Glial Fibrillary Acidic Protein (GFAP) in Oligodendrogliomas: A Reflection of Transient GFAP Expression by Immature Oligodendroglia

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ABSTRACT: Fourteen pure oligodendrogliomas were studied by light- and electronmicroscopy and immunohistochemistry to examine glial fibrillary acidic protein (GFAP) positivity in the tumors. To compare the immunohistochemical staining patterns of neoplastic oligodendroglia and immature oligodendroglia, myelination glia in the white matter of eight normal brains from children under 6 months of age were studied. The tumors possessed light microscopic and ultrastructural features characteristic of oligodendrogliomas. Microtubules were found in the cytoplasm of nine tumors on electronmicroscopy. In one, intermediate filaments and microtubules were observed in occasional tumor cells with polygonal crystalline structures in the cytoplasm. Using the peroxidase-antiperoxidase technique, all specimens were stained for GFAP, vimentin, S-100 and neuron-specific enolase (NSE). In nine tumors, variable numbers of cells with an oligodendroglioma morphology reacted positively for GFAP. All tumors were positive for S-100 and negative for vimentin and NSE. The myelination glia in the eight normal brains stained positively for GFAP but not for vimentin. Vimentin is expressed by developing, reactive and neoplastic astrocytes. Thus, GFAP positivity combined with vimentin negativity in both neoplastic and immature oligodendroglia suggests that GFAP positivity in oligodendrogliomas may reflect the transient expression of this intermediate filament by immature oligodendroglia.

RESUME: Les proteinics fibrillaires acides de la glie (PFAG) dans les oligodendrogliomes; une reflexion de l’expression transitoire de PFAG par l’oligodendroglie immature Quatorze oligodendrogliomes pures ont ete etudies par microscopie optique et electronique et immunohistochimie pour examiner la positivite aux proteinics fibrillaires acides de la glie (PFAG) dans les tumeurs. Afin de comparer les patterns de coloration immunohistochimique de l’oligodendrogliome neoplastique et de l’oligodendrogliome immature, la glie de myelinisation dans la substance blanche de huit cerveaux normaux provenant d’enfants de moins de 6 mois a ete etudiee. A la microscopie optique et electronique, les tumeurs possedeaient des particularites caracteristiques des oligodendrogliomes. On a trouve des microtubules dans le cytoplasme de 9 tumeurs a la microscopie electronique. On a observe dans une tumeur des filaments intermediaires et des microtubules dans quelques cellules tumorales ainsi que des structures cristallines polygonales dans le cytoplasme. En utilisant la technique peroxydase-antiperoxydase, tous les echantillons ont ete colores pour les PFAG, la vimentine, le S-100 et l’enolase specifique aux neurones (ESN). Dans les neuf tumeurs, un nombre variable de cellules possedant une morphologie de type oligodendrogliole avait une reaction positive pour les PFAG. Toutes les tumeurs etaient positives pour le S-100 et negatives pour la vimentine et l’ESN. La glie de myelinisation dans les huit cerveaux normaux prenait une coloration positive pour les PFAG, mais negative pour la vimentine. La vimentine est exprimee par les astrocytes en developpement, reactionnels et neoplasiques. Ainsi, la positivite pour les PFAG associee a la negativite pour la vimentine dans l’oligodendrogliome tant neoplasique qu’immature, suggere que la positivite pour les PFAG dans les oligodendrogliomes peut refleter l’expression transitoire de ce filament intermediaire par l’oligodendrogliome immature.


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Since the initial isolation of the glial fibrillary acidic protein (GFAP) by Eng and co-workers\(^1\) and Bignami et al\(^2\) from multiple sclerosis plaques, and its subsequent recognition as an astrocytic marker,\(^3\) its distribution in various neoplasms of the central nervous system has been widely studied.\(^4,5\) The interpretation of GFAP positivity in oligodendrogliomas has been a subject of much speculation.\(^6,10\) While the presence of an astrocytic component in a mixed astrocytic-oligodendroglial tumor is well known, there is now an increasing recognition of the presence of GFAP in tumor cells with features otherwise typical of oligodendroglia.\(^6-10\) Such cells have been variably interpreted as representing reactive astrocytes, oligodendroglia with astrocytic differentiation, transitional astrocytic-oligoden-
droglial forms or immature oligodendroglia. We studied 14 oligodendrogliomas by light- and electronmicroscopy and immunohistochemically to examine the presence of GFAP positivity in neoplastic oligodendroglia.

**Materials and Methods**

The oligodendrogliomas from the neuropathology files between 1964 and 1984 at Toronto General Hospital and The Hospital for Sick Children, Toronto, were examined. Mixed astrocytic-
oligodendroglial tumors were excluded and only the 14 tumors with tissue for adequate immunohistochemical evaluation were selected.

For light-microscopy, formalin-fixed, paraffin-embedded tissue was cut in 5 μm thick sections that were stained with the hematoxylin-eosin and phosphotungstic acid hematoxylin stains. Morphologic features assessed included cellularity, degree of pleomorphism, mitotic activity, presence of necrosis, calcification, microcystic change, pattern of vascularity and presence of vascular hyperplasia.

For electronmicroscopy (EM), glutaraldehyde-fixed, post-osmicated, epon-embedded tissue was sectioned and examined using a Philips 400 electronmicroscope. EM studies were performed in nine of the 14 cases.

Formalin-fixed paraffin-embedded tissue from all cases was studied immunohistochemically with antisera to GFAP, S-100, vimentin and neuron-specific enolase (NSE), using the peroxidase-

Electronmicroscopic findings in tumors

EM revealed polygonal cells with straight or interdigitating cell membranes (Figure 2). Light and dark cell types were present, the majority being the light cells, which had round-to-

Figure 1 — Photomicrograph of a typical oligodendroglioma with a delicate vascular stroma intersecting nests of tumor cells (case 4). H & E x 200

Figure 2 — Electronmicrograph showing polygonal cells with straight borders and a paucity of cell processes. Light and dark cells are evident (case 4) x 3,960
the light cells. Their cytoplasm contained a few mitochondria, ribosomes, sparse endoplasmic reticulum and Golgi complexes. In both cell types, microtubules were present but intermediate filaments were not found.

In the tumor with cytoplasmic granules, polygonal crystalline structures with a rectangular or hexagonal configuration were identified (Figure 3). These showed linear densities spaced with a periodicity of 150 Å with their axes intersecting at approximately 120 degrees. In the cytoplasm of these cells, both micro-

tubules and intermediate filaments were found and a few autophagic vacuoles were identified.

Immunohistochemical findings

GFAP positivity was observed in nine patients in cells with features typical of oligodendroglia. This was localized to the cytoplasm in a variable number of cells; cells were rare to occasional in eight cases (Figure 4) and numerous in case 9 (Figure 5). The distribution of GFAP-positive cells was random.
Positivity did not correlate with the degree of anaplasia; three high-grade tumors were GFAP-positive while the fourth was negative. The coarse staining of reactive astrocytes in scattered areas was morphologically distinct from the staining of the tumor cells.

Although the pattern was variable, all tumors stained positively with antisera to S-100, the antigen being localized to the nucleus, cytoplasm or both.

None of the tumors stained for vimentin or NSE.

**Immature oligodendroglia**

All immature oligodendroglia (myelination glia) in the developing white matter of the cerebrum and cerebellum showed positive cytoplasmic staining with antisera to GFAP and negative staining for vimentin.

**DISCUSSION**

All tumors in this study showed features of pure oligodendrogliomas with no evidence of a mixed astrocytic component. Ultrastructurally, a two-cell population of light and dark cells was found, as observed by others. Both cell types showed a paucity of cell processes. The cytoplasmic organelles included mitochondria, sparse endoplasmic reticulum, Golgi complexes and free ribosomes. Microtubules were also present. There was some variation in the mitochondrial morphology with occasional giant forms. In case 9, whose tumor had intracytoplasmic PAS-positive granules, crystalline inclusions, which have been described in the literature, were present. They are thought to be related to lysosomes, Golgi apparatus or mitochondria. In the case mentioned by Tani et al intermediate filaments were not present in the cytoplasm, but our case had both intermediate filaments and microtubules in occasional tumor cells. Cervós-Navarro et al reported seven tumors with intermediate filaments in the cytoplasm of cells with features typical of oligodendroglioma.

All the tumors in our patients stained positively for antisera to S-100; however, positivity was present in only some tumor cells, sometimes in the nucleus, at other times in the cytoplasm or more commonly in both. This pattern of S-100 staining has been previously observed. The finding of NSE in tumor cells with features typical of oligodendroglia. In the case mentioned by Tani et al intermediate filaments were not present in the cytoplasm, but our case had both intermediate filaments and microtubules in occasional tumor cells. Cervós-Navarro et al reported seven tumors with intermediate filaments in the cytoplasm of cells with features typical of oligodendroglioma.

GFAP positivity was present in nine tumors, in cells with features typical of oligodendroglioma. In eight cases, only rare-to-occasional cells exhibited GFAP positivity, suggesting that such cells may be missed because of factors of sampling. In the tumor with cytoplasmic granules and crystalline inclusions (case 9), numerous cells expressed GFAP. This was the only tumor in which intermediate filaments were seen on EM. While sampling may be a factor, GFAP positivity does not always correlate with intermediate filament formation.

GFAP-positive cells in oligodendrogliomas have been variably interpreted in earlier studies. Some authors have considered such cells to be astrocytic. Menezes et al proposed that GFAP positivity represented an astrocytic differentiation by the neoplastic oligodendroglia. Van der Meulen et al, who found GFAP positivity in malignant oligodendrogliomas, proposed that a degree of anaplasia or dedifferentiation may be required for GFAP expression. Correlation with malignancy could not be corroborated by us or in the study of Herpers and Budka.

Some authors have proposed that GFAP-positive cells in oligodendrogliomas may represent transitions from oligodendroglia to astrocytes; such tumors have been termed "transitional" oligodendrogliomas. Evidence for the existence of such transitional elements is based on the production of cytoplasmic intermediate filaments by oligodendroglia under various experimental conditions. For example, Hirano and Zimmerman showed intermediate filament formation in the inner and outer loops of myelin sheaths in the rat forebrain after vinblastine implantation. Also, Bunge et al described filaments in trapped cytoplasmic areas with myelin sheaths in spinal cords of animals experiencing remyelination after experimental cord injury.

A further explanation of GFAP positivity in oligodendrogliomas is suggested by the transient presence of GFAP in immature oligodendroglia during the stage of myelination glia. In correlative immunocytochemical and electronmicroscopic studies, Choi and Kim showed GFAP positivity in immature oligodendroglia in the human fetal spinal cord between 12 and 18 weeks gestation, which was no longer evident by 17 to 18 weeks. Raff et al suggested the existence of a bipotential glial precursor cell based on the experiments on rat optic nerves. They demonstrated that a cell in the 7-day-old rat optic nerve had the ability to differentiate into an astrocyte, if cultured in the presence of fetal calf serum, or an oligodendrocyte, if cultured without the calf serum. However, with a common precursor, GFAP-positive oligodendroglia should predominate in mixed glial tumors, rather than in pure oligodendrogliomas as found by Herpers and Budka.

In our patients, the finding of GFAP positivity with negative vimentin further supports the oligodendroglial nature of the cells in question. Vimentin exists in developing, reactive and neoplastic astrocytes. It is expressed in mouse astrocytes in glial precursors well before the onset of GFAP expression, and is the major cytoskeletal component of immature glia in the newborn rat brain. Vimentin also persists in mature astrocytes. In neoplastic astrocytes, both vimentin and GFAP are present but vimentin expression may predominate. The GFAP-positive cells in all our tumors did not express vimentin, suggesting that they are not astrocytic in nature. Similarly, the immature oligodendroglia in the eight normal brains were also vimentin-negative and GFAP-positive.

In conclusion, cells immunoreactive for GFAP may be encountered in otherwise classical oligodendrogliomas. Such cells need not represent an added astrocytic element or astrocytic line of differentiation. More likely they reflect the transient expression of GFAP in immature oligodendroglia during an early phase of development.

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**REFERENCES**


**GFAP in Oligodendrogliomas — Jagadha et al**


