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Rapid Communication

Brain-Specific Expression of Human Microtubule-Associated Protein 1A (MAP1A) Gene and Its Assignment to Human Chromosome 15

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We isolated several cDNA fragments by immunoscreening a human cDNA library with our monoclonal antibody, BG5, that showed neuronal staining on human and rat brain sections. A 1,570 bp sequence of one cDNA fragment showed 75% homology to the rat microtubule-associated protein 1A (MAP1A) cDNA sequence. This rat MAP1A-like human cDNA was highly specific to the adult brain among human tissues tested, and was expressed in various brain regions including white matter. The size of the mRNA detected with Northern blot analysis in adult human brain equaled 10 kb. The gene of this cDNA was assigned to human chromosome 15 that has a syntenic region of mouse chromosome 2, where the mouse MAP1A gene has been assigned. These results indicate that this rat MAP1A-like cDNA is a portion of human MAP1A and is a conserved molecular species among humans and rodents. © 1995 Wiley-Liss, Inc.*

Key words: microtubule-associated protein, molecular cloning, gene mapping, Northern blot, brain

INTRODUCTION

Studies on the molecular mechanisms of Alzheimer disease (AD) have shown that AD occurs by multifactorial causes that may include a mutation of amyloid precursor protein (APP) (Goate et al., 1991), allele ε4 of ApoE protein (Corder et al., 1993), abnormally phosphorylated tau protein (Goedert et al., 1991), and decrease of the levels of functional molecules such as cytochrome oxidase (Chandrasekaran et al., 1994). Yet it is still necessary to isolate several other molecules and antigens to fully understand the molecular mechanism underlying the pathogenesis of AD because at least three responsible genes for familial AD cases have not been isolated (e.g., gene on chromosome 19 for the late onset familial AD; Farrer and Stice, 1993; gene on chromosome 14 for the early onset familial AD; Schellenberg et al., 1992).

In an attempt to identify unique epitopes for AD (Fukuyama et al., 1994a; Fukuyama and Rapoport, 1993), we have isolated various monoclonal antibodies raised against selected brain regions that are susceptible to AD (Rapoport, 1990). One antibody, which showed immunoreactivity to neurons of rat and human brain, was used to isolate corresponding cDNA from a human λgt11 cDNA library. Here we report the partial sequence of clones thus isolated, the brain-specific expression of the mRNA, and the chromosomal assignment of the gene.

MATERIALS AND METHODS

Isolation of Immunoreactive Clones

A λgt11 cDNA library (human amygdala, Clontech, Palo Alto, CA) was screened by a monoclonal antibody BG5 that was obtained in a previous experiment (Fukuyama et al., 1994a) as described by Young and Davis (1983) with minor modification. After tertiary screening of immunoreactive plaques, we obtained two λgt11 clones, BG5i-2 and BG5i-4. Inserted cDNA sizes were determined by polymerase chain reaction (PCR) using λgt11-forward and reverse primers (Clontech). Clones BG5i-2 and BG5i-4 showed a similar sequence in the initial sequencing experiment, and thus were assumed to encode the same protein. The shorter BG5i-2 cDNA was subsequently used for Northern blot analysis

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and gene assignment, whereas the larger BG5i-4 cDNA was sequenced.

Sequencing of cDNA Fragments

The PCR-amplified BG5i-4 cDNA fragment was sequenced using an automated sequencer (DNA Technology, Bethesda, MD). Sequence primers were designed to cover entire portions of the BG5i-4 clone. The BG5i-2 cDNA fragment also was partially sequenced by a cycle sequencing procedure combined with chemiluminescent detection (Circum Vent Phototope Kit, New England Biolabs, Beverly, MA). The sequence data were aligned by an assembly program on a Macintosh computer (AssemblyLIGN, IBI, New Haven, CT). The homologous sequence to that of our cDNA was searched in GenBank (release 83.0) and EMBL (release 38.0) data bases, using appropriate software (Convex, Genetic Computer Group, Madison, WI) on a Macintosh computer.

Northern Blot Analysis

Northern blot filters charged by mRNAs isolated from several different tissues and several brain parts of human were purchased from Clontech. After hybridizing BG5i-2 cDNA under highly stringent conditions using premade Hybrisol I (Oncor, Gaithersburg, MD), filters were washed at 52°C and exposed to X-ray films (Eastman Kodak Company, Rochester, NY) as described (Fukuyama et al., 1994b). Filters were subsequently hybridized with human β-actin cDNA probe (Clontech).

Gene Assignment

A genomic Southern blot filter of the human-rodent monochromosomal hybrid panel was purchased from Oncor. The membrane was charged by 24 kinds of DNAs as well as by host DNAs of human, mouse, and hamster after they were digested with *Pst*I restriction enzyme (Oncor). This panel was hybridized and washed under the same stringent conditions as used with the Northern blot analysis (see above).

RESULTS

Inserted cDNA sizes determined by the PCR technique were 1.5 kb for BG5i-4 clone and 0.8 kb for the BG5i-2 clone, respectively (data not shown). Sequence data of the 1.5 kb BG5i-4 clone and its sequence-data base search showed marked similarity only to the sequence of rat microtubule-associated protein 1A (MAP1A) (GenBank-accession number M83196) (Fig. 1A). Alignment of the sequence to rat MAP1A cDNA showed a similar profile (75% homology) with a relatively divergent region from position at 1 to 400 (56% homology). The sequence from position 400 to the end

A

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   1 ggaattccgg gaacagaagg agaagatccc agaagagaaa gacaaagcct tagatcaaaa
  61 agtcagaagt gttgaacata aggctccgga ggacacggtc gctgaaatga aggacagaga
 121 cctagaacag acagacaaag cccctgaaca gaaacaccag gcccaggaac aaaaggataa
 181 agtctcagaa aagaaggatc aggccttaga acaaaaatac tgggctttgg gacagaagga
 241 tgaagccctg gaacaaaaca ttcaggctct ggaagagaac caccaaactc aggagcagga
 301 gagcctagtg caggaggata aaaccaggaa accaaagatg ctagaggaaa aattccccag
 361 aaaaggtcaa ggccatggaa gagaagttag aagctcttct ggagaagacc aaagctctgg
 421 gcctggaaga gagcctagtg caggagggca gggccagaga gcaggaagaa aagtactgga
 481 gggggcagga tgtggtccag gagtggcaag aaacatctcc taccagagag gagccggctg
 541 gagaacagaa agagcttgcc ccggcatggg aggacacatc tcctgagcag gacaatcggt
 601 attggagggg cagagaggat gtggccttgg aacaggacac atactggagg gagctaagct
 661 gtgagcggaa ggtctggttc cctcacgagc tggatggcca gggggcccgc ccacactaca
 721 ctgaggaacg ggaaagcact ttcctagatg agggcccaga tgatgagcaa gaagtacccc
 781 tgcgggaaca cgcaacccgg agcccctggg cctcagactt caaggatttc caggaatcct
 841 caccacagaa ggggctagag gtggagcgct ggcttgctga atcaccagtt gggttgccac
 901 cagaggaaga ggacaaactg acccgctctc cctttgagat catctcccct ccagcttccc
 961 cacctgagat ggttggacaa agggttcctt cagccccagg acaagagagt cctatcccag
1021 accctaagct catgccacac atgaagaatg aacccactac tccctcatgg ctggctgaca
1081 tcccaccctg ggtgcccaag gacagacccc tccccctgc accccctctcc ccagctcctg
1141 gtcccccac acctgccccg gcatcccata ctcctgcacc cttctcttgg gcgcacagtc
1201 gagtatgaca gtgtggtggc tgcagtgcag gagggggcag ctgagttgga aggtgggcca
1261 tactcccccc tggggaagga ctaccgcaag gctgaagggg aaagggaaga agaaggtagg
1321 gctgaggctc ctgacaaaag ctcacacagc tcaaaggtac cagaggccag caaaagccat
1381 gccaccacgg agcctgagca gactgagccg gagcagagag agcccacacc ctatcctgat
1441 gagagaagct ttcagtatgc agacatctat gagcagatga tgcttactgg gcttggccct
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1561 tccaagccgg aattc
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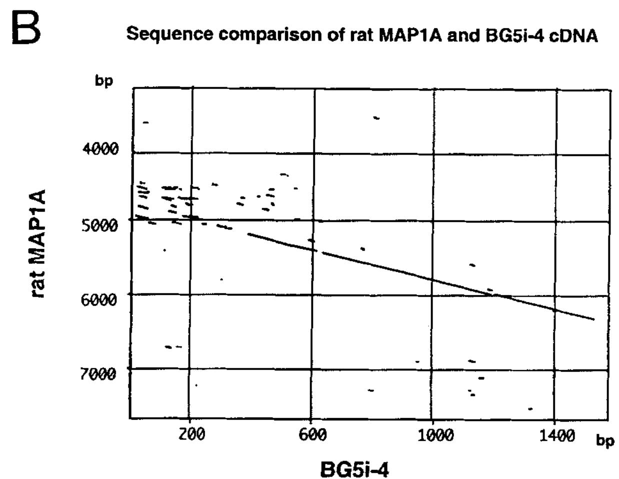

Fig. 1. Sequence data (**A**) and sequence comparison of BG5i-4 clone to corresponding rat MAP1A cDNA (**B**). Conditions for the matrix comparison between them are as follows: window size = 30 bp, minimum % score = 65%, and hash value = 6.

was very similar (82% homology) (Fig. 1B). The sequence of the BG5i-2 clone corresponded to a 295–1,017 portion of BG5i-4 cDNA (data not shown).

Northern blot analysis of mRNAs from various human tissues, using the BG5i-2 clone, showed that this clone was expressed almost exclusively in brain (Fig. 2, BG5i-2, left column). The mRNA size of the hybridized signal was about 10 kb. A minor level of expression of this 10 kb-message was detected in heart and skeletal muscle. The 10 kb-message was expressed in each brain region that was examined (Fig. 2, BG5i-2, right column). Actin cDNA was used to show the level of electrophoresed RNA (Fig. 2, β-actin).

Gene assignment utilizing a human-rodent monochromosomal hybrid-panel whose DNAs were digested first with *Pst*I showed that human-specific 3.6 kb hybridization signal only on hybrid DNA containing human chromosome 15 (15*, Fig. 3).

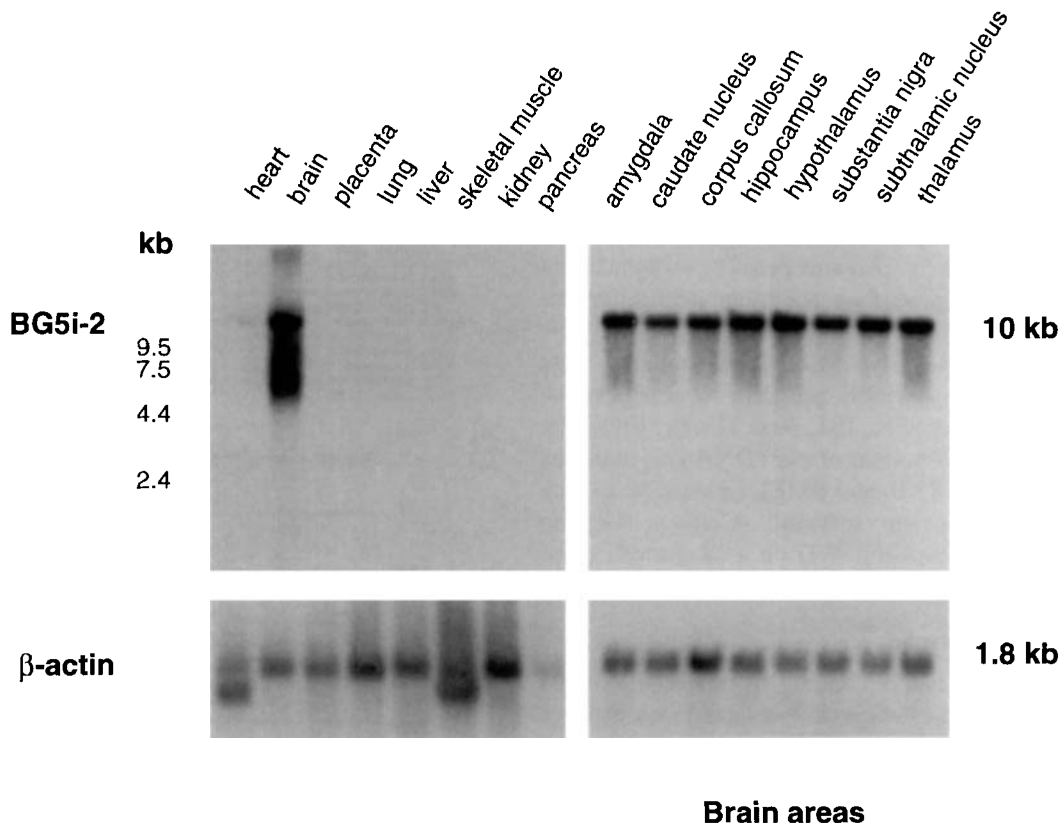

Fig. 2. Northern blot analysis of human BG5i-2 gene expression (**top**). RNAs from various human tissues (**left**) and from various regions of human brain (**right**) were hybridized with a cDNA clone BG5i-2. Same filters then were hybridized with human β-actin cDNA (**bottom**). Sizes (kb) of mRNAs detected with two probes are noted on right. Molecular sizes (kb) of RNA-standards are noted on left.

DISCUSSION

Sequence comparison to the data base showed strong homology of our cDNAs only to rat microtubule-associated protein 1A (MAP1A) cDNA. The mRNA size detected by our cDNA was about 10 kb and was the same size as rat MAP1A mRNA (Langkopf et al., 1992). MAP1A has been known to be the major MAP in brains of adult rats and hamsters (Garner et al., 1990; Langkopf et al., 1992), which is also specific to the brain compared with other tissues of these species (Langkopf et al., 1992; Lewis et al., 1986). Northern blot analysis using BG5 clones agreed with this evidence regarding brain specificity and mRNA size. Because of these results, and of our chromosomal assignment data (see below), we concluded that our cDNA is most likely a portion of human MAP1A.

The sequences of our two cDNA fragments corresponded to the sequence of the central portion of rat MAP1A cDNA. It is known that an amino terminal portion of MAP1A and MAP1B is the binding domain to microtubules, and that a carboxyl terminal portion includes the light chains, LC1 and LC2 (Langkopf et al., 1992). Sequences of MAP1A and MAP1B genes in these portions are well conserved, whereas the central portion between them is not (Garner et al., 1990; Langkopf et al., 1992). This evidence suggests that the central portion of MAP1A also is antigenically diverged and therefore could encode a species-specific epitope that was recognized with our monoclonal antibody in the screening process of cDNAs. Indeed, our cDNAs include the 5′-portion which is divergent from the corresponding sequence of rat MAP1A (56% homology for 400 bp at 5′-portion and 82% for the last, 1,170-bp segment). Thus, it is of interest to map BG5-epitope within this central portion of MAP1A. However, entire sequence comparison of human MAP1A gene to that of rodent MAP1A is necessary to confirm this hypothesis.

In the brain samples tested, levels of mRNA of human MAP1A were about the same. A substantial hybridization signal of human MAP1A also was detected in white matter sample (corpus callosum). Thus, it is likely that MAP1A is expressed in glial cells as well as in

ASSIGNMENT OF HUMAN MAP1A GENE TO CHROMOSOME 15

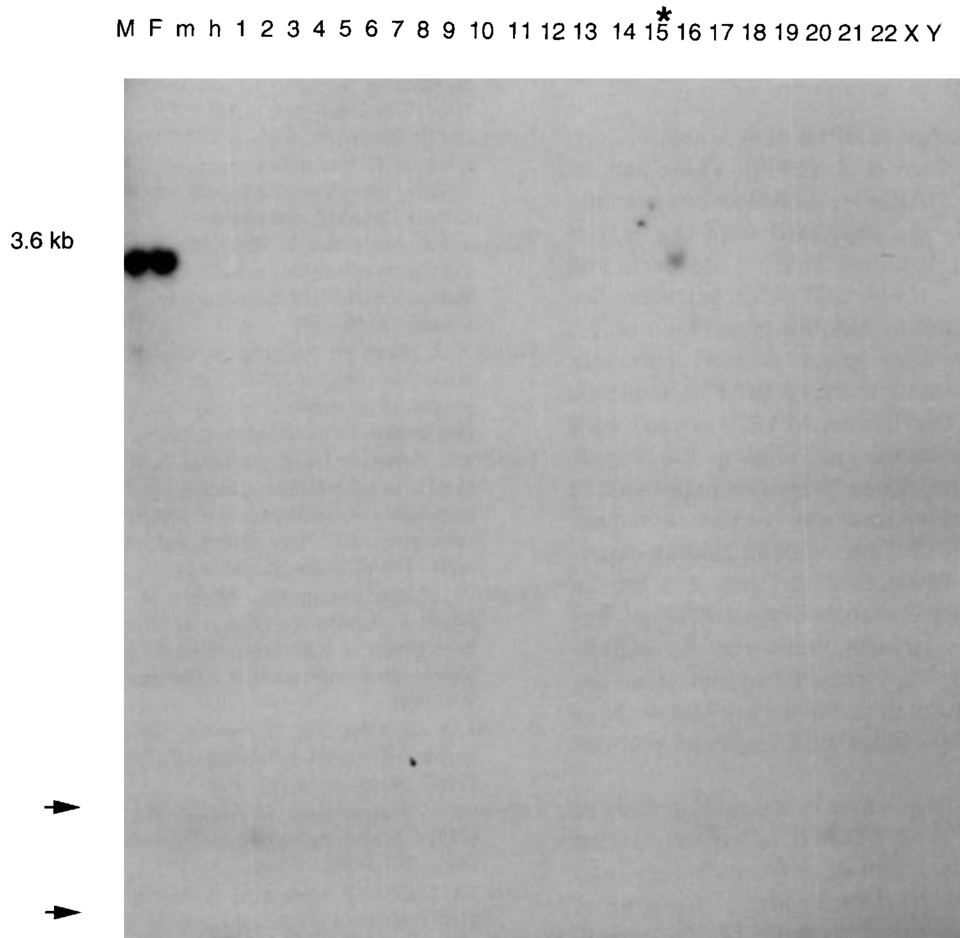

Fig. 3. Assignment of the gene of BG5i-2 cDNA clone to human chromosome 15. Control DNAs of human (M, male; F, female), mouse (m), and hamster (h), as well as DNAs from hybrid cells which have single human chromosomes (1 through Y), are electrophoresed after they are digested with *Pst*I. Human chromosomes 1, 2, 16, 17, 20 and 21 were maintained in human-mouse hybrid cells, whereas the other human chromosomes were held in human-hamster (Oncor). The filter of the hybrid panel was hybridized with a shorter sized clone, BG5i-2. A 3.6 kb human specific *Pst*I band was found only in the hybrid line containing human chromosome 15 (*). Arrows indicate weak rodent signals that cross-hybridized with the cDNA clone.

neurons. Indeed, its mRNA has been detected in oligodendroglia in the rat brain (Tucker et al., 1989).

MAP1B is the major isoform of MAP1 in the embryonic brain. Its expression decreases dramatically during development, while expression of MAP1A increases (Garner et al., 1989, 1990; Oblinger et al., 1994; Safaei and Fischer, 1989; Schoenfeld et al., 1989). Biochemical properties of MAP1A are homologous to those of MAP1B. However, genes for MAP1A and MAP1B are distinct in rodents (Garner et al., 1990; Langkopf et al., 1992). Recently, human MAP1B gene was assigned to human chromosome 5 (Lien et al., 1991). Therefore, assignment of our gene to human chromosome was of interest. We could have assigned the gene of our BG5i-2 cDNA clone to human chromosome 15 by our hybridization experiment using a human-rodent hybrid panel. Therefore, we suggest that MAP1A and MAP1B genes likely are distinct in humans as well as in rodents.

Interestingly, human chromosome 15 is syntenic to a region of mouse chromosome 2 (O'Brien and Marshall-Graves, 1991), where mouse MAP1A gene is located. Human MAP1B gene was assigned to human chromosome 5q13 (Lien et al., 1991), which again includes the mouse syntenic region of chromosome 13 (O'Brien and Marshall-Graves, 1991), on which mouse MAP1B gene was assigned (Garner et al., 1990). These chromosomal

assignments, together with sequence conservation of MAP1A and MAP1B among humans and rodents, suggest that MAP1A and MAP1B are well conserved molecular species that evolved from a common ancestral gene.

The complete human MAP1B cDNA and its gene have been isolated by Lien et al. (1994). These authors also reported a human MAP1B-related gene by searching sequence homology in the expressed sequence tagged library (EST database) with their MAP1B sequence, and found that EST 01683, 01974, and 00368 genes are homologous to some extent to MAP1B gene (Lien et al., 1994). A further homology search showed that these clones have strong similarity to the rat MAP1A sequence (Lien et al., 1994). The human MAP1B-related EST clone was assigned to human chromosome 15q13-qter. Although the authors mentioned its specific expression in the adult brain, they did not show any Northern blot data. Nevertheless, their data obtained without cloning experiments suggested that MAP1B-related gene is a human counterpart of rat MAP1A, namely human MAP1A. Because chromosomal assignment, brain specific expression, and the size of mRNA detected by our clone are very similar to their information, we believe that we have confirmed their MAP1B-related EST clones as portions of MAP1A gene.

MAPs have been implicated in the pathogenesis of AD (Goedert et al., 1991). MAP1B (alternately called MAP5) was shown to be involved in AD pathology, and its expression is altered in AD brain (e.g., Takahashi et al., 1991). However, up to the present, MAP1A expression in AD brain has not been reported. We currently are analyzing the levels of protein and mRNA of MAP1A in control and AD brains, using Western blot and Northern blot techniques.

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