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Correlation of B-FABP and GFAP expression in malignant glioma

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The murine brain fatty acid binding protein (B-FABP) is encoded by a developmentally regulated gene that is expressed in radial glial cells and immature astrocytes. We have cloned the human *B-FABP* gene and have mapped it to chromosome 6q22-23. We show that B-FABP mRNA is expressed in human malignant glioma tumor biopsies and in a subset of malignant glioma cell lines, as well as in human fetal retina and brain. Malignant glioma tumors are characterized by cytoplasmic bundles of glial fibrillary acidic protein (GFAP), a protein normally expressed in mature astrocytes. Establishment of malignant glioma cell lines often results in loss of GFAP. The subset of malignant glioma cell lines that express GFAP mRNA also express B-FABP mRNA. Co-localization experiments in cell lines indicate that the same cells produce both GFAP and B-FABP. We suggest that some malignant gliomas may be derived from astrocytic precursor cells which can express proteins that are normally produced at different developmental stages in the astrocytic differentiation pathway.

Keywords: malignant glioma; glial fibrillary acidic protein; brain fatty acid binding protein; astrocytes; gene mapping

Introduction

Malignant gliomas are the most common brain tumors. They comprise astrocytoma grades III and IV, also called anaplastic astrocytoma and glioblastoma multiforme. These tumors are usually fatal within 2 years of diagnosis in spite of conventional treatment. Malignant gliomas are believed to arise from astrocytes because they are composed of cells that produce bundles of cytoplasmic glial fibrillary acidic protein (GFAP), an intermediate filament protein specifically expressed in differentiated astrocytes (Eng, 1985; Linskey and Gilbert, 1995). Proliferating neuroepithelial stem cells from which both glial and neuronal cells are derived, and radial glial cells from which astrocytes are derived, express the intermediate filament proteins vimentin and nestin rather than GFAP (Schmechel and Rakic, 1979; Hockfield and McKay, 1985; Frederiksen and McKay, 1988; Lendahl *et al.*, 1990). During the development of the central nervous system (CNS), radial glial cells guide the migration of neurons from the ventricular zone towards the cortex (Edmondson and Hatten, 1987; Hatten, 1990). In the adult CNS, astrocytes provide structural support to neighbouring cells as well as influence the physiological properties of neighbouring neurons by secreting growth factors and regulating neurotransmitter levels (Gilman and Schrier, 1972; Janzer and Raff, 1987). Studies using *GFAP*-null mice indicate no gross developmental abnormalities, suggesting that small amounts of other proteins such as vimentin may compensate for lack of GFAP (Gomi *et al.*, 1995). However, recent studies suggest that GFAP is important for astrocytic-neuronal interactions because *GFAP*-null mice have alterations in long-term potentiation (or long-lasting enhancement of synaptic efficacy) in the hippocampus (McCall *et al.*, 1996).

Most malignant glioma tumors have cells that express GFAP. However, histopathological studies have shown that increasing astrocytic anaplasia correlates with reduced GFAP levels; i.e. the number of GFAP positive cells decreases with increasing malignancy (Eng and Rubinstein, 1978; van der Meulen *et al.*, 1978; Velasco *et al.*, 1980; Gottschalk and Szymas, 1987; Russell and Rubinstein, 1989). GFAP expression is often lost in malignant gliomas grown in culture. For example, Rettig *et al.* (1986) found that only six of 22 malignant glioma lines were GFAP positive. Similar results have been reported by other investigators (Bigner *et al.*, 1981). Transfection of a GFAP-deficient malignant glioma line with a functional GFAP cDNA decreased cell proliferation and growth in soft agar (Rutka and Smith, 1993), suggesting a role for GFAP in maintaining a reduced transformed state. Conversely, transfection of a GFAP-positive malignant glioma cell line with a GFAP antisense cDNA construct resulted in undetectable GFAP expression, a greater degree of cell piling, increased proliferative rate/anchorage-independent growth/invasiveness (Rutka *et al.*, 1994), as well as absence of process formation in the presence of granule neurons (Chen and Liem, 1994).

Recently, the gene encoding a new member of the fatty acid binding protein (FABP), called brain (*B-FABP*) or brain lipid binding protein (*BLBP*), has been cloned (Feng *et al.*, 1994; Kurtz *et al.*, 1994). The FABP family consists of structurally related proteins with characteristic cellular, tissue and developmental distribution patterns. Roles proposed for these proteins include the uptake, storage and/or delivery of fatty acids and retinoids (Bass, 1993; Veerkamp and Maatman, 1995; Schnütgen *et al.*, 1996). Some members of this family, including B-FABP, are located in both the nucleus and cytoplasm. It has been proposed that these FABPs deliver ligands to the nucleus, resulting in the modulation of the expression of genes that encode proteins important in lipid metabolism or in cell growth and differentiation (Clarke and Armstrong, 1989; Glatz and van der Vusse, 1990). Liver FABP and heart FABP have been

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implicated in the control of cellular proliferation and differentiation (Keler *et al*., 1992; Burton *et al*., 1994; Yang *et al*., 1994; Huynh *et al*., 1995; Rump *et al*., 1996). In the developing murine CNS, B-FABP is first expressed in neuroepithelial precursor cells and later becomes restricted to radial glial cells and immature astrocytes (Feng *et al*., 1994; Kurtz *et al*., 1994). In the adult brain, B-FABP is present in a selected population of cells (glia limitans, radial glial cells of the hippocampal dentate gyrus, Bergmann glial cells and olfactory bulb) (Kurtz *et al*., 1994; Schnütgen *et al*., 1996). Although B-FABP is generally not expressed in mature astrocytes, this protein has been detected at low levels in GFAP-positive astrocytes found in the post-natal spinal cord (Kurtz *et al*., 1994) and in gomori-positive astrocytes in the brain (Young *et al*., 1996). Antibodies to B-FABP prevent both neuronal and glial cell differentiation of primary cerebellar cells *in vitro* (Feng *et al*., 1994). In these cultures, the extension of the radial glial processes is blocked as well as the migration of neuronal cells along these processes. Based on these observations, it was proposed that the *B-FABP* gene is important for the establishment of the radial glial fiber system that is required for the migration of immature neurons in the developing nervous system (Feng *et al*., 1994). The presence of B-FABP in both the cytoplasm and nucleus suggests a role in a signaling pathway required for glial/neuronal interactions. Recently, Xu *et al*. (1996) identified long chain polyunsaturated fatty acids as possible ligands of murine B-FABP with strongest binding to docosahexaenoic acid (DHA). DHA is enriched in the developing nervous system and is essential for the development of the CNS.

We have cloned the human B-FABP cDNA by screening human fetal retina and fetal brain cDNA libraries with a chick retina FABP cDNA. We have mapped the human *B-FABP* gene and have studied the expression pattern of B-FABP in fetal tissues as well as in malignant glioma cell lines and tumor biopsies. The subset of malignant glioma lines that express GFAP was found to express B-FABP. Indirect immunofluorescence analysis indicates that the same malignant glioma cell can express both GFAP and B-FABP.

Results

Cloning the human B-FABP cDNA

The 709 bp retina FABP cDNA previously isolated from a chick retina cDNA library (Godbout, 1993) was used to screen two human cDNA libraries, one derived from fetal brain and the other from fetal retina. Identical B-FABP cDNA sequences were obtained from both libraries; however a high percentage of the clones obtained from the fetal brain library contained DNA in addition to and unrelated to B-FABP cDNA. The longest B-FABP cDNA clone was isolated from the fetal retina library and contained 757 bp of sequence (GenBank/EMBL U51338). This clone has 55 bp of 5′ flanking DNA, an open reading frame of 132 amino acids (including the start methionine codon) from +56 to +451. The stop codon at +452 is followed by a long 3′ untranslated region of 303 bp. The predicted amino acid sequence of human B-FABP is 92% identical (120/131 amino acids) to chick retina FABP and 87% identical (114/131) to mouse B-FABP (Figure 1).

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Human B-FABP   M V E A F C A T W K L T N S Q N F D E Y M K A L G V G F A T
Mouse B-FABP   - - D - - - - - - - - - - - D - - - - - - - - - - - - - - -
Chicken R-FABP - - - - - - - - - - A D - H - - - - - - - - - - - - - - M

Human B-FABP   R Q V G N V T K P T V I I S Q E G D K V V I R T L S T F K N
Mouse B-FABP   - - - - - - - - - - - - - - - - - G - - - - - - Q C - - - -
Chicken R-FABP - - - - - - - - - - - - - - - - - S - - - - - - - Q - - - -

Human B-FABP   T E I S F Q L G E E F D E T T A D D R N C K S V V S L D G D
Mouse B-FABP   - - - N - - - - - - - E - - S I - - - - - - - - - R - - - -
Chicken R-FABP - - - - - K - - - - - - - - - P - - - - - - - - - T - - - -

Human B-FABP   K L V H I Q K W D G K E T N F V R E I K D G K M V M T L T F
Mouse B-FABP   - - I - V - - - - - - - - - C T - - - - - - - - - V - - - -
Chicken R-FABP - - - - V - - - - - - - - - - - - - - - - - - R - - - - - -

Human B-FABP   G D V V A V R H Y E K A *     131/131 (100%)
Mouse B-FABP   - - I - - - - C - - - - *     114/131 (87%)
Chicken R-FABP - - - - - - - - - - - - *     120/131 (92%)
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Figure 1 Amino acid comparison of human, mouse and chicken brain/retina FABP. The predicted amino sequence (bold letters) derived from the human B-FABP cDNA is compared with the amino acid sequences derived from mouse B-FABP cDNA (Feng *et al*., 1994; Kurtz *et al*., 1994) and chicken R-FABP cDNA (Godbout, 1993). Amino acid identity is indicated by a dash. The asterisks represent the stop codons. The percent identity with human B-FABP is indicated at the end of each sequence. The nucleotide sequence has been deposited to the GenBank/EMBL DNA database under accession number U51338. A human B-FABP cDNA that extends approximately 20 bp upstream of our cDNA has also been isolated by Schnütgen, Börchers and Spener (Institute for Chemical and Biochemical Sensor Research, Münster, Germany) (GenBank/EMBL AJ002962)

Mapping the human B-FABP *gene*

The human B-FABP cDNA was used to probe a human placenta genomic library. One of the bacteriophages that hybridized to this cDNA has a 15 kb insert containing the entire *B-FABP* gene (included within a 4.5 kb DNA fragment), 7 kb of 5′ flanking DNA and 3.5 kb of 3′ flanking DNA. This bacteriophage (huFABP8a) was used to map the *B-FABP* gene by fluorescence *in situ* hybridization (FISH) to normal human lymphocyte chromosomes. As shown in Figure 2, biotinylated huFABP8a DNA specifically hybridized to chromosome band 6q22-23. The regional assignment of *B-FABP* was based on the analysis of 20 well-spread metaphases. Positive signals at 6q22-23 were noted in 19 out of 20 cells. Signals were visualized on both homologues in 75% of the spreads. The band assignment was determined by measuring the fractionated chromosome length and by analysing the banding pattern generated in the DAPI counterstained image.

Tissue distribution of the B-FABP *gene*

We screened RNAs from human fetal tissues to determine the tissue distribution of B-FABP. As shown in Figure 3a, a ~800 nt B-FABP transcript is present in fetal retina (lane 1) and brain (lane 2). B-FABP mRNA levels were approximately 3× higher in the brain than in the retina. The B-FABP transcript appears to be specific to tissues of neuroepithelial origin and was not detected in fetal heart, lung, stomach, liver, kidney and gut. In contrast, GFAP mRNA was barely detectable in fetal brain and was not observed in any of the other fetal tissues tested (Figure 3b). To ensure that similar amounts of RNA were loaded in each lane, the blot was re-probed with

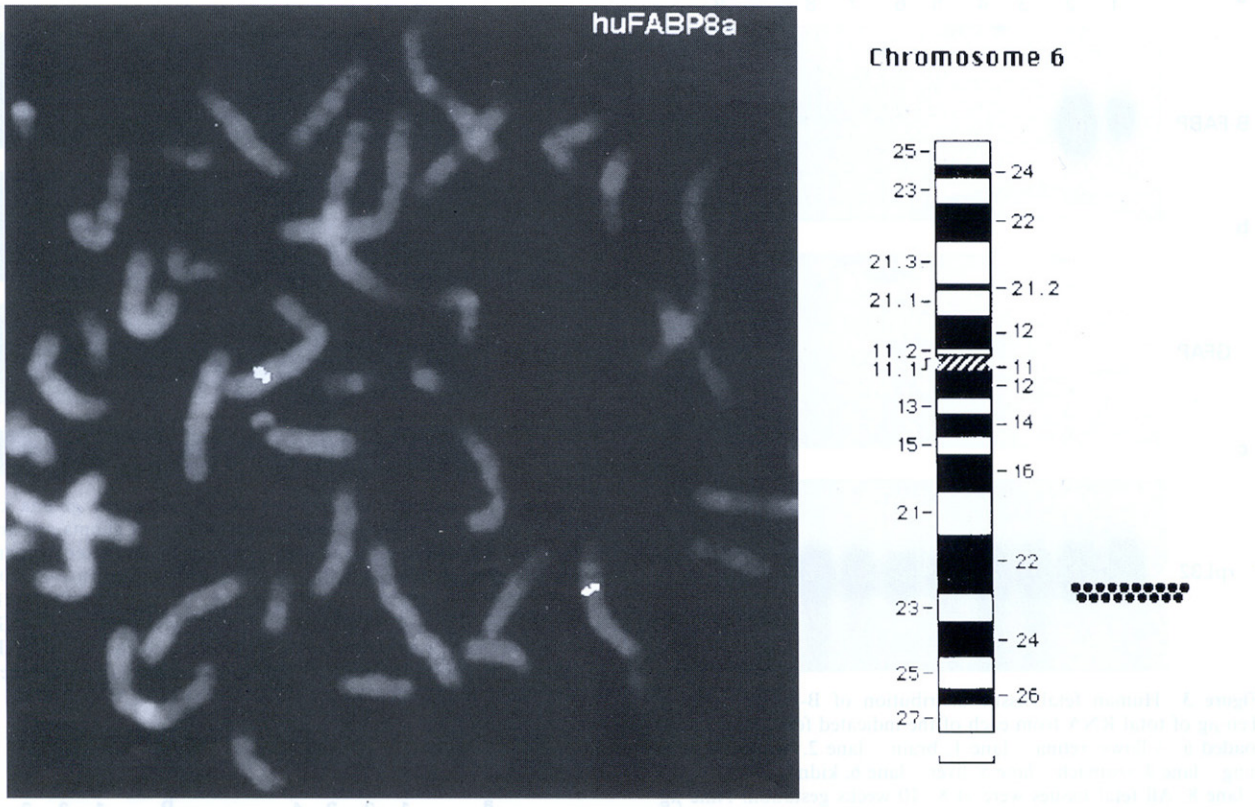

Figure 2 Mapping of human *B-FABP* by FISH. (left) FISH to normal human lymphocyte chromosomes using a genomic DNA clone (huFABP8a) that contains the entire *B-FABP* gene. Clusters of fluorescence resulting from labelling with fluorescein isothiocyanate are clearly visible on the two chromosomes 6. (right) Schematic diagram indicating that the *B-FABP* gene maps to 6q22-23

the transcribed rpL32/4A pseudogene which hybridizes to its encoded ribosomal protein RNA. Figure 3c indicates that similar amounts of RNA were loaded in lanes 1–8. Adult brain RNA (lane 9) was included in this analysis as a positive control for the GFAP probe.

Expression of B-FABP and GFAP mRNA in malignant glioma

Murine B-FABP is expressed in the developing CNS in radial glial cells, the precursors of the astrocytes from which malignant gliomas are believed to originate (Feng *et al*., 1994; Kurtz *et al*., 1994). We therefore screened 15 human malignant glioma cell lines to determine whether B-FABP mRNA is expressed in these tumor cells. Of the 15 lines tested, four (lane 4 – M016, lane 6 – M049, lane 14 – U138MG, lane 15 – U251MG) expressed elevated levels of B-FABP mRNA (Figure 4a). M059J (lane 7) had intermediate levels, while M067K and A1235 had low levels. No B-FABP mRNA was detected in the remaining eight lines.

In contrast to B-FABP, GFAP is expressed in differentiated astrocytes. As described earlier, GFAP is expressed in malignant glioma biopsies as well as in some malignant glioma cell lines. We found a good correlation between B-FABP and GFAP mRNA levels in our 15 malignant glioma lines. High levels of GFAP mRNA were observed in M016, M049, U138MG and U251MG while M059J expressed intermediate levels of GFAP mRNA (Figure 4b). No GFAP mRNA was detected in the remaining 10 lines, including M067K and A1235 which express low levels of B-FABP mRNA. Both the rpL32 and actin probes indicate that similar amounts of RNA were loaded in each lane with the possible exception of M059J which had low levels of both these control RNAs (Figure 4c and d). U138MG has previously been reported to be negative for GFAP mRNA and protein (Bigner *et al*., 1981; Tohyama *et al*., 1993). The U138MG line that we tested was obtained from Dr Fogh (Sloane Kettering Institute, NY) in 1973 and may represent an earlier version of the cell line tested by others or may represent a selected subpopulation which is positive for GFAP.

Western blot analysis of B-FABP and GFAP in malignant glioma

To assay whether malignant glioma cell lines that express B-FABP transcripts also produce the protein, we carried out Western blot analysis on four malignant glioma lines analysed for B-FABP and GFAP mRNA expression. Total cell extracts were prepared from U251MG and M049 which express elevated levels of B-FABP and GFAP mRNA, and U87MG and M002 lines with undetectable B-FABP and GFAP mRNA. Western blot analysis was carried out using antiserum prepared against recombinant chick retina FABP. This antiserum is specific to chick retina and chick brain FABP and also recognizes the FABP expressed in human brain (Godbout *et al*., 1995 and our unpublished data). As shown in Figure 5a, the FABP antiserum detected a single band at ~15 kDa in U251MG and M049 extracts but not in U87MG or

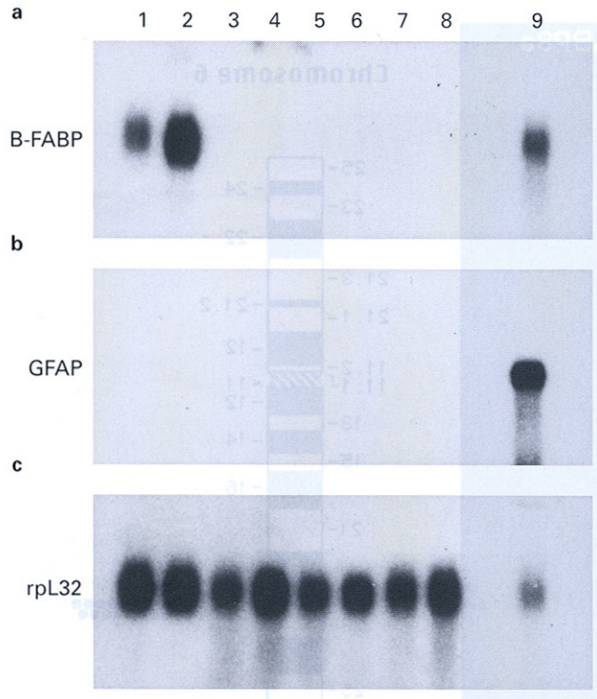

Figure 3 Human fetal tissue distribution of B-FABP mRNA. Ten μg of total RNA from each of the indicated fetal tissues were loaded as follows: retina – lane 1, brain – lane 2, heart – lane 3, lung – lane 4, stomach – lane 5, liver – lane 6, kidney – lane 7, gut – lane 8. All fetal tissues were at 8–10 weeks gestation. Nine μg of total RNA from adult brain were loaded in lane 9. The filter was sequentially hybridized with: (**a**) B-FABP cDNA, (**b**) GFAP cDNA, and (**c**) rpL32/4A, a ribosomal protein gene (Dudov and Perry, 1984). The exposure time for the filter hybridized with B-FABP cDNA was 3 days while that of the filter hybridized with GFAP cDNA was 10 h. The specific activity of each probe was 10^8 c.p.m./μg of nick translated DNA

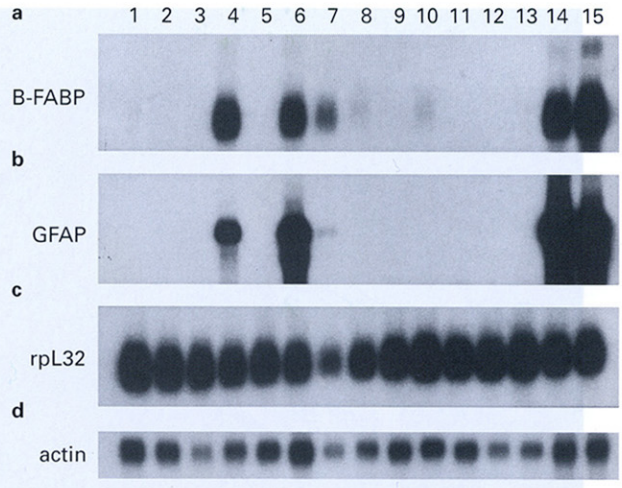

Figure 4 Northern blot analysis of 15 malignant glioma cell lines. Two μg of poly(A)$^+$ RNA from malignant glioma cell lines were loaded as follows (lane numbers are indicated after the name of the cell line): M002 – 1, M010 – 2, M012 – 3, M016 – 4, M021 – 5, M049 – 6, M059J – 7, M067K – 8, M125 – 9, A1235 – 10. A172 – 11, T98 – 12, U87MG – 13, U138MG – 14, U251MG – 15. The filter was sequentially hybridized with: (**a**) B-FABP cDNA, (**b**) GFAP cDNA, (**c**) rpL32/4A ribosomal protein gene and (**d**) actin cDNA

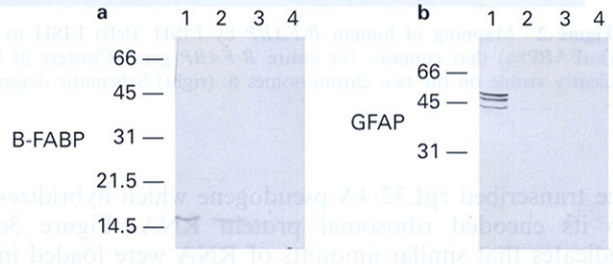

Figure 5 Western blot analysis of B-FABP and GFAP in malignant glioma cell lines. Total cell extracts were prepared from U251MG, M049, U87MG and M002. Equal amounts of protein (10 μg) were loaded in each lane: 1 – U251MG, 2 – M049, 3 – U87MG, 4 – M002 and electrophoresed in a 15% acrylamide-SDS gel. Proteins were transferred to nitrocellulose, blocked and incubated with: (**a**) anti R-FABP antiserum at a 1:500 dilution and (**b**) anti-GFAP monoclonal antibody at a 1:200 dilution. The signal was detected using alkaline phosphatase-linked goat anti-rabbit IgG. The molecular weight standards (kDa) are indicated on the left

M002 extracts. Similarly, commercial monoclonal antibody to GFAP specifically recognized GFAP present in U251MG and M049 but not in U87MG or M002 extracts (Figure 5b). We consistently obtained three bands in GFAP-positive cell lines using this antibody preparation. The band with the slowest migration rate was strongest and indicated a molecular mass slightly greater than 50 kDa. The bands with faster migration rates may represent breakdown products of GFAP. The presence of multiple bands on Western blots using GFAP antiserum has been reported by others (Tohyama *et al*., 1993).

Indirect immunofluorescence analysis

Although some glial cells such as Bergmann glial cells appear to co-express GFAP and B-FABP (Kurtz *et al*., 1994), in general B-FABP is expressed in radial glial cells and immature astrocytes of the developing CNS, while GFAP production correlates with astrocyte differentiation. Our Northern and Western blot analyses indicate that the same cell lines can express these two transcripts/proteins. We carried out double labeling immunofluorescence to answer another question: whether the same cells in malignant glioma cultures can express both GFAP and B-FABP. Analysis of U251MG using anti-GFAP antibody revealed the characteristic staining pattern observed by other investigators using this cell line; i.e., GFAP (indicated by the red color) was found throughout the filamentous network of U251MG cells (Figure 6). In contrast, B-FABP (green) was located in the body of the cells as well as in the nucleus and was not found in network fibers. The majority of individual cells were positive for both proteins. The results with M016 were similar although only a subset (approximately half) of B-FABP-positive cells expressed GFAP. These results are in agreement with the Western blot and Northern blot analyses which indicated that B-FABP mRNA and protein levels are higher than GFAP mRNA and protein in M016 in comparison with U251MG. As a negative control, we repeated these experiments with T98 which has undetectable B-FABP and GFAP transcripts. Labeling of T98 was barely detectable using both anti-GFAP antibody and anti-R-FABP antibody.

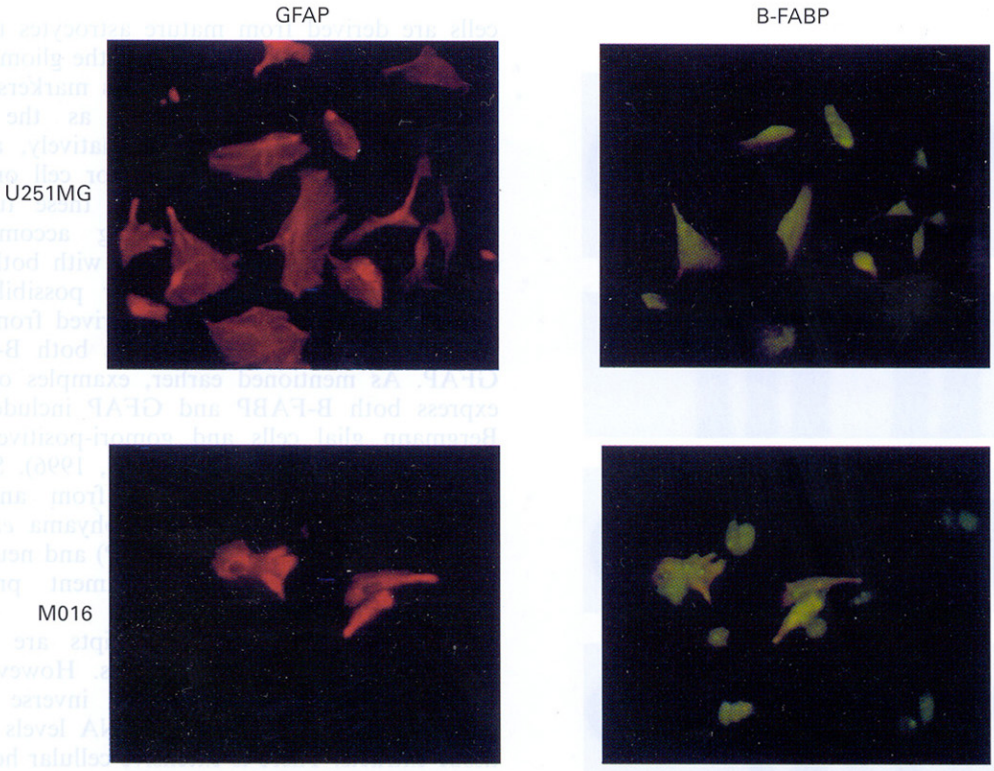

Figure 6 Co-expression of GFAP and B-FABP in malignant glioma cells. Indirect double labeling immunofluorescence staining of U251MG (top) and M016 (bottom) cells. (left) The red color (rhodamine) indicates the presence of GFAP in the cytoplasmic network fibers. (right) The green color (fluorescein) indicates the presence of B-FABP in the cytoplasm and nucleus of the cells

GFAP and B-FABP RNA levels in tumor biopsies

To ensure that expression of B-FABP in malignant glioma was not a cell culture artifact, we studied the expression of this transcript in seven malignant glioma biopsies, five of which were diagnosed as astrocytoma grade IV (tumors 1, 2, 3, 5, 6). Tumor 4 was identified as virtually all oligodendroglioma (recurrent tumor) while tumor 7 was an anaplastic (grade III) astrocytoma. B-FABP mRNA was present in all seven samples albeit at different levels (Figure 7a). Similarly, GFAP mRNA was observed in all the samples analysed with significant variations from sample to sample (Figure 7b). There was no consistent pattern in the amounts of B-FABP and GFAP mRNA expressed in individual tumors. Tumors 1, 5 and 6 had the highest levels of B-FABP mRNA while tumors 2, 3 and 5 had the highest levels of GFAP mRNA. The oligodendroglioma (tumor 4) had low levels of both GFAP and B-FABP mRNA. Tumors 2 and 7 had particularly low levels of B-FABP mRNA while very low levels of GFAP mRNA were found in tumor 1. It should be noted that some variation was also observed using either the rpL32 or the actin control probes. This may reflect the cellular heterogeneity and gene expression abnormalities observed in malignant glioma tumor biopsies.

Discussion

B-FABP and GFAP are normally expressed in the same cell lineage, but at different developmental stages. B-FABP is expressed in radial glial cells which are precursors to astrocytes, while the intermediate filament protein GFAP is specifically expressed in mature astrocytes. B-FABP is also found in neuroepithelial precursor cells and in some neuronal cells, such as migrating cerebellar granule neurons, neither of which express GFAP (Feng and Heintz, 1995). Some cells do co-express B-FABP and GFAP; e.g., the Bergmann glial cells of the cerebellum which are radial glial cells that persist in the adult nervous system, the radial glial cells of the hippocampal dentate gyrus and the gomori-positive astrocytes of the brain (Kurtz *et al*., 1994; Young *et al*., 1996). Here, we show that B-FABP is expressed in the subset of malignant glioma cell lines that also express GFAP.

Neuronal and glial cells constitute the two major cell types in the brain. These cells are derived from precursor cells which are in turn derived from the multipotential neuroepithelial stem cells of the neural tube. In primate brain, there is a slow but measurable turnover of glial cells (Rakic, 1985). In the adult mouse brain, it has been estimated that $\sim 10^5$ glial cells are generated each day (Smart and Leblond, 1961). Clonal analyses indicate that astrocytes can originate from either multipotent precursor cells or from progenitor cells restricted to the astrocytic lineage (Levison and Goldman, 1993; Luskin and McDermott, 1994). Of note, a number of studies indicate that there are stem cells in the adult mammalian brain that can differentiate into neurons as well as glial cells although the adult mammalian brain is generally believed to lack the capacity to generate new neurons (McKay, 1997). Taken together, these results suggest the presence of different types of precursor cells in the adult brain, including multipotential precursor cells.

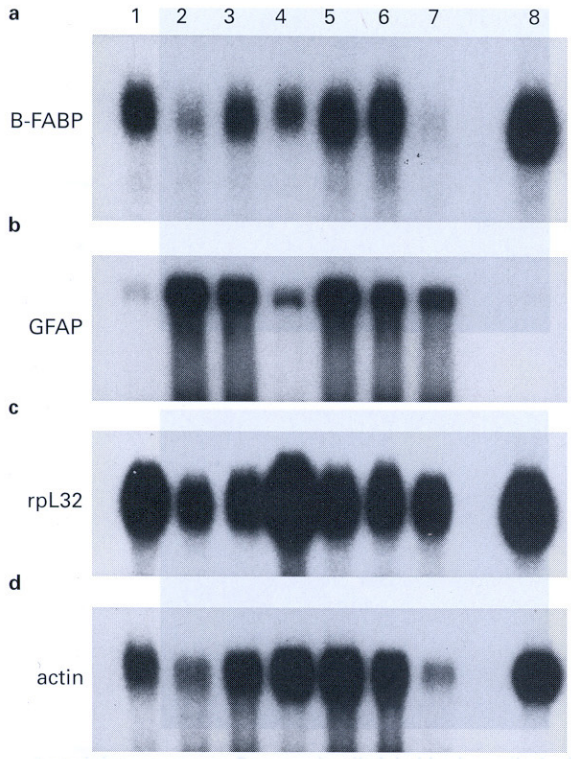

Figure 7 Northern blot analysis of malignant glioma tumor biopsies. Ten μg of total RNA from seven malignant glioma tumor biopsies (lanes 1–7) were loaded in each lane. Two μg of poly(A)$^+$ RNA from fetal brain were loaded in lane 8. The filter was sequentially hybridized with: (**a**) B-FABP cDNA, (**b**) GFAP cDNA, (**c**) rpL32 DNA and (**d**) actin cDNA

The cellular origin of malignant glioma remains controversial. These brain tumors occur mainly in adults, demonstrate considerable cellular heterogeneity and express the astrocyte-specific marker GFAP with the more aggressive tumors expressing lower levels of GFAP than the less aggressive and more differentiated tumors (Eng and Rubinstein, 1978; van der Meulen *et al*., 1978; Velasco *et al*., 1980; Gottschalk and Szymas, 1987; Russell and Rubinstein, 1989). Because most malignant gliomas express GFAP in at least some of their cells, these tumors have been postulated to originate from the glial astrocytic lineage, either from mature astrocytes that express GFAP or from an astrocyte precursor cell or stem cell that is induced to express GFAP during tumor formation. We have found that the *GFAP* and *B-FABP* genes are co-expressed in five of 15 malignant glioma lines. Neither gene is expressed in eight of the 15 lines while the remaining two lines have low levels of B-FABP mRNA and undetectable GFAP mRNA. We have also shown that both GFAP and B-FABP can be expressed in the same cell. For example, all U251MG cells produced GFAP and B-FABP, while approximately half of B-FABP-positive M016 cells produced GFAP. Although the significance of cell- or tissue-specific gene expression in cancer cells remains poorly understood, the discovery of a radial glia/neuroepithelium gene that is co-expressed with the astrocyte-specific *GFAP* gene in a subset of malignant glioma lines may provide new insight into the nature of the cell of origin of these tumors. If malignant glioma cells are derived from mature astrocytes that express GFAP, one would postulate that the glioma cells have acquired the ability to co-express markers specific to different developmental stages as the result of malignant transformation. Alternatively, a precursor cell (either an astrocytic precursor cell or stem cell) may be the cell of origin for these tumors with malignant transformation being accompanied by expression of markers associated with both immature and mature astrocytes. Another possibility is that certain malignant gliomas are derived from a type of glial cell that normally expresses both B-FABP and GFAP. As mentioned earlier, examples of cells that express both B-FABP and GFAP include cerebellar Bergmann glial cells and gomori-positive astrocytes (Kurtz *et al*., 1994; Young *et al*., 1996). Support for malignant gliomas originating from an immature precursor cell is provided by Tohyama *et al*. (1993) who found that both glial (GFAP) and neuronal (NF-L, -H, -M) intermediate filament proteins are produced by U251MG.

GFAP and B-FABP transcripts are present in malignant glioma tumor biopsies. However, we did not find either a direct or an inverse correlation between GFAP and B-FABP RNA levels using total tissue extracts. There is extensive cellular heterogeneity in malignant glioma biopsy material. As well, normal tissue is sometimes included in the tumor biopsies. *In situ* hybridization or immunohistochemical analysis will be required to determine whether there is a correlation between B-FABP and GFAP expression in malignant glioma tumor biopsies. It will also be important to assess whether B-FABP expression, like GFAP, is associated with a less aggressive phenotype.

The human *B-FABP* gene is located at 6q22-23. A number of genes have been mapped to this region, including the proto-oncogene c-*myb* (Harper *et al*., 1983) and the tumor protein gene *TPD52* (Byrne *et al*., 1996). Restriction fragment length polymorphism analysis has indicated frequent allelic losses on chromosome 6 in glial neoplasms (Liang *et al*., 1994). Studies using comparative genomic hybridization (CGH) have shown that there is loss of heterozygosity on chromosome 6q16-ter in some malignant gliomas (Mohapatra *et al*., 1995). Other regions shown to undergo loss of genetic material include chromosomes 9p, 10, 11, 13, 14, 17p, 18 and 22q (James *et al*., 1989; Fults *et al*., 1990; Miyakoshi *et al*., 1990; Mohapatra *et al*., 1995; Schlegel *et al*., 1996; Weber *et al*., 1996). Although loss of heterozygosity is not common in the region where *GFAP* maps, 17q21 (Bongcam-Rudloff *et al*., 1991), it will be interesting to determine whether there is any correlation between loss at 6q16-ter and B-FABP levels in malignant glioma.

In summary, we have shown that human B-FABP mRNA is expressed in tissues of neuroepithelial origin as well as in malignant glioma tumors and in a subset of malignant glioma cell lines. We have mapped the *B-FABP* gene to chromosome band 6q22-23, a region which undergoes loss of heterozygosity in malignant glioma. Analysis of malignant glioma cell lines indicates that B-FABP is often co-expressed with GFAP and that the same cell can express both proteins. These results suggest that the cell origin of malignant glioma may be an astrocytic precursor cell that has the potential of expressing both proteins

normally or as the result of tumor formation. Additional analysis using malignant glioma tumor biopsy material will be required in order to provide more definite information regarding the expression of B-FABP and GFAP, and malignancy.

Materials and methods

Cell lines

The source or references for the cell lines are: M002, M010s, M012, M016, M021, M049, M059J, M067K (Miyakoshi *et al.*, 1990; Godbout *et al.*, 1992); M125 (this work); A1235, A172 (Stuart A Aaronson, NCI, Bethesda, MD, USA); T98 (Walter Nelson-Rees, Naval Biomedical Research Station, Oakland, CA, USA); U87MG, U138MG, U251MG (Jorgen Fogh, Sloane Kettering Institute, Rye, NY, USA). Cells were cultured in Dulbecco's modification of Eagle's MEM plus 10% fetal calf serum and antibiotics.

Screening of cDNA and genomic libraries for B-FABP cDNA and genomic clones

The human fetal retina and fetal brain cDNA libraries purchased from Stratagene (La Jolla, CA) were screened with the chick R-FABP cDNA insert under low stringency (i.e. filters were washed at room temperature in 0.1 × SSC, 0.1% SDS). Positive clones were purified and the inserts sequenced using the dideoxynucleotide chain-termination method with T7 DNA polymerase (Pharmacia, Uppsala, Sweden) as modified for double-stranded DNA templates (Mierendorf and Pfeffer, 1987). A human placenta genomic library (Clontech, Palo Alto, CA) was screened at high stringency using the human B-FABP cDNA insert. Positive plaques were purified and the genomic DNA analysed using restriction enzymes and by Southern blotting.

Fluorescence in situ *hybridization (FISH) mapping*

Conventional chromosome spreads were prepared from normal human lymphocytes. The FISH method used was based on published methods (Lichter *et al.*, 1990). Biotinylated probe was detected with avidin-fluorescein isothiocyanate (FITC). The chromosome preparations were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). The FISH mapping was carried out by the Human Genome FISH Mapping Resource Centre, Hospital for Sick Children, Toronto, Ontario, Canada.

Northern blot analysis

Procedures for extraction of poly(A)$^+$ RNA and conditions for probe hybridization, washing filters, and stripping filters have been described (Godbout *et al.*,1993; Godbout, 1993). Poly(A)$^+$ RNA from cell lines was isolated using the hot phenol method (Godbout *et al.*, 1993) while total RNA was isolated from malignant glioma tumor biopsies using phenol and lithium chloride (Godbout, 1993). The following probes were used for hybridization: a 700 bp human B-FABP cDNA insert described in the Results section, a 2.3 kb human GFAP cDNA insert from the American Type Culture Collection, Rockville, MD) (GenBank/EMBL No. M78090), a 1.3 kb *Hind*III fragment from the ribosomal protein gene rpL32/4A (Dudov and Perry, 1984) and mouse α-actin cDNA (Minty *et al.*,1981).

Western blots

The preparation of the R-FABP antiserum has been previously described (Godbout *et al.*, 1995). Total cell extracts (10 μg protein/lane) were run in a 15% SDS-polyacrylamide gel. Western blot analysis was carried out using the standard protocol for protein transfer onto nitrocellulose described by Schleicher and Schuell (Keene, NH, USA). Duplicate filters were exposed to anti-GFAP monoclonal antibody (1:200 dilution) obtained from Sigma and anti-R-FABP antibody (1:500 dilution) (Godbout *et al.*, 1995). The signal was detected using alkaline phosphatase-linked goat anti-rabbit IgG.

Indirect immunofluorescence

Malignant glioma cells were grown on coverslips. Cells were fixed in −20°C methanol and blocked using normal goat serum. The coverslips were incubated with a 1:100 dilution of the primary antibodies. After washing in phosphate buffered saline, the coverslips were incubated with a 1:100 dilution of the secondary antibodies. The primary antibodies used were anti-GFAP monoclonal antibody (Sigma) and anti-R-FABP polyclonal antibody (Godbout *et al.*, 1995). The secondary antibodies were anti-rabbit IgG-fluorescein (for labeling B-FABP) and anti-mouse Ig-rhodamine (for labeling GFAP) (Boehringer Mannheim). For the co-localization experiments, both the anti-GFAP and anti-B-FABP primary antibodies were added to the same coverslip.

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