCTGGAAAGGAAAGGAAGAGTGGGGAGGCAGGATGGGGCACAGGTGAGCTGGGACAAGATGGGAGACTGCTGT     320
 Q  R  P  G  K  E  R  K  S  G  E  A  G  W  G  T  G  E  L  G  Q  D  G  R  L  L  S
CCTCCACCCTCTCCCTCAGTAGTAACAGGTCCTTGGGCCAGCGCCAGAACTCTCCGCTGCCCTTTCAATGGAGAATCACA     400
   S  T  L  S  L  S  S  N  R  S  L  G  Q  R  Q  N  S  P  L  P  F  Q  W  R  I  T
CACAGCTTCCGCTGGATGGCCCAGGTGTTGGCCTCTGAGCTCAGCCTGGTTGCCTTTATCCTACTATTGGTCATGGCCTT     480
H  S  F  R  W  M  A  Q  V  L  A  S  E  L  S  L  V  A  F  I  L  L  L  V  M  A  F
CTCCAAGAAATGGCTGGACCTCTCTAGGAGCCTCTTCTACCAGCGCTGGCCCGTGGATGTCAGCAACAGAATCCACACAT     560
 S  K  K  W  L  D  L  S  R  S  L  F  Y  Q  R  W  P  V  D  V  S  N  R  I  H  T  S
CAGCCCACGTTATGTCCATGGGGCTCCTGCACTTTTGCAAATCCAGGAGCTGTTCTGACTTAGAGAATGGGAAAGTCACC     640
  A  H  V  M  S  M  G  L  L  H  F  C  K  S  R  S  C  S  D  L  E  N  G  K  V  T
TTCATCTTCTCCACCCTCATGCTATTCCCCATTAACATCTGGATCTTCGAGTTGGAAAGGAATGTATCCATCCCCATAGG     720
F  I  F  S  T  L  M  L  F  P  I  N  I  W  I  F  E  L  E  R  N  V  S  I  P  I  G
CTGGAGCTATTTCATTGGTTGGCTGGTGCTTATCCTATACTTCACCTGCGCGATCCTTTGCTACTTCAACCATAAAAGTT     800
 W  S  Y  F  I  G  W  L  V  L  I  L  Y  F  T  C  A  I  L  C  Y  F  N  H  K  S  F
TCTGGAGTCTGATTCTGAGCCACCCCAGTGGTGCCGTGTCCTGCAGCAGCAGTTTCGGCTCAGTAGAAGAATCTCCAAGG     880
  W  S  L  I  L  S  H  P  S  G  A  V  S  C  S  S  S  F  G  S  V  E  E  S  P  R
GCACAGACGATCACAGACACCCCCATCACCCAGGAGGGAGTCCTGGATCCTGAGCAGAAGGATACACATGTGTAATCTTT     960
A  Q  T  I  T  D  T  P  I  T  Q  E  G  V  L  D  P  E  Q  K  D  T  H  V  *
TCTGAACTCCTGGCACCAAGTTCTGTCCATTCATCTGACCCCATCTCCTCATCCTCCCCCAGCCCTTGAATAGGTTGGTC    1040
CTCATCATTGCAAGGAATGAGAAAGGGAGGATTTTGCACTCCTCTGCTTTCTCCCTGCCTTGATTGAGCTTGAGTGATGT    1120
GGAATAAATTGTCCGTCTCTTCTTTCTC                                                        1149
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Fig. 1. Nucleotide sequences of full-length human *OPPO1* cDNA. This 1149bp cDNA spans an open reading frame (ORF) from nucleotide 183–956, encoding a putative 257-amino acid protein.

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MDAEYSGNEFPRSEGERDQHQRPGKERKSGEAGWGTGELGQDGRLLSSTL      50
|  |   ||    | |          ||                 |    |
M--EPDLNE----E-ES--------ERI---------------R----T-      15

SLSSNR-SLGQRQNSPLPFQWRITH-SFRWMAQVLASELSLVAFILLLVM      98
|   || ||  | || |||||  |  | |||||| ||| ||||| |||||
SR--NRRSLEHRRNSLLPFQWKATNNS-RWMAQVVASEFSLVAFLLLLVM      62

AFSKKWLDLSRSLFYQRWP--VD--V--SNRIHTSAHVMSMGLLHFCKSR     142
 ||||||  | | | || |  |   |  |  || |   || |||  | |
VFSKKWLYPSKSRFHQRYPQNVTKRVYTS--IH-S---MSTGLLYICVSK     106

SC-S-DL-E-N----------G--K-----------V-T-----------     153
|| | |  | |          |  |            | |
SCPSSDNGEDNFKMWTIHPVFGVAKISFTLAIGLGFVLTTWLHLPYLPCL     156

----F---IF---S----TLM-L----FPINIWIFELER-NVSIPIGWSY     183
    |   |    |    ||  |    || | || || | | | ||||||
QRMPFFGLIGIILSFCEVTLIFLTLLLFPVNLWIYEL-RKNISVPIGWSY     205

FIGWLVLILYFTCAILCYFNHKSFWSLILSHPSG----AVSCSSSFG--S     227
|||||||||||| |||| |||  |||| |  |     |  |||  |  |
FIGWLVLILYFTCGILCYLNHKNYWSLIMS--STTINTA--CSSL-GPES     250

-VEESPRAQTITDTPITQEGVLD-PE--QK----DTHV.............     257
 |  ||  ||    |  ||     |   ||    |  |
LV--SPS-QT----PSSQENSQESPKDDQKPSSPDKVVSPPQPDTTG...     290
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Fig. 2. The comparison of amino acid sequences between human and mouse OPPO1. Upper sequences are human and the others are mice. Vertical lines indicate identical sequences. There is 42% homology between them. The bold letters are the position of the sequences common to the flagella basal body rod protein of *Salmonella*.

cDNA sequence with genomic sequence found in the HTGS database. A BLAST search with the *OPPO1* cDNA sequence showed identical regions in chromosome 17 sequence (Hs17_10875 in GenBank). The ORF region of the human *OPPO1* has three exons and two introns. As shown in Fig. 2, human OPPO1 protein has homology to mouse OPPO1 (42% identity overall). The sequence of mouse OPPO1 protein has a 22-amino acid sequence common to the flagella basal body rod protein of *Salmonella* (bold letters) (Fig. 2). However, the human protein does not have any known motifs. To determine the expression patterns of the human *OPPO1* in normal tissues, RT-PCR was done with various tissues as templates. Nested RT-PCR was performed with primers, HOPPO1F1, HOPPO1F2, HOPPO1R1, and HOPPO1R2. The 356bp-sized band was clearly detected specifically in the testis with very weak expression in pancreas (Fig. 3). No bands could be detected in the other 13 tissues. On the other hand, the partial cDNA fragments of *ZNF8*, *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200* (see Fig. 4) were amplified according to the Materials and Methods. The mouse *Znf8* is highly expressed in the testis and the level of expression of the human *ZNF8* is also high. In mouse, the expression of the *GR194*, *GR219*, *GR093*, *GR046*, GR163, and *GR200* are limited to the testis and ovary. However, the human *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200* are expressed in various tissues (Fig. 4).

DISCUSSION

In this study, we report the isolation and characterization of the human cDNA encoding OPPO1. The mouse protein of the *Oppo1* has a 22-amino acid sequence common to the flagella basal body rod protein of *Salmonella*. The flagellum is the organelle of motility for *Salmonella typhimurium* and many other bacterial species. Its known structural features are the basal body, hook, hook-associated proteins, and

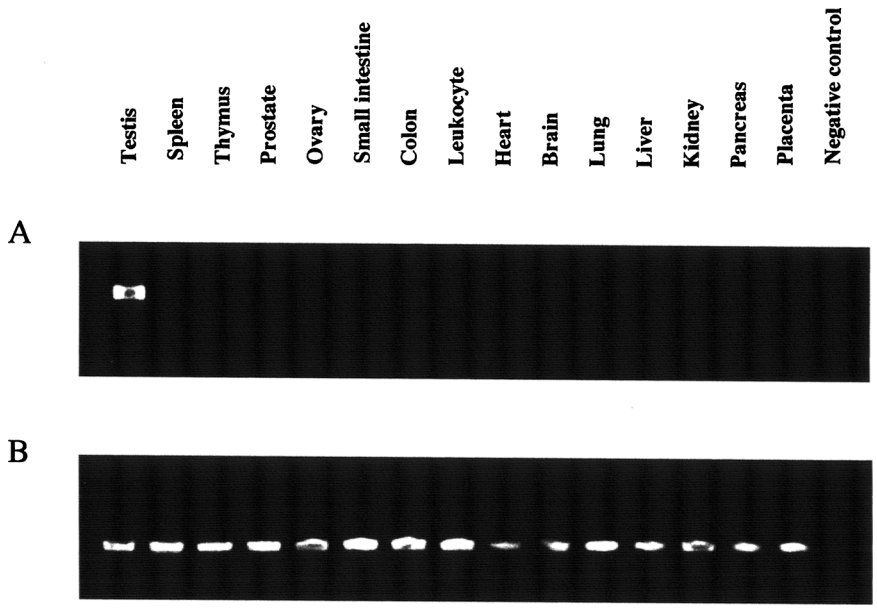

Fig. 3. RT-PCR analysis of human *OPPO1* cDNAs. Distribution patterns of them in 15 adult human tissues were examined by RT-PCR. A strong band was detected in testis and very weak band was in pancreas on the human *OPPO1*. β-Actin was used as a positive control.

helical filament. The assembly of the bacterial flagellum in *Salmonella* is considered to have three distinct stages: formation of the basal body, the hook, and the filament (9–11). Although there are large differences between the flagella of bacteria and those of mammals, it was speculated that these proteins had very important roles in the formation and function of the flagellum (3). OPPO1 is a new flagella protein that may be concerned with flagella basal body formation in mouse. However, deduced protein of the human *OPPO1* has no flagella basal body rod protein nor any domains (Fig. 2). Thus, it is suggested that the human *OPPO1* has different functions from mouse one.

The *Oppo1* is expressed specific in the testis in adult mouse. The transcript is detected from the age of 11 days through to adulthood (3). In situ hybridization demonstrated specific staining in spermatocytes and spermatids from late pachytene to the terminal elongated spermatids in the seminiferous tubules. The storage of transcripts and translational control would be common phenomena in the late stage of spermiogenesis in transcriptionally inactive elongating spermatids (12). The human *OPPO1* is predominantly expressed in the testis with slight expression in pancreas. In mouse, the examined tissues did not include pancreas, thus, the mouse *Oppo1* may also be weakly expressed in pancreas.

Smad proteins are known to be the direct downstream mediators of BMP/TGF-β signaling from the receptors to the nucleus (13). BMP signaling plays critical roles in male germ cell development, deletion of *Bmp8a* or *Bmp8b* causes severe defects in spermatogenesis, and mutation in *BMP7* enhances the mutant phenotype of *Bmp8a* (14–16). Functional analysis suggested that the mouse *Znf8* negatively regulated the TGF-β/BMP signaling pathway in vivo. In mouse, the *Znf8* is expressed ubiquitously during mouse embryogenesis. In contrast, the transcription of mouse *Znf8* is high in adult mouse testis, with the same cell- and stage-specific transcription pattern as *Smad1* but low to moderate in other tissues (4). Thus, it was suggested that mouse *Znf8* played critical roles in mediating BMP signaling during spermatogenesis. From the high level of the expression in the testis, it is suggested that the human *ZNF8* may play roles in spermatogenesis.

In mouse, the *GR194*, *GR219*, *GR093*, *GR046*, *GR163* and *GR200* are expressed only in the testes and ovaries. However, we demonstrate that the human genes are expressed in a number of tissues. Thus, they may have another biological function from their expression patterns.

In summary, the present study shows that the human *OPPO1* is predominantly expressed in the testis. It is not known which cells in the testis it is expressed.

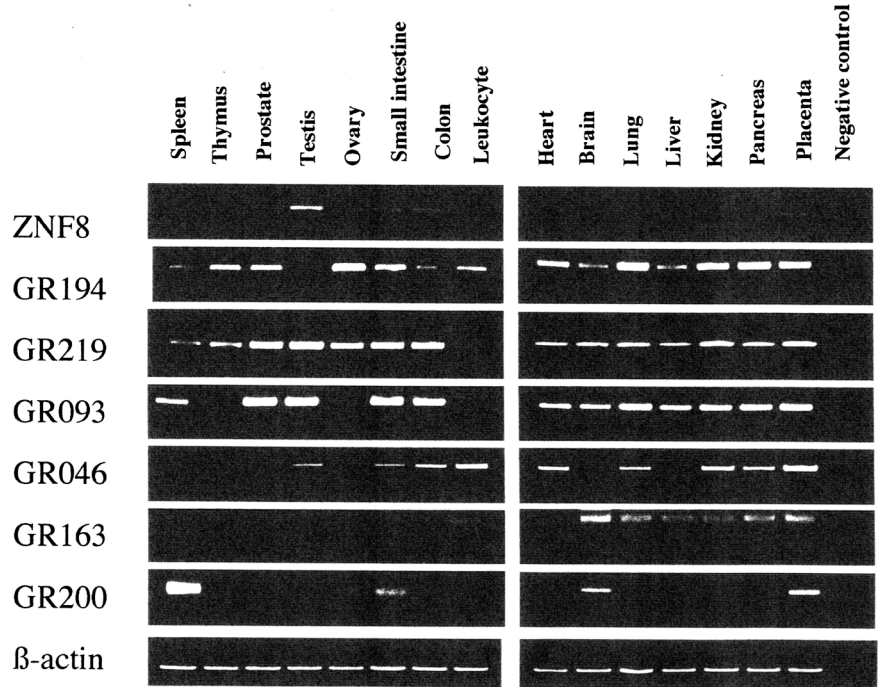

Fig. 4. RT-PCR analyses of human *ZNF8*, *GR194*, *GR219*, *GR093*, *GR046*, *GR163*, and *GR200* cDNAs. Distribution patterns of them in 15 adult human tissues were examined by RT-PCR. The strong band was detected in testis on the human *ZNF8* and strong bands were detected on the other genes in the various tissues. β-Actin was used as a positive control.

However, from the expression patterns and homology of amino acid sequences between mouse and human, it is suggested that the human *OPPO1* may play some roles in human spermatogenesis. In addition, it is also suggested that the human *ZNF8* may play roles in human spermatogenesis.

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