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SHORT COMMUNICATION

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Isolation, tissue expression, and chromosomal assignment of human *RGS5*, a novel G-protein signaling regulator gene

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Abstract The regulator of G-protein signaling (RGS) proteins have recently been identified as signal transduction molecules which have structural homology to SST2 of *Saccharomyces cerevisiae* and EGL-10 of *Caenorhabditis elegans*. Multiple genes homologous to SST2 are present in higher eukaryotes, and the group of these genes is termed the RGS family. RGS proteins are involved in the regulation of heterotrimeric G-proteins by acting as GTPase-activators. A putative new member of the RGS family was isolated from a neuroblastoma cDNA library. The amino acid sequence deduced from the cDNA possessed all consensus motifs of the RGS domain and showed closest homology to mouse RGS5 (90% identical), indicating that it was human *RGS5* (*hRGS5*). The messenger RNA of *hRGS5* was abundantly expressed in heart, lung, skeletal muscle, and small intestine, and at low levels in brain, placenta, liver, colon, and leukocytes. The chromosome localization of the gene in the 1q23 region was determined by a monochromosomal hybrid panel and a radiation hybrid panel.

Key words G-protein · *RGS5* · Chromosome mapping · 1q23 · Neuroblastoma · cDNA library

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Introduction

Heterotrimeric G-proteins comprise a superfamily which is involved in the transmission of extracellular signals of hormones and neurotransmitters across the cell membrane to regulate various physiological processes. Recently, novel regulatory molecules for these G-protein signaling pathways, termed RGS, have been identified (Koelle and Horvits 1996; Koelle 1997; Dohlman and Thorner 1997).

The function of the RGS family was initially discovered from the genetic analysis of two independent model organisms, *Saccharomyces cerevisiae* and *Caenorhabditis elegans*. In *S. cerevisiae*, SST2, the prototype for the newly recognized RGS family, is involved in the desensitization of mating pheromone, which operates through a heterotrimeric G-protein signalling pathway (Dohlman et al. 1996; Druey et al. 1996). Rat RGS 1–4 have been shown to partially complement a defect of SST2 in yeast, indicating an evolutional conservation of structure and function (Druey et al. 1996). Another line of functional evidence for RGS has emerged from studies of the neurotransmitter-modulated egg-laying behavior of *C. elegans.* Goa-1, a Go alpha subunit regulates this behavior, and EGL-10, an RGS protein, regulates this behavior in an opposite manner, and genetic analysis has suggested that EGL-10 regulates G-protein signaling pathways (Koelle and Horvits 1996). Further biochemical analysis of the RGS family has assigned their function as GTPase-activating proteins of heteromeric G-proteins (Berman et al. 1996; Chatterjee et al. 1997; Popov et al. 1997).

hRGS5 cDNA was cloned from a full-length enriched cDNA library constructed from a neuroblastoma (NB) sample using the oligo-capping method, as described previously (Maruyama and Sugano 1994; Suzuki et al. 1997). Here we report the sequence features, expression profile, and chromosomal assignment of the *hRGS5* gene. This information should prove valuable in designing studies to evaluate its cellular function in G-protein signaling.

Materials and methods

Isolation and sequencing of human *RGS5*

We constructed a full-length enriched cDNA library derived from the NB specimen, using the oligo-capping method (Maruyama and Sugano 1994; Suzuki et al. 1997). A cDNA clone revealed from one-pass sequence that it had closest homology to mouse and rat *RGS5*. The entire sequence of this clone was determined by a shot-gun strategy (Seki et al. 1997) and searches of the public databases were performed routinely with the FASTA program of the UWGCG package (Pearson and Lipman 1988).

Chromosome mapping

Chromosomal assignment of the *hRGS5* was done by polymerase chain reaction (PCR) analysis of human and rodent somatic cell hybrid panels. DNA of the human-rodent somatic cell hybrid panel (Mapping panel no. 2) and radiation hybrid panel (Genebridge 4) were purchased from the National Institute of General Medicine Service, Coriell Cell Repositories (Camden, NJ, USA) and Research Genetics (Huntsvill, AL, USA), respectively. Primers used for PCR amplification correspond to the nucleotide (n. t.) 1573 to n. t. 1593 (5′-TGGCTAATTGTCCTATGATGC-3′) and the n. t. 1741 to n. t. 1761 (5′-GTGAAGGAATACTGGGGTTGC-3′)(PCR product size, 189 bp). PCR was carried out in the manner described previously (Saito et al. 1997; Seki et al. 1997).

Northern blot analysis

Northern blot analysis of hRGS5 mRNA in various human tissues. Northern blot filters containing adult human poly $(A)^+$ RNAs (2μg/lane) were purchased from Clontech Laboratories (Palo Alto, CA, USA) and hybridization and washing were performed following the manufacturer's instructions. The 2.1-kb cDNA fragment containing the entire open reading frame was labelled with [α-^{32}P] dCTP and used as a hybridization probe.

Results and discussion

The isolated hRGS5 cDNA clone was 2076 bp in length and had an open reading frame of 181 amino acids. The predicted protein of 181 amino acids had a calculated molecular weight of approximately 21-kDa. The nucleotide sequence data reported here will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB008109. A homology search of the conceptual translated amino acid sequence of the isolated cDNA revealed that it was most homologous to mouse RGS5 (Chen et al. 1997), having 90% identity at the amino acid level, and thus we judged this clone was derived from the human *RGS5* (*hRGS5*) gene. The alignment of the deduced amino acid sequences of hRGS5, mouse RGS5, and rat RGS5 (partial sequence) (Druey et al. 1996) is shown in Fig. 1.

We determined the chromosomal location of the *RGS5* gene using a somatic cell hybrid panel. A PCR product of the expected size of 189 bp was detected in human genomic DNA, control cDNA, and somatic cell hybrids containing only human chromosome 1 (data not shown). We performed PCR analysis of a radiation hybrid panel with the same primers as those used in the assay for the somatic cell hybrid panel. Statistical analysis of the radiation hybrid data was performed using the (RHMAPPER) software package (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). The data vector for the *hRGS5* gene was 1001010001 0010121210 0000010100 0011000010 1010101001 0101000000 0010000000 0101100000 0100000000 021, and the consequent report indicated the gene was mapped between markers GHCL.GATA70D01 and WI-4182, both of which have been cytogenetically mapped to 1q23. The position is 0.00 cR from GHCL.GATA70D01 (lod > 3.0). To confirm the PCR-based chromosome mapping by an inde-

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mRGS5      1  MCKGLAALPHSCLERAKEIKIKLGILLQKPDSAVDLVIPYNEKPEKPANGHKPSLEEVLQ
rRGS5      1  ............................................................
hRGS5      1  MCKGLAALPHSCLERAKEIKIKLGILLQKPDSVGDLVIPYNEKPEKPAKTQKTSLDEALQ

mRGS5     61  WRQSLDKLLQNSYGFATFKSFLKSEFSEENLEFWVACENYKKIKSPIKMAEKAKQIYEEF
rRGS5      1  ..................................VACENYKKIKSPIKMAEKAKQIYEEF
hRGS5     61  WRDSLDKLLQNNYGLASFKSFLKSEFSEENLEFWIACEDYKKIKSPAKMAEKAKQIYEEF

mRGS5    121  IQTEAPKEVNIDHFTKDITMKNLVEPSPRSFDLAQKRIYALMEKDSLPRFVRSEFYKELI
rRGS5     27  IQTEAPKEVNIDHFTKDITMKNLVEPSPHSFDLAQKRIYAL..................
hRGS5    121  IQTEAPKEVNIDHFTKDITMKNLVEPSLSSFDMAQKRIHALMEKDSLPRFVRSEFYQELI

mRGS5    181  K
rRGS5        .
hRGS5    181  K
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Fig. 1 Alignment of human (*h*) RGS5 (accession no., AB008109), mouse (*m*) RGS5 (accession no., U67188), and rat (*r*) RGS5 (accession no., U32435). Identities are indicated by *black background*, and *similar residues are shadowed*

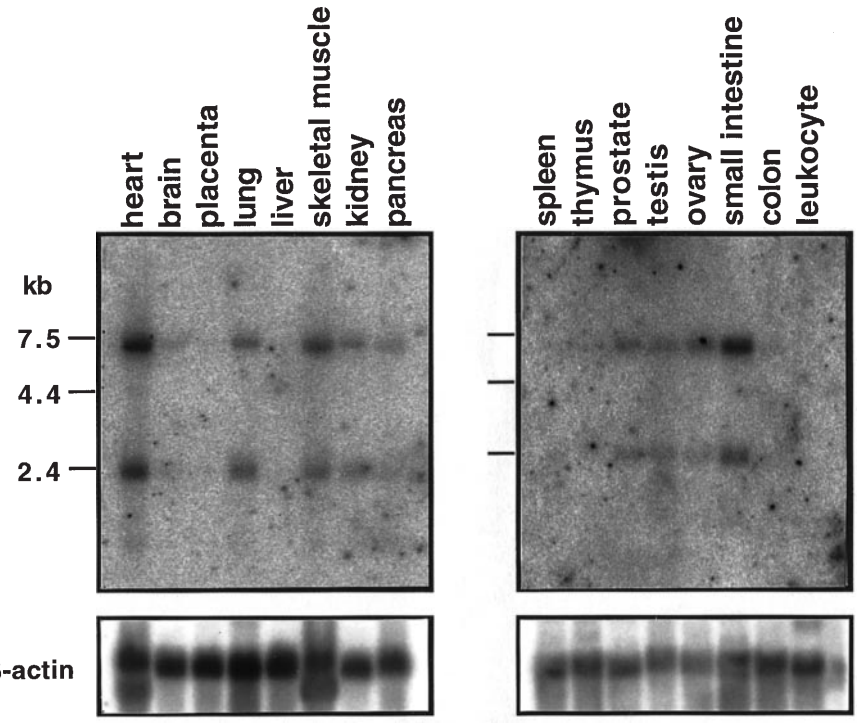

Fig. 2 Northern blot analysis of human RGS5. Northern blot filters containing adult human poly $(A)^+$ RNAs (2μg/lane) were purchased from Clontech Laboratories (Palo Alto, CA, USA), and hybridization and washing were performed following the manufacturer's instructions. The 2.1-kb cDNA fragment containing the entire open reading frame was labelled with [α-^{32}P] dCTP and used as a hybridization probe. The *bottom panel* shows the result with a β-actin probe used to adjust for the amount of RNA loaded. Size markers (*left*) are in kilobases (*kb*)

pendent approach, we performed R-banding fluorescence in-situ hybridization (FISH), using a P1 phage DNA, as described previously (Seki et al. 1997). Clear doublet signals were consistently demonstrated on the q23 region of chromosome 1 (data not shown). Therefore, the gene was mapped on the q23 region of chromosome 1.

We examined the tissue distribution of RGS5 in various tissues by northern blot analysis. Hybridization of human RNAs from multiple tissues (Clontech) with a *RGS5* cDNA probe detected two mRNA species, which migrated as 2.1-kb and 7.0-kb bands (Fig. 2). The size of the band is consistent with the notion that our clone is full length. The *hRGS5* was abundantly expressed in heart, lung, skeletal muscle, and small intestine, and at low levels in brain, placenta, liver, colon, and leukocytes. These observations are similar to findings for mouse *RGS5* (Chen et al. 1997). Recently, the localization of nine rat RGSs, including RGS5, was investigated systematically in rat brain, using an in-situ hybridization technique; the RGS5 mRNA was expressed at lower density in rat brain than in the other tissues examined (Gold et al. 1997).

A large number of RGSs have been identified through a database search for expressed sequence tags (ESTs) and screening by a PCR-assisted method (Druey et al. 1996; Koelle 1997). In addition a yeast two-hybrid system using Gai3 has identified GAIP (De Vries et al. 1995), another member of the RGS family (Berman 1996). Also recently identified is axin, a gene regulating the embryonic axis in the mouse, and this was found to have structural similarities with RGSs (Zeng et al. 1997).

RGS proteins can now be seen to be associated with a large gene family, with an ancestral, origin in lower eukaryotes; the number of members of the family is still rapidly expanding. The physiological significance and biochemical properties of different RGS proteins remain to be explored.

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