Journal of Assisted Reproduction and Genetics, Vol. 19, No. 11, November 2002 (© 2002)

SHORT COMMUNICATION

HOKKAIDO, JAPAN

Isolation and Expression Analysis of the Testis-Specific Gene, *STRA8*, Stimulated by Retinoic Acid Gene 8

Submitted May 28, 2002; accepted May 30, 2002

Retinoids are known to be required for vertebrate reproduction, and in the male, for the maintenance of normal testicular structure and function. Previously several novel retinoic acid responsive genes, collectively designated as the *Stra* genes, had been isolated in the mouse. The *Stra8* gene encodes a cytoplasmic protein and is expressed specific to the developing male gonad during mouse embryogenesis. In adult mouse, its expression is restricted to the premeiotic germ cells. Thus it has been suggested that the mouse Stra8 protein may play a role in the premeiotic phase of spermatogenesis.

Recently a lot of genes that are expressed only in male germ cells have been isolated in the mouse. The mouse *Stra8*, *Rnh2*, *Piwil2*, *Tex17*, and *Tuba7* were identified as testis-specific expressed genes. In addition, the *Figla* was known to be a testis- and ovary-specific gene. Recently we had reported the isolation of the human *RNH2* cDNA and its expression, which is limited to the human testis. In the present study, we have isolated full-length cDNA of *STRA8* and partial cDNAs of *PIWIL2*, *FIGLA*, *TEX17*, and *TUBA7*, and analyzed their expression patterns in human tissues.

KEY WORDS: Spermatogenesis; STRA8; testis.

INTRODUCTION

Retinoids have been shown to regulate various physiological functions (1,2). Retinoids are known to be required for vertebrate reproduction, and in the male, for the maintenance of normal testicular structure and function. Retinol deficiency leads to cessation of spermatogenesis and degeneration of the seminiferous tubules (3,4). Previously a number of novel retinoic acid (RA)-responsive genes, collectively designated as the *Stra* genes, had been isolated in the mouse (5–9). The *Stra8* gene encodes a cytoplasmic protein and is expressed specific to the developing male gonad during mouse embryogenesis. In adult mouse, its expression is restricted to the premeiotic germ cells. Thus it has been suggested that the mouse Stra8 protein may play a role in the premeiotic phase of spermatogenesis (10).

Recently a lot of genes that are expressed only in male germ cells have been isolated in the mouse (11). In the present work a systematic search was carried out for genes expressed in mouse spermatogonia but not in somatic tissues. The mouse *Stra8*, *Rnh2*, *Piwil2*, *Tex17*, and *Tuba7* were identified as testis-specific expressed genes. In addition, the *Figla* was known to be a testis- and ovary-specific gene. Previously we had reported the isolation of the human *RNH2* cDNA and its expression, which is limited to the human testis (12). In this study, we have isolated full-length cDNA of *STRA8* and partial cDNAs of *PIWIL2*, *FIGLA*, *TEX17*, and *TUBA7*, and analyzed their expression patterns in human tissues.

Materials and Methods

Isolation of Human *RNH2* cDNA. The mouse *Stra8* cDNA was isolated as done previously (10). Using mouse amino acid sequences (NM_009292 in GenBank), we found the region including homology in amino acid level in the human genome sequences (AC009330 in GenBank). The primers encompassing introns, STRA81F, STRA82F, STRA81R, and STRA82R, were made using homology, and nested RT-PCR was performed with mouse testis cDNA library (Clontech) as a template. The resultant PCR product was sequenced in both directions. 5′RACE and 3′RACE were carried out with the primers STRA85RACE1, STRA83RACE1, and ST RA83RACE3. The used oligonucleotides were the following: STRA81F; 5′-GCACAGCTGCAGGAGC TTGAG-3′, STRA82F; 5′-GGAGACGGCTGTCC CAGG-3′, STRA81R; 5′-GCTGAAACTTCTCCTC TGGG-3′, STRA82R; 5′-CTCTGGGTTTTCTGGG TTGC-3′, STRA85RACE1; 5′-ACAGGTGGGAGA TGGCCGCGGAGACG-3′, STRA83RACE1; 5′-ATCGTCTCCGCGGCCATCTCCCACCTG-3′, ST RA83RACE3; 5′-AGAAACACCGCGGCCCTGC GACCCTG-3′. Both RACE products were sequenced in both directions. The isolated full-length cDNA sequences were compared to human genome sequences.

Expression Analysis by RT-PCR. For the expression analyses of the human *STRA8*, *PIWIL2*, and

1058-0468/02/1100-0531/0 © 2002 Plenum Publishing Corporation

FIGLA, RT-PCR was performed with the primers STRA81F, STRA82F, STRA81R, and STRA82R (*STRA8*); PIWIL2F1 and PIWIL2R1 (*PIWIL2*); FIGLA1F, FIGLA2F, and FIGLA1R (*FIGRA*). The used oligonucleotides were the following: PIWIL2F1; 5′-TCATGTACGGCAGGGCTGTG-3′, PIWIL2R1; 5′-CTACAGGGTTGGAGAAAGGG-3′, F1FIGLA 1F; 5′-TGCCGGCTCAAGCGGCTG-3′, FIGLA2F; 5′-CTACTCGTCCACTGAAAACC-3′, FIGLA1R; 5′-TGCCCAAGGCCCTTCCTCTTC-3′. The analyzed human cDNA were the following: spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocyte, brain, heart, kidney, liver, lung, and pancreas (Clontech).

Expression Analysis by Northern Blot. To detect expression patterns in a variety of tissues, the isolated partial-cDNAs of the human *TEX17*, and *TUBA7* were hybridized to ready-made filters (an RNA panel from the heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) for northern blot analysis (Multiple Tissue Northern Blot, Clontech). The probes were made by RT-PCR with the primers that include no introns: TEX17F1, TEX17 F2, TEX17R1, and TEX17R2 (*TEX17*); TUBA7F1, TUBA7F2; TUBA7R1 and TUBA7R2 (*TUBA7*). The used oligonucleotides were the following: TEX 17F1; 5′-CAGATCTTTTTGCCTGGTGC-3′, TEX 17F2; 5′-CTGTCAAAGATGCTTTGGC-3′, TEX17 R1; 5′-ACTGTGTTGAAACGGGAC-3′, TEX17R2; 5′-AGAA CGCATTGGGTGGTGTGTG-3′, TUBA 7F1; 5′-CCCTGCTCATAGAACGTCTC-3′, TUBA 7F2; 5′-GCAAGAAGTCCAAGCTGGAG-3′, TUB A7R1; 5′-TCAGCAGAAATGACAGGGG C-3′, T UBA7R2; 5′-GCCAGAGGGAAGTGGAT GTG-3′.

RESULTS

We found partial nucleotide sequences representing a putative human *STRA8* gene in the human genome sequences (*Homo sapiens* clone RP11-83M17 from 7q31). To isolate human *STRA8* cDNA, nested RT-PCR was performed with primers STRA81F, STRA82F, STRA81R, and STRA82R. The resultant nested-PCR product was sequenced in both directions. Based on the sequences, the primers were made and 5′RACE and 3′RACE were carried out. Both PCR products were sequenced. The *STRA8* cDNA containing whole open reading frame (ORF) is 993 bp (GenBank accession no. AF513502). The genomic structure of *STRA8* was determined by comparison of the cDNA sequence with genomic sequence found in the HTGS database. A BLAST search with the *STRA8* cDNA sequence showed identical regions in chromosome 7 sequence (Hs7_7845 in GenBank). The ORF region of the human *STRA8* has nine exons and eight introns. The putative protein of *STRA8* consists of 330 amino acid residues. As shown in Fig. 1, human STRA8 protein has some homology to mouse stra8 (55% identity overall) and several putative phosphorylation sites for protein kinase A and C, casein kinase 2, and proline-dependent kinases (13). However, the human protein does not have glutamine acid rich domain different from that of mouse (bold letters) (Fig. 1).

To determine the expression patterns of the human *STRA8* in normal tissues, RT-PCR was performed with various tissues as templates. RT-PCR was carried out with primers STRA81F, STRA82F, STRA81R, and STRA82R. The 591-bp-sized band was clearly detected specifically in the testis with *STRA8* cDNA (Fig. 2(A)). No bands could be detected in the other 15 tissues. Then, the human *STRA8* is expressed specific to testis like the mouse *Stra8*. On the other hand, the partial cDNA fragments of *FIGRA* and *PIWIL2* were amplified as described under Materials and Methods. In mouse, the *Piwil2* is expressed specific to the testis and the expression of the *Figla* is limited to the testis and ovary. However, the human *FIGRA* is expressed not only in the testis but also in the lung, liver, and pancreas. In addition, the human *PIWIL2* is expressed in the various tissues (Fig. 2(A)). Similarly it was demonstrated by northern blot hybridization analysis that the human *TEX17* and *TUBA7* are expressed in the various tissues (Fig. 2(B)).

DISCUSSION

In this study, we report the isolation and characterization of human cDNA encoding stimulated retinoic acid 8, STRA8. The mouse protein of the *Stra8* has glutamic acid rich domain that is found in several proteins such as the centromere autoantigen protein B, troponin T, or neurofilaments L, M, and H. However, deduced protein of the human STRA8 had no glutamic acid rich domain. Then, it was suggested that the function of human STRA8 protein might be different from that of the mouse. However, human STRA8 protein also has several putative phosphorylation sites like the protein of the mouse (Fig. 1).

In mouse, the *Stra8*, *Piwil2*, *Tuba7*, and *Tex17* are expressed only in the testes, and they especially they

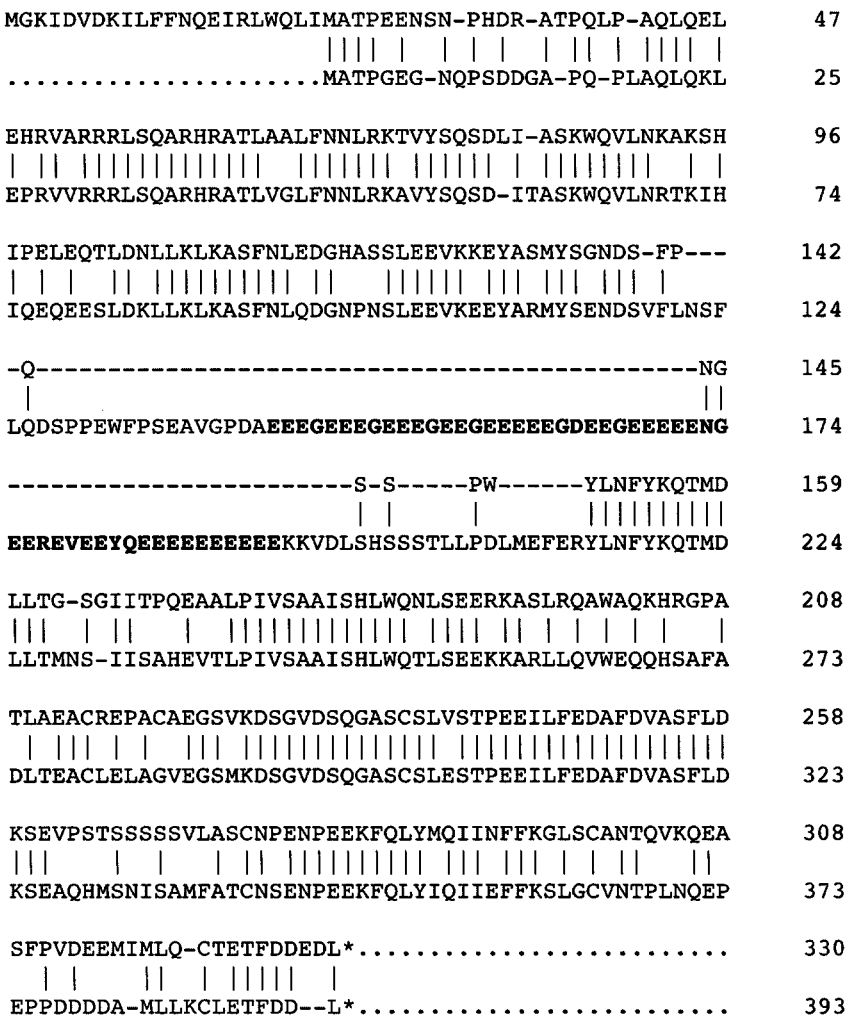

Fig. 1. The comparison of amino acid sequences between human STRA8 and mouse Stra8. Upper sequences are those of human STRA8 and the others is those of mouse Stra8. Vertical lines indicate identical sequences. There is 55% homology between them. The bold letters are the position of glutamine acid rich domain.

are limited to their spermatogonia (11). In addition, the mouse *Figla* is also expressed only in the testis and ovary. However, in this study it we found that these human genes, except for *STRA8* were expressed not only in the germinal tissues but also in the various somatogenic tissues. The amino acid sequences had some homologies; however, they may have other biological functions from their expression patterns.

The mouse *Stra8* gene is expressed specific to the testis. In addition, the expression is limited to the germ cell and not found in the somatic cells (11). The expression was strongly detected in spermatogonia. In this study, the expression analysis on histological level was not performed on the human *STRA8*. Then, it has not been unclear whether the expression of human *STRA8* is germ-cell-specific or not.

In summary, the present study suggests that the human *STRA8* can be expressed only in the testis. It is not known whether its expression is specific to germ cell or not. However, in the male, retinoids are required for the maintenance of normal testicular structure and function. Retinol deficiency leads to cessation of spermatogenesis. Then, from the expression patterns and homology of amino acid sequences between mouse and human, it is suggested that the human *STRA8* may play some roles in human spermatogenesis.

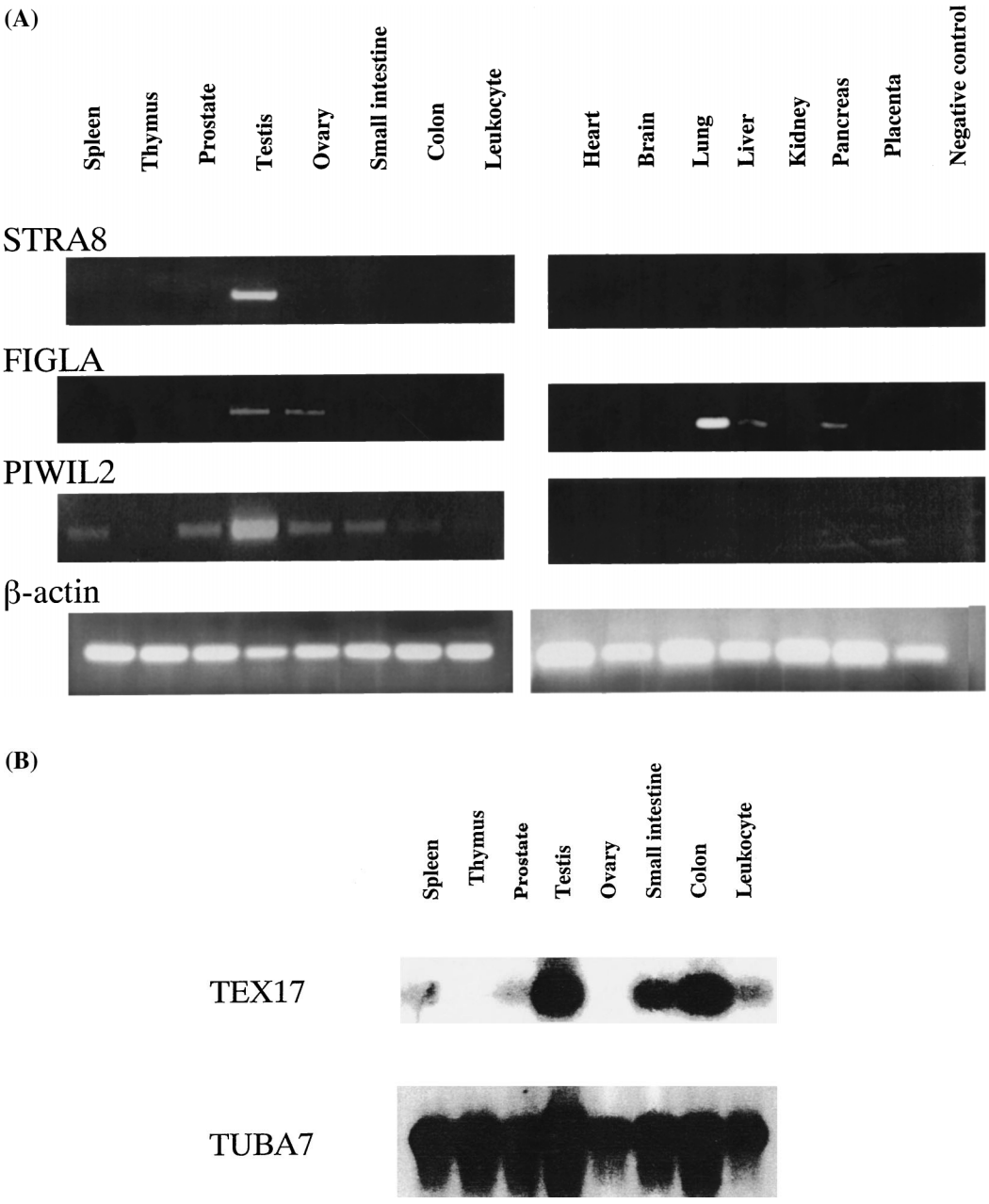

Fig. 2. (A) RT-PCR analyses of human *STRA8*, *FIGLA*, and *PIWIL2* cDNAs. Distribution patterns of them in 14 adult human tissues were examined by RT-PCR. β-Actin was used as a positive control. (B) Northern blot hybridization analysis of human *TEX17* and *TUBA7*. At least three clear bands (testis, small intestine, and colon) were detected in the human *TEX17* and all bands of the examined tissues were seen in the human *TUBA7*.

REFERENCES

1. Kastner P, Manuel M, Chambon P: Nonsteroid nuclear receptors: What are genetic studies telling us about their role in real life. Cell 1995;83:859–869
2. Sporn MB, Roberts AB, Goodman DS: The Retinoids: Biology, Chemistry and Medicine. New York, Raven Press, 1994, p. 679
3. Thompson NJ, Howell JM, Pitt GAJ: Vitamin A and reproduction in rats. Proc R Soc Ser B 1964;159:510–535
4. Howell JM, Thompson JN, Pitt GAJ: Histology of the lesions produced in the reproductive tract of animals fed a diet deficient in vitamin A alcohol but vontaining vitamin A acid. I: The male rat. J Reprod Fertil 1963;5:159–167
5. Bouillet P, Oulad-Abdelghani M, Vicaire S, Garnier JM, Schuhbaur B, Dolle P, Chambon P: Efficient cloning of cDNAs

of retinoic acid-responsive genes in P19 embryonal carcinoma cells and characterization of a novel mouse gene, Stra1 (mouse LERK-2). Dev Biol 1995;170:420–433
6. Bouillet P, Chazaud C, Oulad-Abdelghani M, Dolle P, Chambon P: Sequence and expression pattern of the Stra7 (Gbx-2) homeobox-containing gene induced by retinoic acid in P19 embryonal carcinoma cells. Dev Dyn 1995;204:372–382
7. Oulad-Abdelghani M, Bonillet P, Chazaud C, Dolle P, Chambon P: AP-2.2: A novel AP-2-related transcription factor induced by retinoic acid during differentiation of P19 embryonal carcinoma cells. Exp Cell Res 1996;255:338–347
8. Roy B, Taneja R, Chambon P: Synergistic activation of retinoic acid (RA)-responsive genes and induction of enbryonal carcinoma cell differentiation by an RA receptor α (RARα)-, RARβ-, or RARγ-selective ligand in combination with a retinoid X receptor-specific ligand. Mol Cell Biol 1995;15:6481–6487
9. Taneja R, Thisse B, Rijli F, Thisse C, Bouillet P, Dolle P, Chambon P: The expression pattern of the mouse receptor tyrosine kinase gene MDK1 is conserved through evolution and requires Hoxa-2 for rhombomere-specific expression in mouse embryos. Dev Biol 1996;177:397–412
10. Oulad-Abdelghani M, Bouillet P, Decimo D, Gansmuller A, Heyberger S, Dolle P, Bronner S, Lutz Y, Chambon P: Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol 1996;135:469–477
11. Wang PJ, McCarrey JR, Yang F, Page DC: An abundance of X-linked genes expressed in spermatogonia. Nat Genet 2001;27:422–426
12. Miyamoto T, Hasuike S: Isolation and expression analysis of the human RNH2 gene encoding ribonuclease inhibitor 2. J Assist Reprod Genet 2002;19:394–397
13. Kemp BE, Pearson RB: Protein kinase recognition sequence motifs. Trends Biochem Sci 1990;15:342–346

Toshinobu Miyamoto,[1,2] Kazuo Sengoku,[1] Naoyuki Takuma,[1] Shiga Hasuike,[1] Hiroaki Hayashi,[1] Tomofumi Yamauchi,[1] Tsuyoshi Yamashita,[1] and Mutsuo Ishikawa[1]

[1] Department of Obstetrics and Gynecology, Asahikawa Medical College, Midorigaokahigashi 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan.
[2] To whom correspondence should be addressed; e-mail: toshim@asahikawa-med.ac.jp.