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SHORT COMMUNICATION

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Isolation and Expression Analysis of the Human RNH2 Gene Encoding Ribonuclease Inhibitor 2

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Ribonuclease inhibitor 1 (RI-1) is a cytoplasmic protein that inhibits a variety of pancreatic-type mammalian Rnases by forming a very tight, reversible 1:1 complex. Recently a novel gene encoding ribonuclease inhibitor 2, Rnh2 has been isolated in mouse. The expression pattern of the mouse *Rnh2* is specific to testis, especially in the spermatogonia. Then it has been suggested that the mouse *Rnh2* gene may play critical roles in mouse spermatogenesis. In this study, we isolated the human *RNH2* cDNA and analyzed its expression pattern. The human RNH2 is also expressed limited to testis, and then it is suggested that the human *Rnh2* gene may also play critical roles in human spermatogenesis.

KEY WORDS: RNH2; testis; spermatogenesis.

INTRODUCTION

Ribonuclease inhibitor 1 (RI-1) is a cytoplasmic protein that inhibits a variety of pancreatic-type mammalian Rnases by forming a very tight, reversible 1:1 complex (1). Three possible functions have been suggested. The first one is regulation of the activity of cytoplasmic Rnases involved in RNA turnover. The second one is safeguarding against secreted, noncytoplasmic Rnases that mislocalize to the cytoplasm. The last one is regulation of the activity of RNases, such as angiogenin or eosinophil-derived neurotoxin, that have physiological activities other than the digestion of RNA in food (2). The primary structure of RI-1 revealed that the molecule is built entirely from two types of alternating homologous leucine-rich repeats (3–5). This motif has been found in a large variety of proteins, and is characterized in particular by leucine residues at conserved positions (6).

Recently a novel gene encoding ribonulease inhibitor 2, Rnh2, has been isolated in mouse (7). The work was carried out by a systematic search for genes expressed in mouse spermatogonia but not in somatic tissues. The expression pattern of the mouse *Rnh2* is specific to testis. Then it has been suggested that the mouse *Rnh2* gene may play critical roles in mouse spermatogenesis.

MATERIALS AND METHODS

Isolation of Human RNH2 cDNA

The mouse RNH2 cDNA was isolated recently (7). Using the mouse RNH2 amino acid sequences (AF285581 in GenBank), we found the region including homology in amino acid level in the human genome sequence (Hs 19_11376 in GenBank). The primers encompassing introns, RNH2F and RNH2R, were made using homology and RT-PCR was performed with mouse 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 5RACE1, 5RACE2, 3RACE1, and 3RACE2. The used oligonucleotides are the following: RNH2F; 5′TCCCTGGCTGCAGAGGGTATG-3′, RNH2R; 5′-TCTCTTCCTCAGCCGTCAG-3′, 5RACE1; 5′-GAACACGTAGGAGCTCTCACGCTCCCCG-3′, 5RACE2; 5′-CCCCATTTCTCCGGAGGTCGTCT TCAC-3′, 3RACE1; 5′-ACCTTGAACACCTTGG ACCACACAGGGG-3′, and 3RACE2; 5′-GGTT GTACTCTGTGAGGCCCTGAGACAC-3′. Both 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 the expression analysis of the mouse *RNH1* and *RNH2*, RT-PCR was done with the primers RNH2F, RNH2R, RNH1F, and RNH1R. The used oligonucleotides are the following: RNH1F; 5′-GACATCAGCTCTGCACTTCG-3′ and RNH1R; 5′-CAGCCTCATTGATGTCGTTG-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).

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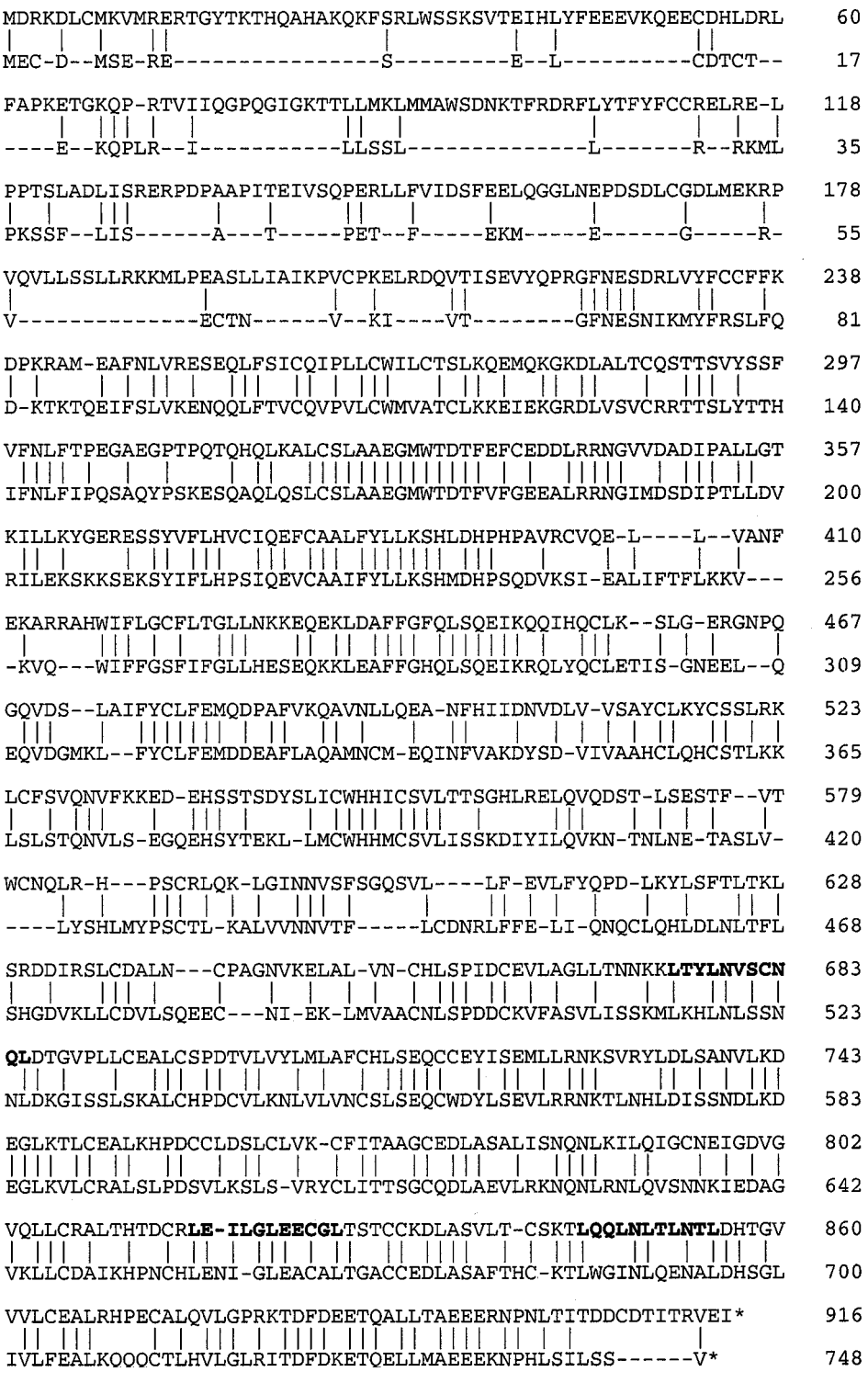

Fig. 1. The comparison of amino acid sequences between human and mouse RNH2. Upper sequences are human and the other is mouse. Vertical lines indicate identical sequences. There is 43% homology between them. The bold letters are the positions of leucine-rich repeats.

RESULTS

We found partial nucleotide sequences representing a putative human *RNH2* gene in the human genome sequence (chromosome 19). To isolate human *RNH2* cDNA, RT-PCR was performed with primers RNH2F and RNH2R and the resultant PCR product sized 1.65 kb was sequenced. Based on the sequence, the primers were made and 5′RACE and 3′RACE were carried out. Both PCR products were sequenced. The full length of *RNH2* cDNA is 3234 bp (GenBank accession no. AF482706), which contain the whole open reading frame. The genomic structure of *RNH2* was determined by comparison of the cDNA sequence with genomic sequence found in the HTGS database. A BLAST search with the *RNH2* cDNA sequence showed identical regions in chromosome 19 sequence (Hs19_11376 in GenBank). Human *RNH2* has nine exons and eight introns. The putative protein of RNH2 consists of 916 amino acid residues and it has at least three leucine-rich repeats (Fig. 1). As shown in Fig. 1, human RNH2 protein has some homology to mouse RNH2 (43% identity overall). However, the identity between human RNH1 (GenBank accession no. XM_006139) and RNH2 is only 26% in the amino acid sequences.

To determine the expression patterns of the human *RNH2* and *RNH1* in normal tissues, RT-PCR was done with various tissues as templates. RT-PCR was performed with primers RNH2F, RNH2R, RNH1F, and RNH1R. On the *RNH1*, the 417 bp-sized bands were detected in all examined tissues except for testis (Fig. 2(A)). On the other hand, on the *RNH2*, the 1.65 kb-sized band was clearly detected specifically in the testis (Fig. 2(B)). No bands could be detected in the other 15 tissues. The human RNH2 is expressed specific to testis.

DISCUSSION

In this study, we report the isolation and characterization of human cDNA encoding ribonuclease inhibitor 2, RNH2. The protein of *RNH2* has at least three leucine-rich repeats. Leucine-rich repeats (LRRs) are relatively short in comparison to other repeats' families. They are associated with an astonishing variety of functions, including signal transduction, transmembrane receptors, DNA repair, cell adhesion, and extracellular matrix proteins (8). The common function among LRRs is that they form complexes with other proteins. For example, the LRRs of ribonuclease A inhibitor bind to ribonuclease A (9). Then it is suggested that human RNH2 protein has the function of forming complexes with other proteins.

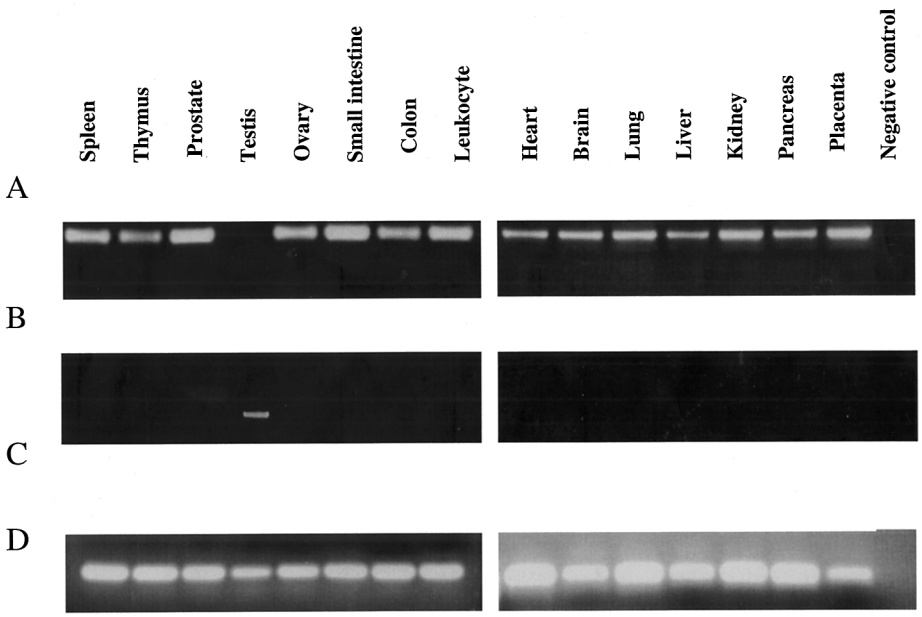

Fig. 2. RT-PCR analyses of human RNH1 and RNH2 cDNA. Distribution patterns of RNH1 and RNH2 in 14 adult human tissues were examined by RT-PCR. A displays the product of RNH1 and B does the one of RNH2. β-Actin was used as positive control.

Both of human RNH1 and RNH2 have several LRRs, and so they must form protein–protein interactions. However, another part of amino acid sequence between human RNH1 and RNH2 is much different. In addition, the expression patterns of the human *RNH1* and *RNH2* genes are completely different. Then it was proposed that the human RNH2 has another biological role compared to that of RNH1.

The mouse *RNH2* gene is expressed specific to the testis. In addition, the expression is limited to the germ cell but not in the somatic cells (7). The expression was strongly detected in spermatogonia. In this study, the expression analysis on histological level was not performed on human RNH2. Then, it is not clear if the expression of human RNH2 is germ cell specific or not.

In summary, the present study suggests that the human RNH2 be also expressed only in the testis. It seems to have the function of protein–protein interaction by its LRRs. It is not known that its expression is specific to germ cell or not. However, from the expression patterns and homology of amino acid sequence between mouse and human, it is suggested that the human RNH2 may play some roles in human spermatogenesis.

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