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SHORT COMMUNICATION

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Cloning, expression analysis, and chromosomal localization of *HIP1R*, an isolog of huntingtin interacting protein (HIP1)

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Abstract Huntington disease (HD) is an inherited neurodegenerative disorder which is associated with CAG expansion in the coding region of the gene for huntingtin protein. Recently, a huntingtin interacting protein, HIP1, was isolated by the yeast two-hybrid system. Here we report the isolation of a cDNA clone for *HIP1R* (huntingtin interacting protein-1 related), which encodes a predicted protein product sharing a striking homology with HIP1. RT-PCR analysis showed that the messenger RNA was ubiquitously expressed in various human tissues. Based on PCR-assisted analysis of a radiation hybrid panel and fluorescence *in situ* hybridization, *HIP1R* was localized to the q24 region of chromosome 12.

Key words Huntingtin interacting protein (HIP1) · Leucine zipper · Chromosome 12q24 · Full-length enriched cDNA library · Neuroblastoma

Introduction

Huntington disease (HD) is inherited as an autosomal dominant disease that gives rise to progressive, selective neural cell death associated with choreic movements and dementia (Harper 1991). The disease is associated with increases in the CAG triplet repeat length present in a gene called *huntingtin* located on chromosome 4p16.3 (Ross et al. 1995; Zoghbi et al. 1996). In HD, CAG expansion beyond 35 repeats usually results in a clinical phenotype (HDCRG 1993; Kremer et al. 1994). There is a strong inverse relationship between CAG size and age of onset of this disease (Andrew et al. 1993; Duyao et al. 1993; Snell et al. 1993). Wanker et al. (1997) hypothesized that binding proteins, with a restricted pattern of expression, specific to the elongated polyglutamine stretch of the huntingtin protein, could cause selective vulnerability in particular cells. At present, three huntingtin interacting proteins have been identified, such as huntingtin-associated protein (Li et al. 1995), the glycolytic enzyme GAPDH (Burke et al. 1996), and the ubiquitin-conjugating enzyme hE2-25K, also named HIP2 (Kalchman et al. 1996). Recently, another huntingtin interacting protein, HIP1, was isolated by yeast two-hybrid screening. HIP1 shares homology with Sla2p, a component of cytoskeleton in *Saccharomyces cerevisiae* (Kalchman et al. 1997; Wanker et al. 1997).

In the present study, cDNA for a HIP1-related protein (HIP1R) was cloned from a full-length enriched cDNA library constructed from a neuroblastoma sample using the oligo-capping method as described previously (Maruyama and Sugano 1994; Suzuki et al. 1997). Here we report the sequence feature, expression profile, and chromosomal assignment of the *HIP1R* gene.

Results and discussion

The HIP1R cDNA clone was revealed from a one-pass sequence to be the most homologous to HIP1 (accession number U79734). The entire sequence of this clone was determined by a shot-gun strategy (Seki et al. 1997). The isolated *HIP1R* cDNA clone was 3876 bp in length and had a single ORF of 890 amino acids. The predicted protein had a calculated molecular weight of approximately 99 kDa. The nucleotide sequence data reported here will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number of AB013384. A canonical

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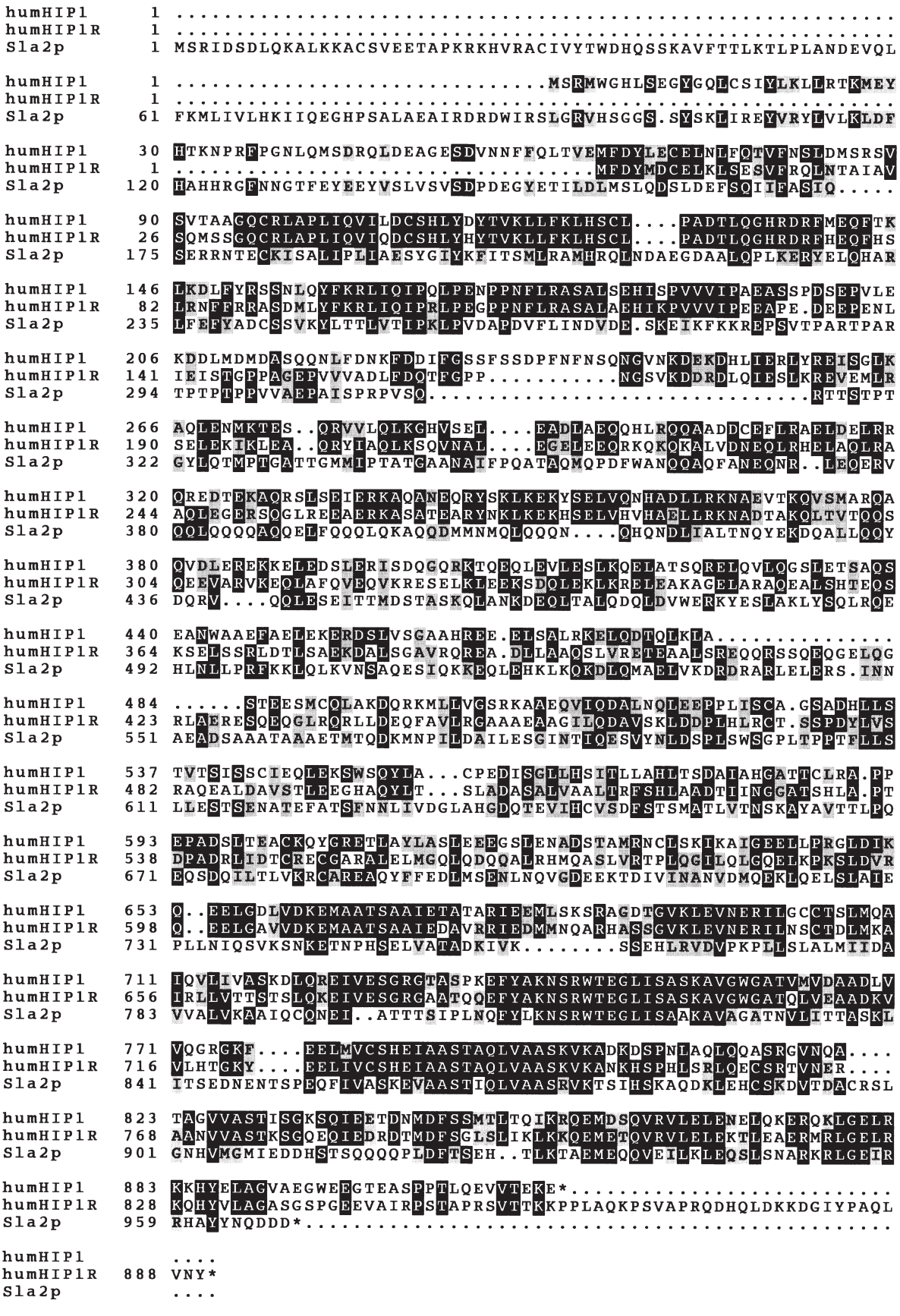

Fig. 1 Alignment of HIP1 (accession number U79734), HIP1R (accession number AB013384), and Sla2p (accession number Z22811). Identities are indicated by *black background* and similar residues are *shaded*. An *asterisk* denotes the termination codon

polyadenylation signal, AATAAA, was located 23 bp upstream of a poly A. Homology search of the conceptual translated amino acid sequence of the isolated cDNA revealed that it was most homologous to HIP1 (Kalchman et al. 1997), having 56% identity at the amino acid level, and thus we named the gene for the cDNA huntingtin interacting protein-related (HIP1R). The alignment of the predicted amino acid sequences of HIP1, HIP1R, and Sla2p is shown in Fig. 1. A homology search for HIP1 revealed that the HIP1 gene product shares significant similarity with the Sla2 gene product from *S. cerevisiae*, which is known to code for an essential cytoskeletal-associated protein (Kalchman et al. 1997; Wanker et al. 1997). Kalchman et al. (1997) suggested that an increased polyglutamine tract could disturb the normal interaction of huntingtin with HIP1 which, in turn, could lead to an alteration of biological events on the membrane causing premature cell death and ultimately the clinical manifestations of HD.

Since HIP1R has a high sequence homology to HIP1 in the amino terminal region, which was shown to be responsible for the interaction with huntingtin (Wanker et al. 1997), it is conceivable although not proven that HIP1R also interacts with huntingtin in its amino terminal region. Our sequence analysis revealed a highly conserved motif consistent with a leucine zipper encompassing the amino acid residues 329–357 (LEEKSDQLEKLKRELEAKAGELARAQEAL) of HIP1R. In HIP1 and Sla2p, a leucine zipper motif is found at the region of amino acid residues 412–433 (LESLKQELGTSQRELQVLQGSL) and 481–502 (LAKLYSQLRQEHLNLLPRFKKL), respectively. Leucine zippers are known to mediate protein-protein interactions occurring in the cytoskeleton, or to act in transcriptional activations by allowing the formation of homo- or hetero-dimers of transcription-regulating proteins (John et al. 1994; Pearlman et al. 1994). The leucine zipper motifs conserved among the HIP1 family could contribute to some important role(s) in interaction with other proteins.

We examined the tissue distribution of *HIP1R* transcript in various tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR). Primers used for RT-PCR correspond to the coding region of the gene. Considering its ubiquitous expression in a wide variety of human tissues (Fig. 2), HIP1R described in the present study seems to be involved in a basic housekeeping function of cells.

We determined the chromosomal location of the *HIP1R* gene using a radiation hybrid panel (Genebridge 4, Research Genetics, Huntsville, AL, USA) in the same manner of previous reports (Saito et al. 1997; Seki et al. 1997). Primers used for PCR amplification correspond to the 3′ UTR region of the gene (5′-CCTGAAGGTCGTGGATGGATG-3′) and (5′-CTGGTAGAATCGGTGCCCCAG-3′) (102-bp PCR product). The radiation hybrid mapping data was processed using the RHMAPPER software package (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). The data vector for the *HIP1R* gene was 0010100001 1000100000 0100100101 0001010010 0000110000 1000000000 0000001010 1001100000 0100011010

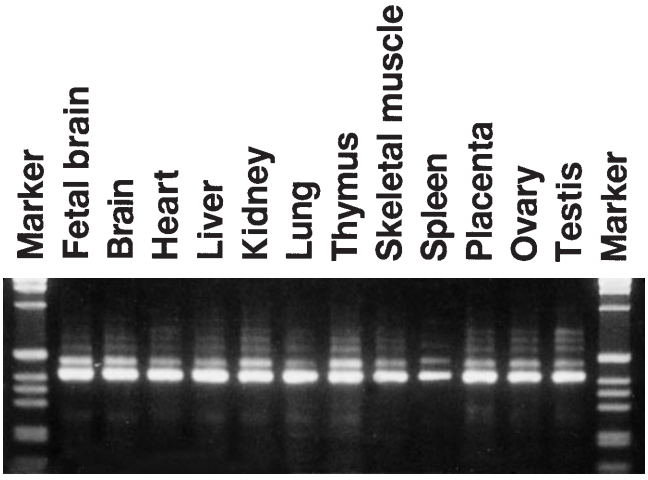

Fig. 2 Primers used for RT-PCR were to amplify the 425 bp cDNA of the transcript. The primers were (5′-GAAATGCTCCGCTCTGAACTG-3′) and (5′-AACTCCGACTCCCGCTTCACC-3′). The primer set gave the longer PCR product from genomic DNA which was easily distinguished from the 425 bp product from the mRNA. The templates of the human tissues of poly(A)$^+$ RNAs were purchased from Clontech (Palo Alto, CA, USA). The cDNA templates for RT-PCR were synthesized from 2 μg of poly (A)$^+$ using excess amounts of Superscript II reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA) and random hexamer primers. PCR was carried out in a final volume of 10 μl containing 1 × LA-PCR buffer (Takara, Kyoto, Japan), 2 μM each primer, 200 μM each dNTP, 1 μl of template DNA and 0.01 units of LA-Taq DNA polymerase (Takara, Kyoto, Japan). Temperature and time schedule were 30 cycles of 95°C for 20 s and 62°C for 1 min. PCR products were separated on 2.5% Nusieve GTG agarose gel (FMC, Rockland, ME, USA) with a 1 kb ladder DNA marker (Gibco)

0000001010 1001100000 0100011010 0000110000 1000000000 0100100101 0001010010 1000100000 0010100001 110 and the consequent statistical report indicated the gene was mapped to 4.81 cR distal from WI-6021 (lod >3.0) which maps cytogenetically to the 12q24 region. To confirm the PCR-based chromosome mapping by an independent approach, we performed fluorescence *in situ* hybridization (FISH) using a P1 phage DNA probe as described previously (Seki et al. 1997). The P1 clone containing the *HIP1R* sequence was isolated by the method described previously (Ohira et al. 1997). The clear doublet signals were consistently demonstrated on the q24.2–q24.3 region of chromosome 12 (Fig. 3). Therefore, the gene was mapped on the q24.2–q24.3 region of chromosome 12. Some of the neurodegenerative disease was mapped to the vicinity of this region including scapuloperoneal syndromes (Isozumi et al. 1996), the distal hereditary motor neuropathy (distal HMN) or the spinal form of Charcot-Marie-Tooth (CMT) disease (Timmerman et al. 1996). Homology between HIP1R and HIP1 implies the possible involvement of HIP1R in these neurodegenerative diseases. Our precise chromosomal positioning data of the *HIP1R* gene might contribute to the ongoing positional candidate approaches for the disease genes which are linked to the genomic region.

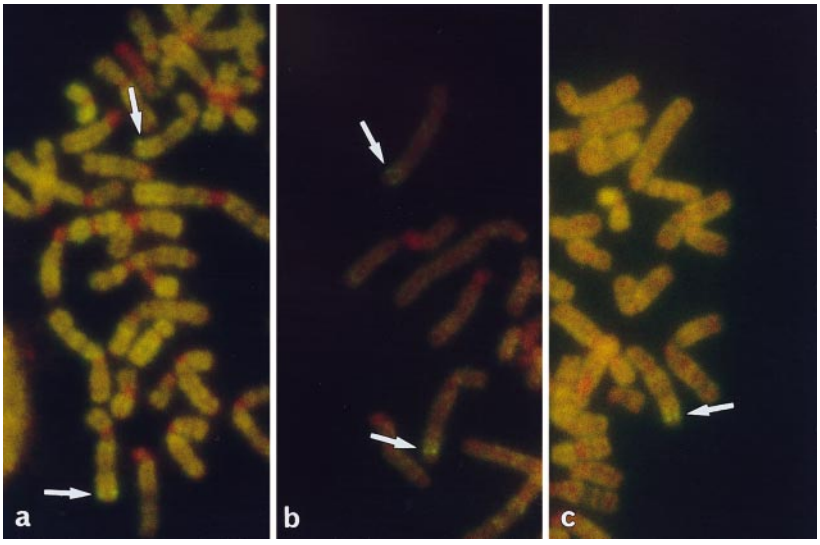

Fig. 3a–c Fluorescence *in situ* hybridization (FISH) of the *HIP1R* gene. FISH was carried out using a biotinylated hybridization probe made from P1 phage clone harboring the *HIP1R* gene. *Arrows* indicate the hybridization signals on human chromosome 12q24.2–q24.3. The metaphase spreads were photographed with a Nikon (Tokyo, Japan) B-2A filter

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