

SHORT COMMUNICATION

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A novel human gene whose product shares homology with bovine brain-specific protein p25 is expressed in fetal brain but not in adult brain

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Abstract We have cloned a novel human gene (*CI4orf5*) from a fetal brain cDNA library that is located on chromosome 14 and consists of 4 exons. It encodes a protein of 170 amino acids that shares homology with human p25 alpha and bovine p25. Reverse transcription-polymerase chain reaction analysis indicated that it is highly expressed in liver and pancreas. Its transcripts could not be detected in adult brain but could be found in fetal brain.

Key words Bovine brain-specific p25 · Human p25 alpha · cDNA cloning

A brain-specific protein, p25, was originally isolated from the partially purified fraction of bovine tau protein kinase II (Takahashi et al. 1991). The immunohistochemical studies showed that p25 was localized in oligodendrocytes and neuropils (Takahashi et al. 1993). Its complete cDNA sequence has been determined, but its function is still unknown (Shiratsuchi et al. 1995). Its counterpart in humans, p25 alpha, with which it shares 90% identity at the amino acid level, has been cloned, and it was found to be expressed ubiquitously rather than to be brain specific (Seki et al. 1999).

During the large-scale sequencing analysis of a human fetal brain cDNA library constructed in our lab (Xu et al. 2001), we isolated a novel cDNA clone that is 742 bp long. It has an open reading frame from 168 to 638 bp that encodes an 18.5 kDa protein. The first ATG satisfies the Kozak consensus A/GXXATGG and the 5' untranslated region contains two in-frame stop codes. The polyadenylation signal (AATAAA) is located at 707 to 712 bp, 16 bp before the

polyA tail. BLASTX searching with its putative amino acid sequence against the nonredundant sequence database in GenBank showed that it has 57% identity with both bovine p25 and human p25 alpha at the amino acid level. Additionally, it shares homology with five other putative proteins from human, mouse, fruit fly, and *C. elegans* (Fig. 1). There are several sites conserved in all these proteins, the most notable among them is the Rossmann motif GXGXXG, an ATP-binding site of protein kinases (Kemp and Pearson 1990), in that it is the only motif that can be found in all these proteins. In accordance with the suggestion of the Human Gene Nomenclature Committee, the gene was named *CI4orf5*. Its sequence has been submitted to GenBank (accession number AY072034).

BLAST searching with its nucleotide acid sequences against the nonredundant sequences database in GenBank found a perfect match with human bacterial artificial chromosome (BAC) C-2552B11 (AL157687) and R-998D10 (AL161668) on chromosome 14. On the basis of these data, we established the exon–intron structure of the gene according to the AG–GT rule (Table 1), which shows that the gene has 4 exons. The BLAST result also revealed a sequence tagged site (STS) marker (STS65398) within the cDNA sequence (from 577 to 701 bp), by which the gene could be mapped to position 9.20 on Chromosome 14 of the GM 99 GB4 Map (cR 3000) (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search/).

Human multiple tissue cDNA panel I and the tumor panel (Clontech, Palo Alto, CA, USA) were used as templates to investigate the expression pattern of the gene, according to the manufacturer's protocol. The polymerase chain reaction (PCR) primers were designed for positions 472 and 690 bp (sequences: CAGTGGACCGTTTGACAGACACAGCA and GGTGTAAACATGCAGGGTCAGGGGGC). A clear band between 200 and 300 bp can be detected in several tissues and tumor cell lines (The bands smaller than 100 bp are primer dimers). Among the normal tissues, the gene is highly expressed in liver and pancreas and has a moderate expression level in heart, skeletal muscle, and kidney. However, its transcript could not be detected in lung, placenta, or brain, even when the PCR cycle number was increased to

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Table 1. Exon-intron structure of *C14orf5*

3' Splice acceptor	Exon	Size (bp)	5' Splice donor	Intron	Size (bp)
attactccacagTCCTCCCTCC	1	56	GTACTCTAAGgtacataaaaga	1	210
ctaactgaccagGGCCAAGAAC	2	242	GCAAAGTCAAgtaggagccaa	2	259
cctgctgagcAAAGCAACAA	3	154	TGGCGCTACTgtgagtacagc	3	728
	4	276			

The exon-intron boundaries were identified according to the Blastn results of the DNA sequence of *C14orf5* against AL161668. All the boundaries are in accordance with GT-AG (in bold) rules. The residues of the exons are capitalized

Fig. 1. Multiple alignment of *C14orf5* with its seven homologs. NP_057224: brain specific protein CGI-38 (*Homo sapiens*); XP_043104: hypothetical protein (*Homo sapiens*); XP_003662: brain-specific protein p25 alpha (*Homo sapiens*); NP_080757: RIKEN cDNA 2700055K07 gene (*Mus musculus*); CAA59741: novel brain-specific protein p25 (*Bos taurus*); AAF49479: CG4893 gene product (*Drosophila melanogaster*); T25598: hypothetical protein C32E.3 (*Caenorhabditis elegans*). Similar residues in at least three fourths of the sequences are shadowed (the residues conserved in all the sequences are indicated by a black background). The asterisks indicate the ATP-binding site of the protein kinases

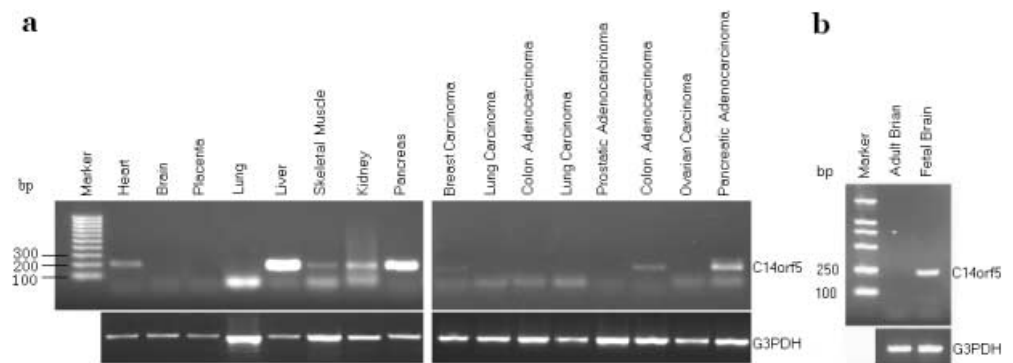
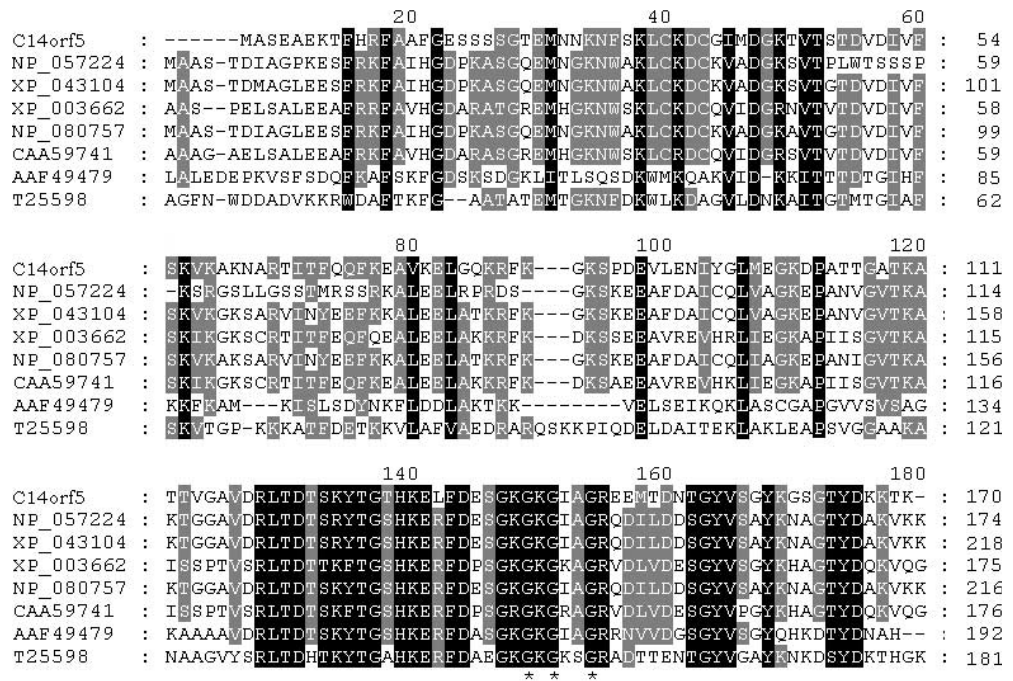


Fig. 2. **a** The expression pattern of *C14orf5* in normal tissues and tumor cell lines. Twenty-five cycles (for *G3PDH*) or 38 cycles (for *C14orf5*) of amplification (94°C, 30s and 68°C, 90s) were performed by using an Advantage 2 Kit (Clontech). The polymerase chain reaction (PCR) products of *C14orf5* and *G3PDH* were then electrophoresed on

a 1.8% agarose gel (FMC, Philadelphia, PA, USA). **b** The distribution of *C14orf5* in fetal and adult brain. The PCR conditions are the same as for **a**. The products of 30 cycles for *G3PDH* and 38 cycles for *C14orf5* of amplification were electrophoresed

40. And among the tumor cell lines, the signal was detected only in breast carcinoma, pancreas adenocarcinoma, and one colon adenocarcinoma (Fig. 2a). In contrast to its homologs, such as p25 alpha, which is ubiquitously expressed (Seki et al. 1999), or bovine p25, which is brain specific

(Takahashi et al. 1991), the gene has a completely different expression pattern. Because the gene is not expressed in adult brain but was isolated from fetal brain, we used reverse transcription (RT)-PCR to examine its expression level in the latter. The PCR product could be detected only

in fetal brain (Fig. 2b), which confirmed that it is expressed in fetal brain but not in adult brain. Further experiment is necessary to determine whether the gene plays a role in brain development.

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