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Cloning, Sequence, and Developmental Expression Analysis of C4-2, a Potential Brain Tumor-Suppressor Gene

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Background: Previously, we reported the isolation of C4-2 as a potential tumor suppressor gene in human brain tumors. To understand the function of this gene, we investigated its molecular characterization and expression during development.

Methods: Human fetal brain library screening and 5′RACE-PCR method was used to isolate the full-length cDNA. The coding region of C4-2 was used for in situ hybridization to study its expression during development.

Results: We report here the complete sequence of this gene. Sequence analysis indicated that C4-2 has a 94% sequence identity to a family of cAMP-regulated phosphoproteins (ARPP-16/19) in the coding region. C4-2 has a 3.1 Kb long 3′UTR with variable identity to ARPP-16 and ARPP-19. Northern blot analysis indicated that C4-2 is expressed at high levels in normal brain compared to other tissues. Zoo blot analysis demonstrated that the coding region of C4-2 is highly conserved among different animals. In situ hybridization using C4-2 coding region demonstrated that it follows a unique expression pattern during mouse brain development. High level of C4-2 expression was also observed in the spinal cord and somites of the developing embryo.

Conclusion: Expression analysis during brain development strongly suggests that this family of proteins may play an important role not only in normal functioning of the brain, but also during brain development.

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KEY WORDS: **brain; tumor suppressor gene; phosphoprotein; cAMP; development**

INTRODUCTION

During 1996, brain tumors were the cause of death of several thousand people in the United States [1], and the number is increasing. Brain tumors affect young children as well as adults [1]. Glioblastoma multiforme (GM) is

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among the most malignant of brain tumors [2]. Despite extensive progress in research and current treatments available, the average life span after being diagnosed with GM is ~1–2 years. Thus it is important to develop techniques by which brain tumors can be diagnosed at an early stage for effective treatment. To do so, it is important to understand the cellular and molecular events that take place during brain tumor initiation and progression.

The series of events that transform normal cells into tumor cells are not precisely known. It has been suggested that either loss of tumor suppressor genes, overexpression of oncogenes, or a combination of both may be involved in cell transformation [3]. The effect of either tumor suppressor genes or oncogenes is evident at the chromosomal level or at the gene expression level. It has been demonstrated that events such as loss or mutations of tumor suppressor genes (*p53, p15, p16, RB-1, DCC*), or overexpression of oncogenes (*met, EGFR, CDK4, myc, SAS*) may play an important role in the initiation and progression of brain tumors [4–6]. Despite tremendous progress, the exact series of events that lead to initiation and progression of malignant brain tumors are not known at present. Thus, isolation of new candidate genes and defining their role in the complex pathways of brain tumorigenesis would be very useful in developing protocols for early brain tumor detection and perhaps therapy.

Differential display-polymerase chain reaction (DD-PCR) is a modified PCR technique first developed in 1992 and recently modified and improved to increase PCR specificity and efficiency [7,8]. DD-PCR has been utilized to isolate differentially expressed genes in a number of different applications and in several different kinds of tumors [7,9–12].

We have reported previously the isolation of a potential tumor suppressor gene, C4-2, using the technique of DD-PCR [13]. C4-2 is expressed in normal brain tissue (NBT) at high levels as compared to GMT. Low levels of C4-2 expression were observed in a wide variety of tumor types studied. Using the technique DD-PCR, we were able to clone only 250 bases of C4-2. Sequence identity analysis indicated that C4-2 had a 66% sequence identity to a family of cAMP regulated phosphoproteins, ARPP-16/19 [14,15]. We report here the cloning, sequence, and expression analysis of a unique transcript (C4-2, 3.32 Kb) for the ARPP-16/19 family of proteins.

MATERIALS AND METHODS

Library Screening

A normal human fetal brain library was purchased from Stratagene (San Diego, CA). A previously isolated 250 base pair (bp) fragment for C4-2 was labelled using the mega prime labelling kit from Amersham (Arlington Heights, IL), and 1×10^6 independent clones were screened using the protocol recommended by Stratagene. Several positive clones were isolated and insert size determined. One of the positive clones had a 2.4 Kb insert and was selected for characterization. This clone was completely sequenced by Sequetech (Mountain View, CA). On the basis of sequence identity analysis (BLAST program of the National Center for Biotechnology Information (NCBI), NIH, Bethesda, MD) of this clone, we have made a 3′ primer (5′agtgaattcaacagttaatgcacatg3′) that was close to the 5′ end of this 2.4 Kb insert. Using this primer of the C4-2 clone, we performed a 5′RACE-PCR (rapid amplification of complementary DNA end-polymerase chain reaction) protocol using the 5′RACE kit from Gibco/BRL (Baltimore, MD). We also designed a 5′ primer (5′gcagacaagcttatggaagataaagtgactagtccagag3′) against the translational initiation site of the previously published bovine ARPP-16 sequence [14]. The first round of PCR was done using the primers mentioned above. For a second round nested PCR, we used a C4-2 primer (5′gcaaaactactgtatttacaaaaaatggcac3′) that was upstream of the primer used in the first round. A 0.9 Kb product was isolated as a result of the 5′RACE-PCR method and was then cloned into the pCRII vector from Invitrogen (Carslbad, CA).

Northern Blot Analysis

To study the expression of the C4-2 (3.32 Kb) transcript in human tissues, a multiple tissue Northern blot membrane containing 2 μg of polyA+ pure RNA in each lane was purchased from Clonetech (Palo Alto, CA). The 2.4 Kb fragment for C4-2 isolated from the library was used as a probe for hybridization using the method recommended by Clonetech. After using C4-2 probe, the membrane was treated with 0.1% SDS (sodium dodecyl sulfate) solution at 94°C for 30 minutes. Human β *actin* (a housekeeping gene) cDNA fragment, provided by Clonetech, was then used as a probe to hybridize the membrane. Quantitation of Northern blot was performed using the ImageQuaNT™ volume quantitation program from the Molecular Dynamics Phosphor Imager (Sunnyvale, CA), which calculates the volume under the surface created by a 3D plot of pixel locations and pixel values. We quantitated the volume (the integrated intensity in all the pixels in the spot excluding the background) of C4-2 bands in Northern blot. These pixel values were then normalized with pixel values in the bands of a housekeeping gene β *actin* and are referred to as relative expression in Figure 3.

Zoo Blot Analysis

A zoo blot membrane containing 5 μg of predigested genomic DNA was purchased from Clonetech. The zoo blot was prehybridized according to the method recommended by Clonetech. First, the coding region of C4-2 was analyzed. The coding region of C4-2 was PCR amplified using gene specific primers (5′gcagacaagcttatg-

gaagataaagtgactagtccagag 3′ and 5′ cgtctatctagatcagccagccagtttgctagcaacaagaga 3′).

The identity of the PCR product was confirmed using sequencing and Southern blot analysis. The PCR amplified fragment was then labelled with dATP^{32} (3,000 Ci/mmole) using the megaprime labelling kit from Amersham. The C4-2 coding region probe was then hybridized for 18 hours under the conditions recommended by Clonetech. After exposing the membrane to the autoradiogram, the probe was removed by incubating the membrane in 0.1% SDS at 90°C for 30 minutes. To analyze if the 3′UTR (un-translated region) of the C4-2 is conserved, the last 500 bases of the C4-2 were amplified using C4-2 specific primers (5′caagctcgaaattaaaccctcac-

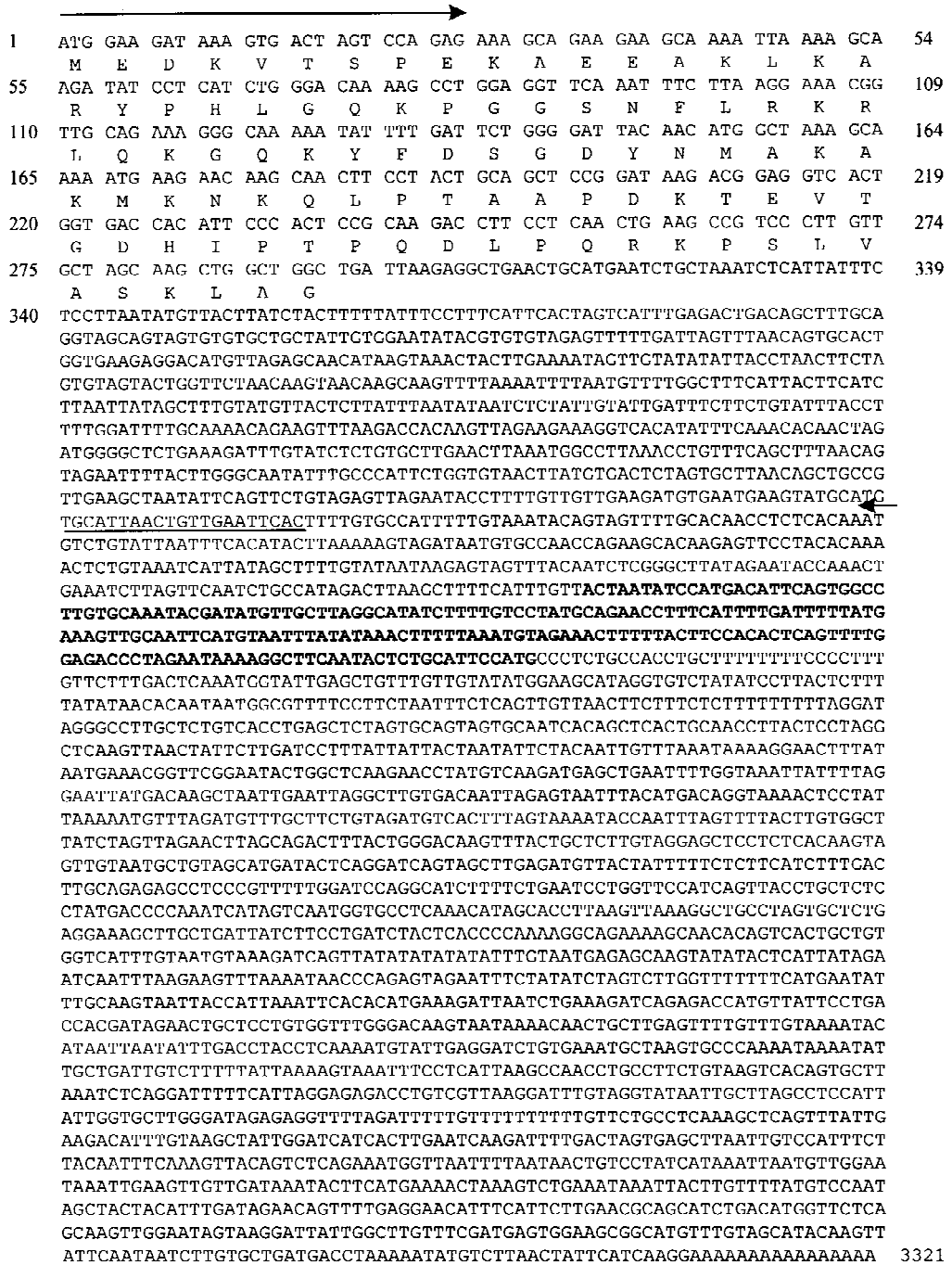

Fig. 1. Sequence of clone C4-2 (3.32 Kb). The 250 bp sequence indicated in bold letters is the original clone isolate by DD-PCR. C4-2 (3.32 Kb) was isolated from a human fetal brain library, and 5′RACE-PCR was done using the primers indicated by arrows. Deduced amino acids are shown by single letter.

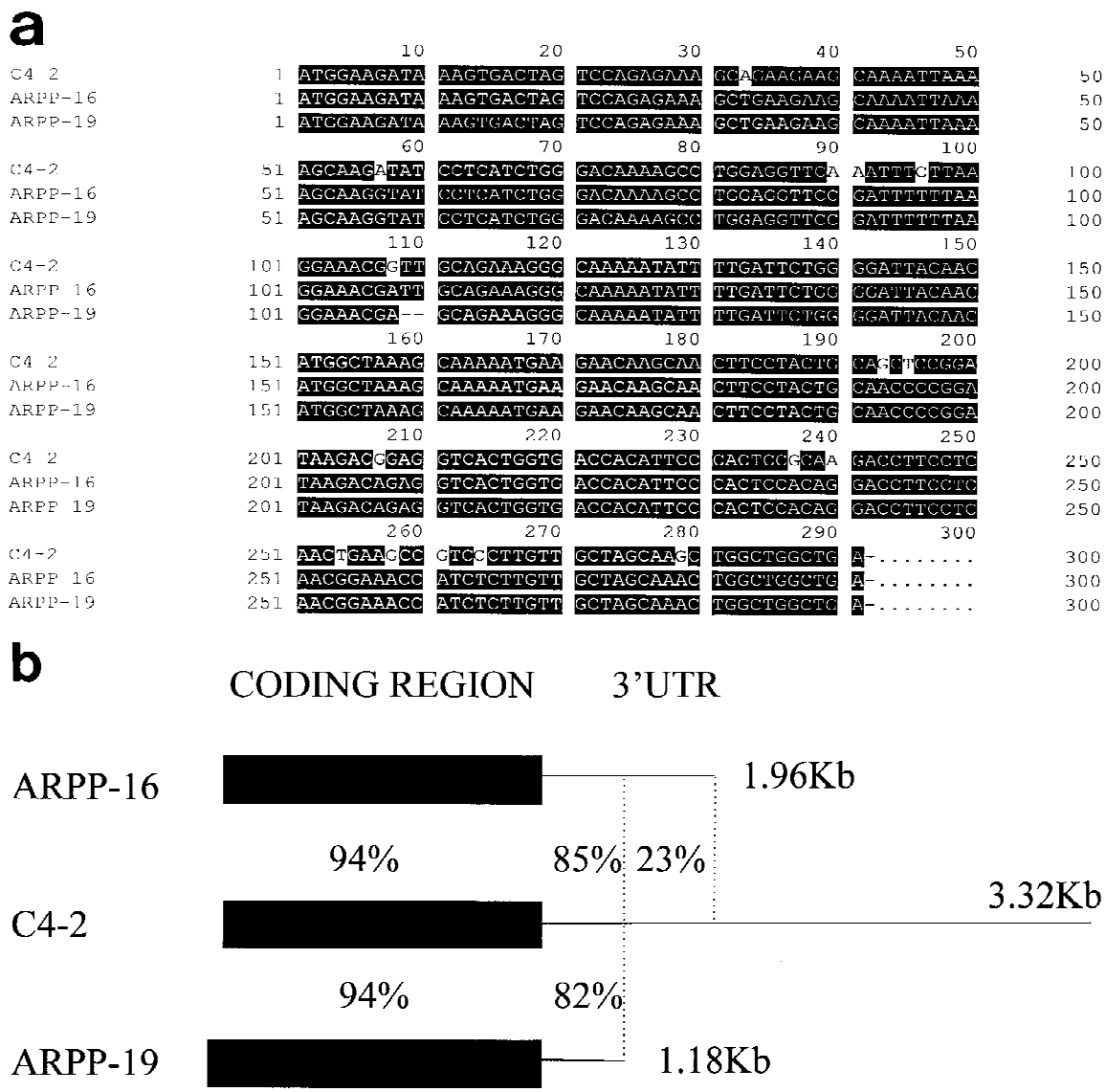

Fig. 2. Sequence identity analysis of C4-2 (3.32 Kb) with bovine ARPP-16/19 genes. Panel (**a**) shows sequence identity of the coding region of C4-2 (3.32 Kb), ARPP-16, and ARPP-19. Black boxes show regions of identity between sequences. Panel (**b**) shows a summary of sequence identity analysis between C4-2 (3.32 Kb), ARPP-16 and ARPP-19 sequences.

taaaggggtaagtcacagtgcttaaatcctca 3′ and 5′cacttaactaatacgactcactatagggttgtatgctacaaacatgccgctt 3′). The PCR product was then gel purified and labelled as noted above. Hybridization and washing of the membrane was carried out as recommended by Clonetech.

In Situ Hybridization

In situ hybridization was performed as described previously [16]. Briefly, 6 μm, 4% paraformaldehyde-fixed, paraffin-embedded mouse embryo sections from Novagen (Madison, WI) were deparaffinized by two washes in xylene, followed by rehydration through graded concentrations of ethanol from 100% to 70%. They were then washed in PBS (phosphate-buffered saline) and treated with proteinase K (25 mg/ml for 10 min), followed by fixation in 4% paraformaldehyde. After incubation in 0.25% acetic anhydride/0.1 M TEA (tri ethanol amine), sections were dehydrated through graded concentrations of ethanol from 70% to 100% and prehybridized for 2 hours at 55°C in 50% formamide, 5 × SSC (sodium chloride, sodium citrate) at pH 4.5, 50 μg/ml tRNA, 50 μg/ml heparin, and 1% SDS.

Sections were hybridized with 1 μg/ml DIG labeled antisense or sense probes for 18 hours at 55°C. Probes were synthesized with the Genius 4 kit from Boehringer Mannheim (Indianapolis, IN) using the T3 and T7 promoters of a PCR template-derived from cDNA cor-

responding to bases 112–402. The PCR template was amplified using primers 5′caagctcgaaattaaccctcactagaagggatggaagataaagtgactagtccagag 3′ and 5′cactaactaatacgactcactatagggtcagccagccagtttgctagcaacaagaga 3′, which contain the T3 and T7 promoters added to sequences corresponding to bases 112–138 and 373–402 of ARPP-16. Following hybridization, slides were washed in 50% formamide, 2 × SSC at pH 4.5, 1% SDS at 50°C, treated with 5 μg/ml RNase A for 30 min at 37°C, and washed in 50% formamide, 2 × SSC pH 4.5 at 50°C. Sections were preblocked in 10% normal sheep serum (Sigma, St. Louis, MO) and incubated with a 1:2,000 dilution of alkaline phosphate conjugated antidioxigenin Fab fragments (Boehringer Mannheim) for 18 hours at 4°C. For detection, slides were incubated with NBT (Nitroblue Tetrazolium Chloride/BCIP (5-bromo-4-chloro-3-indilyl-phosphate, 4-toluidine salt) in the dark for 28 hours. After counterstaining with eosin Y, slides were mounted with Permount and visualized using an Axioskop (Carl Zeiss, Thornwood, NY) routine microscope.

RESULTS

Cloning of Human C4-2 (3.32 Kb) Transcript

The previously described C4-2 clone (0.25 Kb) was used for screening a human fetal brain library (Stratagene) for isolation of a full-length cDNA. The result of this screening was the isolation of a 2.4 Kb cDNA fragment. Sequence analysis of this clone showed that it has strong sequence identity to the 3′ end of both ARPP-16 and ARPP-19 genes. To investigate if C4-2 contained coding regions like ARPP-16 or ARPP-19, a specific primer to the 5′ end of the 2.4 Kb fragment of C4-2 was used for carrying out 5′RACE-PCR. We also made an additional primer that was specific to the translational start site of the ARPP-16 gene to carry out PCR from the 5′ RACE-PCR product (Fig. 1). The result of the 5′RACE-PCR was a 0.9 Kb fragment with overlapping sequence to the 2.4 Kb cDNA. We completely sequenced the 2.4 Kb and 0.9 Kb fragments and deduced a 3.32 Kb cDNA sequence (Fig. 1). This clone is referred to as C4-2 (3.32 Kb) in the text. Next, we performed sequence identity analysis of both the coding region and the 3′UTR of the C4-2 (3.32 Kb). The coding region of C4-2 (3.32 Kb) is 94% identical to bovine ARPP-16 (Accession# M33617) and ARPP-19 (Accession# M33618). The major characteristic of C4-2 (3.32 Kb) is the presence of a very long 3′UTR (3.1 Kb). We next performed sequence identity analysis between bovine ARPP-16, ARPP-19 and human C4-2 (3.3 Kb) 3′UTR (Fig. 2). ARPP-16 had 85% and ARPP-19 (complete 3′UTR) had 82% identity to the C4-2 (3.32 Kb). The remaining 581 bases of the ARPP-16 have a 23% identity to clone C4-2 (3.32 Kb). The C4-2 (3.32 Kb) clone has an additional 1977 bases of 3′UTR with no identity to ARPP-16 or ARPP-19.

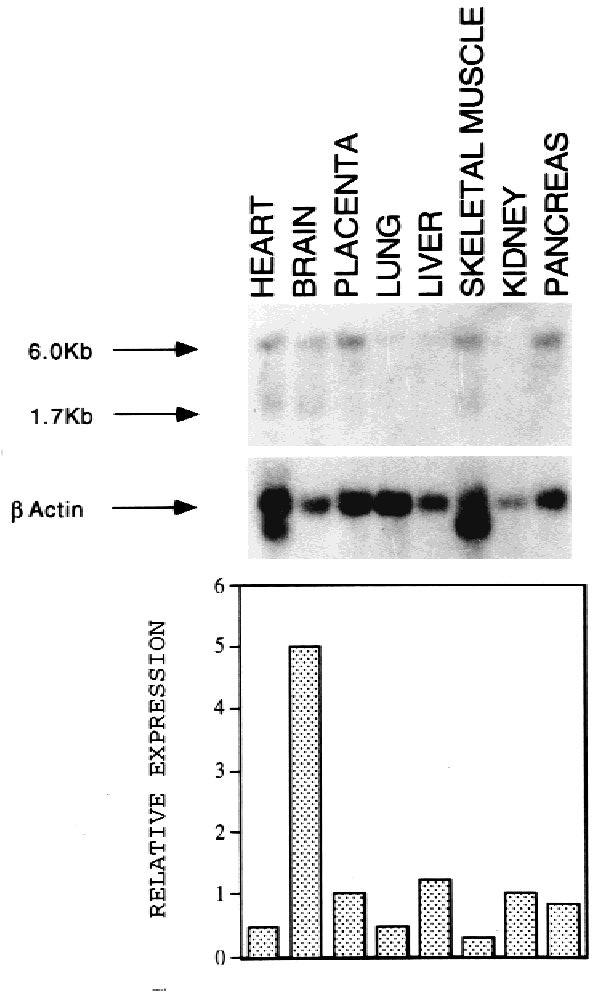

Fig. 3. Expression of C4-2 in normal human tissues. Shown is an autoradiogram of a Northern blot of C4-2 expression (**upper panel**), and of β *actin* expression (**middle panel**), which serves as an internal control for gel loading in various normal human tissues. The bar graph is the relative expression of C4-2 after correction for gel loading based on β actin expression. C4-2 expression in normal tissues is highest in the normal brain. The 6.0 and 1.7 Kb transcripts of C4-2 are indicated by arrows.

Expression of C4-2 (3.32 Kb) in Normal Human Tissues

It has been reported previously by Northern blot analysis of human brain RNA that the ARPP-16/19 probe resulted in two potential transcripts of 1.7 Kb and 6.0 Kb [14,15]. The 1.7 Kb transcript encodes for the ARPP-16 protein, but the 1.18 Kb transcript that may encode for the ARPP-19 has not been observed on the Northern blot. We performed Northern blot analysis on 2 μg polyA+ RNA isolated from eight different tissues. As shown in Figure 3, we observed the 6.0 Kb transcript expressed in brain as compared to other tissues. A low level of the 1.7 Kb transcript was observed, and this may be due to cross reactivity of the probe used.

Zoo Blot Analysis of Coding and Noncoding Regions of C4-2 (3.32 Kb) Transcript

We were next interested in understanding the role of C4-2 during mouse brain development. To do so, we first wanted to determine if the coding and 3′UTR region of the human C4-2 is conserved. To answer this question, we performed zoo blot analysis using coding region and 3′UTR of C4-2. As shown in Figure 4, when the coding region of the C4-2 was used as probe, a strong positive signal was observed in the human, monkey, rat, mouse, dog, cow, rabbit, and chicken, and none with the yeast DNA. After removing the probe, the same blot was used for hybridization with a 500 bp probe generated against the 3′ end of the C4-2 (3.32 Kb) transcript. As shown in Figure 4, a positive signal was observed in DNA from the human, monkey, and cow. A low level of positive signal was observed with rat, rabbit, and chicken DNA. No signal was observed with mouse or dog DNA. This data suggests that C4-2, 3′ UTR is more conserved in higher mammals than rodents.

Expression of C4-2 Coding Region During Mouse Development

To understand the role of C4-2 (3.32 Kb) during brain development, we used the technique of in situ hybridization, which has been used for studying changes in gene expression [17]. On the basis of zoo blot results described in the previous section, the coding region of C4-2 was used as either a sense or antisense probe to study the expression. Mouse embryos fixed in paraformaldehyde and embedded in paraffin from six different developmental stages were used for this analysis. As shown in Figure 5 (panels a–h), an increasing level of C4-2 expression was observed from the day 10 embryo to the day 13 embryo. Expression of C4-2 was uniform in the brain and in some cases confined to specific regions

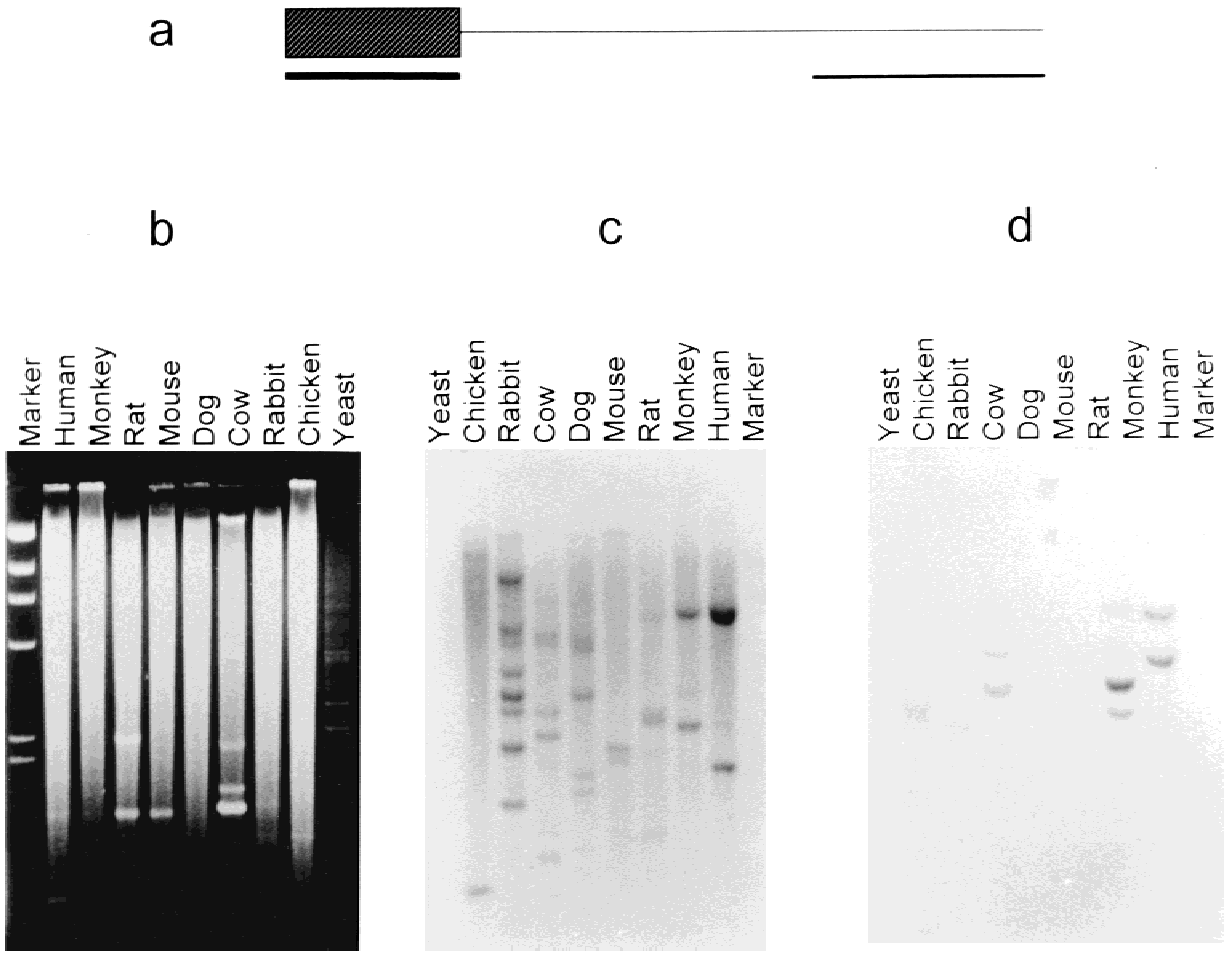

Fig. 4. Zoo blot analysis of C4-2 gene. Panel **(a)** shows the schematic of the C4-2 gene with coding region shown as a hatched box and the noncoding region as a line. The probes used for coding and noncoding regions in the zoo blot are indicated by thick and thin lines respectively. Panel **(b)** shows the ethidium bromide stained gel of the DNA digested with a restriction enzyme (EcoRI). Panels **(c)** and **(d)** show the autoradiogram of the zoo blot hybridized with C4-2 coding region and noncoding regions, respectively. Refer to Materials and Methods for details.

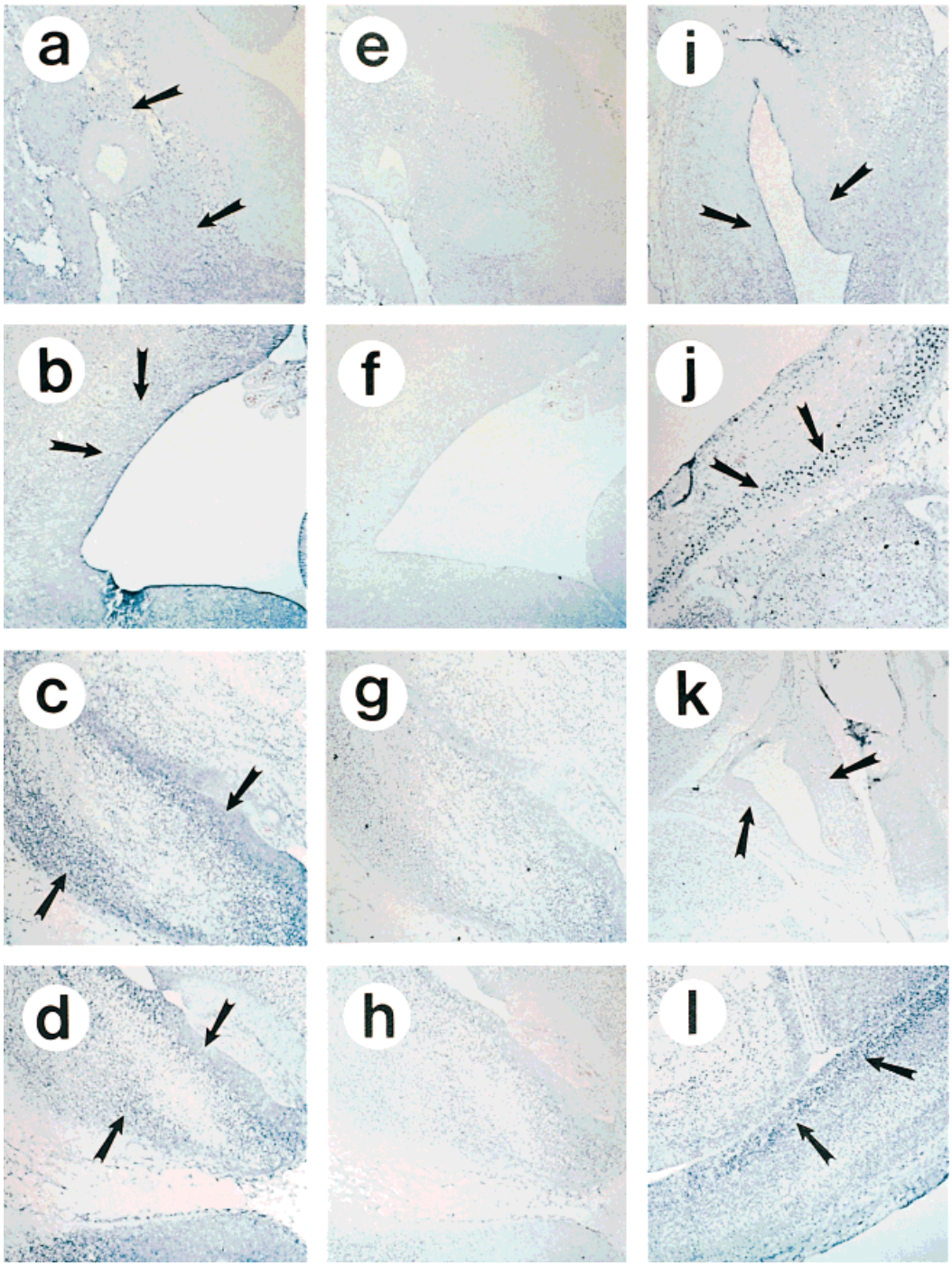

Fig. 5. Expression of C4-2 during mouse brain development. Panels **a, b, c, d, i, j, l,** and **k** show expression in embryos, 10, 12, 13 (fore brain), 13 (hind brain), 14, 15 (hind brain), 15 (fore brain), and 16 days old, using antisense probe. Panels **e, f, g,** and **h** are the serial sections of those used in panels a, b, c, and d, but hybridized with C4-2 sense probe. Arrows indicate specific regions of C4-2 expression. Serial sections corresponding to panels i, j, k, and l were hybridized to C4-2 sense probe, and no signal was observed (data not shown).

(Fig. 5, panels c, d, j, i). Low or no expression of C4-2 was detected when a C4-2 sense probe was used on serial sections (Fig. 5, panels e, f, g, h). Panels i, j, k, and l in Figure 5 show that the expression of C4-2 decreases as the development proceeds with expression confined to specific regions. No signal was observed when a C4-2 sense probe was used on the same developmental stage serial sections (data not shown). Another unique observation was the expression of C4-2 at very high levels in the areas surrounding the spinal cord (Fig. 6). A high

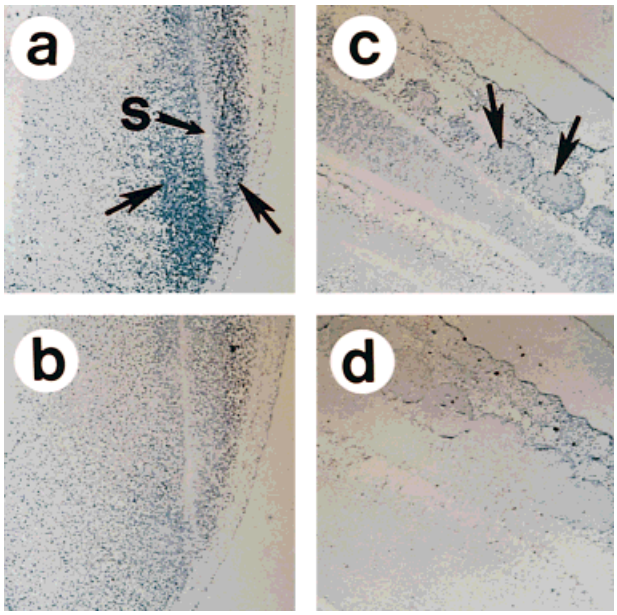

Fig. 6. Expression of C4-2 in developing spinal cord and somites. Panels **(a)** and **(c)** show spinal cord and somite region of 11- and 13-day-old embryos hybridized with C4-2 antisense probes. Panels **(b)** and **(d)** are serial sections of those in (a) and (c), hybridized with C4-2 sense probe. Arrows indicate specific region of expression. Letter S in panel (a) shows the spinal cord.

level of C4-2 expression was observed in developing somites (Fig. 6). No signal was observed on serial sections when the C4-2 sense probe was used (Fig. 6). No expression of C4-2 was detected in the remainder of the embryo (data not shown).

DISCUSSION

To understand the process of cell transformation and tumorigenesis, it is important to understand the cellular and molecular events that underline these processes. Genes that are overexpressed in tumor cells, whose overexpression in normal cells cause transformation are termed ''oncogenes.'' Tumor suppressor genes, however, are genes that control normal cell growth, differentiation, and progression through the cell cycle. Their expression is low in tumor cells as compared to the normal cells, and if they are overexpressed in tumor cells, the cell reverts back to normal. Several oncogenes and tumor suppressor genes have been suggested to play some role in brain tumor initiation and progression, but the exact mode of action is not known at present [4–6]. On the basis of previous studies, it is thought that the series of events that may lead to tumor formation are complex and the number of genes involved may be more than those already isolated and studied [18]. Thus isolation and characterization of new genes would be very useful, not only in understanding the process of tumorigenesis but also for therapy.

The technique of DD-PCR that we utilized to isolate differentially expressed genes, have been used extensively to isolate potential oncogenes and tumor suppressor genes with altered expression [13,19]. Recently DD-PCR technique was used successfully for isolation of tumor suppressor genes from brain tumors [13,20]. We reported previously the isolation of C4-2 using this technique. This gene was shown to be expressed at very low levels, not only in cell lines derived from brain tumors but also in primary breast and prostate tumors. We also demonstrated that C4-2 expression is high in a normal brain as compared to other tissues [13]. We report here the gene organization, full-length cDNA sequence, and developmental expression analysis of C4-2, a potential tumor suppressor gene.

Sequencing of the C4-2 (3.32 Kb) cDNA indicated that it is a novel transcript of the previously cloned family of cAMP-regulated phosphoproteins (ARPP-16 and ARPP-19). The coding region of the C4-2 (3.32 Kb) is almost identical to the ARPP-16, but the untranslated region showed variable homology to both ARPP-16 and ARPP-19. Specifically, the last 1.97 Kb of the C4-2 (3.32 Kb) does not show any homology to ARPP-16/19. On the basis of C4-2 sequencing, we conclude that C4-2 (3.32 Kb) is part of the 6.0 Kb transcript previously observed on the Northern blot. Northern blot analysis demonstrated that the expression of C4-2 is brain specific. Zoo blot analysis DNA from different species using the coding and noncoding regions strongly suggest that the coding region is highly conserved, whereas the noncoding region is more conserved in mammals. This evolutionary conservation of sequence strongly suggests its important functional role. Developmental expression of C4-2 strongly suggests that it may have a role in normal brain development. High level of C4-2 expression around the embryonic spinal cord may validate the previously proposed function of ARPP-16 in neurotransmission [15]. More work is necessary specifically to address the C4-2 function. For example, by developing a mouse lacking the C4-2 gene, we may gain some insight into this gene's normal function. To address the role of C4-2 as a tumor suppressor gene, we have recently transfected several brain tumor cell lines with C4-2 in both sense and antisense direction. A similar approach has been used with the connexin 43 gene to demonstrate its role as a potential tumor suppressor gene [21]. We are currently studying the growth rate in culture and in soft agar of the brain tumor cell lines transfected with C4-2 clone.

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REFERENCES

1. Parker S, Tong T, Bolden S, Phyllis A, Wingo PA: Cancer statistics, 1997. CA Cancer J Clin 1997;47:5–27.
2. Laws ER Jr, Thapar K: Brain tumors [Review]. CA Cancer J Clin 1993;43:263–271.
3. Furnari FB, Huang HJ, Cavence WK: Genetics and malignant progression of human brain tumours [Review]. Cancer Surv 1995; 25:233–275.
4. Mikkelsen T: Recent advances in brain tumor molecular biology [Review]. Curr Opin Oncol 1994;6:229–234.
5. Bögler O, Huang HJ, Kleihues P, Cavenee WK: The p53 gene and its role in human brain tumors [Review]. Glia 1995;15:308–327.
6. Westermark B, Nister M: Molecular genetics of human glioma [Review]. Curr Opin Oncol 1995;7:220–225.
7. Liang P, Pardee AB: Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. [Comment in: Science 1995;267(5201):1186–1187.] Science 1992;257(5072): 967–971.
8. Yeatman TJ, Mao W: Identification of a differentially-expressed message associated with colon cancer liver metastasis using an improved method of differential display. Nucleic Acids Res 1995; 23:4007–4008.
9. Nishio Y, Aiello LP, King GL: Glucose induced genes in bovine aortic smooth muscle cells identified by mRNA differential display. FASEB J 1994;8:103–106.
10. Liang P, Averboukh L, Keyomarsi K, et al: Differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells. Cancer Res 1992;52:6966–6968.
11. Sehgal A, Presente A, Dudus L, Engelhardt JF: Isolation of differentially expressed cDNAs during ferret tracheal development: Application of differential display PCR. Exp Lung Res 1996;22: 419–434.
12. Hadman M, Adam BL, Wright GL Jr, Bos TJ: Modifications to the differential display technique reduce background and increase sensitivity. Anal Chem 1995;226:383–386.
13. Sehgal A, Keener C, Boynton AL, Young RF, et al.: Characterization of C4-2 as a tumor-suppressor gene in human brain tumors. J Surg Oncol 1997;64:102–108.
14. Horiuchi A, Williams KR, Kurihara T, et al.: Purification and cDNA cloning of ARPP-16, a cAMP-regulated phosphoprotein enriched in basal ganglia, and of a related phosphoprotein, ARPP-19. J Biol Chem 1990;265:9476–9484.
15. Brené S, Lindefors N, Ehrlich M, et al.: Expression of mRNAs encoding ARPP-16/19, ARPP-21, and DARPP-32 in human brain tissue. J Neurosci 1994;14:985–998.
16. Wilkinson DG: ''In Situ Hybridization, A Practical Approach.'' New York: Oxford University Press, 1992.
17. Huynh DP, Tran TM, Nechiporuk TT, Pulst SM: Expression of neurofibromatosis 2 transcript and gene product during mouse fetal development. Cell Growth Differ 1996;7:1551–1561.
18. Fields C, Adams MD, White O, et al.: How many genes in the human genome [Review]. Nature Gene 7:345–346, 1994.
19. Sehgal A, Keener C, Boynton AL, et al.: Isolation and characterization of a novel gene from human glioblastoma multiforme tumor tissue. Int J Cancer 1997;71:565–572.
20. Uchiyama CM, Zhu J, Carroll RS, et al.: Differential display of messenger ribonucleic acid: A useful technique for analyzing differential gene expression in human brain tumors [Review]. Neurosurgery 1995;37:464–470.
21. Chen SC, Pelletier DB, Ao P, Boynton AL: Connexin 43 reverses the phenotype of transformed cells and alters their expression of cyclin/cyclin-dependent kinases. Cell Growth Differ 1995;6:681–690.