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Microtubule-associated protein-2 immunoreactivity: a useful tool in the differential diagnosis of low-grade neuroepithelial tumors

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Abstract Complex and variable morphological phenotypes pose a major challenge to the histopathological classification of neuroepithelial tumors. This applies in particular for low-grade gliomas and glio-neuronal tumors. Recently, we and others have identified microtubule-associated protein-2 (MAP2) as an immunohistochemical marker expressed in the majority of glial tumors. Characteristic cell morphologies can be recognized by MAP2 immunoreactivity in different glioma entities, i.e., process sparse oligodendroglial versus densely ramified astrocytic elements. Here, we describe MAP2-immunoreactivity patterns in a large series of various neuroepithelial tumors and related neoplasms (n=960). Immunohistochemical analysis led to the following conclusions: (1) specific pattern of MAP2-positive tumor cells can be identified in 95% of glial neoplasms; (2) ependymal tumors do not express MAP2 in their rosette-forming cell component; (3) tumors of the pineal gland as well as malignant embryonic tumors are also characterized by abundant MAP2 immunoreactivity; (4) virtually no MAP2 expression can be observed in the neoplastic glial component of glio-neuronal tumors, i.e. gangliogliomas; (5) malignant glial tumor variants (WHO grade III or IV) exhibit different and less specific MAP2 staining patterns compared to their benign counterparts (WHO grade I or II); (6) with the exception of melanomas and small cell lung cancers, MAP2 expression is very rare in metastatic and non-neuroepithelial tumors; (7) glial MAP2 expression was not detected in 56 non-neoplastic lesions. These data point towards MAP2 as valuable diagnostic tool for pattern recognition and differential diagnosis of low-grade neuroepithelial tumors.

Keywords Microtubule-associated protein-2 · Neuropathology · Gliomas · Astrocytomas · Precursor cells

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Introduction

The WHO classification system for tumors of the nervous system and its coverings [12] has established a taxonomy of neoplastic entities, which defines morphological phenotypes and predicts the biological behavior. Studies on the molecular pathogenesis have substantially contributed to this classification. However, elucidating the differential diagnosis, cellular origin and pathogenesis of low-grade neuroepithelial tumors will require a substantial amount of additional work. This applies in particular for the characterization of epilepsy-associated glio-neuronal tumors [3]. Furthermore, criteria and pathways for the development of astrocytic, oligodendroglial or mixed phenotypes remain to be elucidated. Some of these issues are of considerable clinical importance [13]. As an example, patients suffering from anaplastic oligodendrogliomas with allelic loss of chromosome 1p and 19q benefit from polychemotherapy regimens [7].

At the microscopic level, nuclear atypia, cellular and vascular proliferation as well as tumor necrosis are relevant findings to predict malignant progression of diffuse gliomas [1, 6]. Specific immunohistochemical markers have been established to better characterize the nature of neuroectodermal neoplasms. Pattern recognition in astrocytic gliomas relies on GFAP-positive glial cell elements with diffuse arborization of cellular processes. In contrast, oligodendroglial markers have, so far, not been commercially available for paraffin-embedded histological sections. Recently, we and others described MAP2 as a helpful marker for the differential diagnosis of low-grade gliomas, in particular oligodendrogliomas [4, 20, 21].

Table 1 MAP2 immunoreactivity in various tumor entities (n=960) and non-neoplastic conditions (n=56). All tumors were diagnosed according to the WHO classification 2000. MAP2 positivity refers to staining of the major neoplastic component of the tumor or the neuronal element in glioneuronal neoplasms (*MAP2* microtubule-associated protein-2)

Tumor entity	MAP2-positive/ total cases	
Astrocytic tumors	395/428	92.3%
Diffuse astrocytoma (WHO grade II)	73/79	92.4%
Anaplastic astrocytoma (WHO grade III)	86/89	96.6%
Glioblastoma (WHO grade IV)	129/139	92.8%
Gliosarcoma (WHO grade IV)	6/8	75%
Glioblastoma with oligodendroglioma component (WHO IV)	5/5	100%
Pilocytic astrocytoma (WHO grade I)	85/95	89.5%
Pleomorphic xanthoastrocytoma (WHO grade II)	7/8	87.5%
Subependymal giant cell astrocytoma (WHO grade I)	4/5	80%
Oligodendroglial tumors	111/111	100%
Oligodendroglioma (WHO grade II)	43/43	100%
Anaplastic oligodendroglioma (WHO grade III)	68/68	100%
Mixed gliomas	126/127	99.2%
Oligoastrocytomas (WHO grade II)	40/40	100%
Anaplastic oligoastrocytomas (WHO grade III)	86/87	98.9%
Ependymal tumors	19/62	30.6%
Ependymoma (WHO grade II)	8/30	26.7%
Anaplastic ependymoma (WHO grade III)	11/29	37.9%
Myxopapillary ependymoma (WHO grade I)	0/1	0
Subependymoma (WHO grade I)	0/2	0
Neuronal and mixed neuronal-glial tumors*	88/89	98.8%
Gangliocytoma (WHO grade I)	2/2	100%
Desmoplastic infantile astrocytoma/ganglioglioma (WHO I)	2/3	66.7%
Dysembryoplastic neuroepithelial tumor (WHO grade I)	31/31	100%
Ganglioglioma (WHO grade I)	37/37	100%
Papillary glioneuronal tumor (WHO grade I)	2/2	100%
Atypical ganglioglioma (WHO grade I)	2/2	100%
Central neurocytoma (WHO grade II)	12/12	100%
Pineal parenchymal tumors	10/10	100%
Pineocytoma (WHO grade I)	4/4	100%
Pineoblastoma (WHO grade IV)	4/4	100%
Pineal parenchymal tumor of intermediate differentiation (WHO grade III)	2/2	100%
Embryonal tumors	57/61	93.4%
Medulloblastoma (WHO grade IV)	31/32	96.9%
Supratentorial primitive neuroectodermal tumor (WHO grade IV)	21/24	87.5%
Atypical teratoid/rhabdoid tumor (WHO grade IV)	5/5	100%
Tumors of meningothelial cells	3/17	17.6%
Meningioma (WHO grade I)	0/4	0%
Atypical meningioma (WHO grade II)	0/6	0%
Anaplastic meningioma (WHO grade III)	3/7	42.9%
Mesenchymal, non-meningothelial tumors	0/12	0%
Lipoma	0/1	0%
Sarcoma	0/11	0%
Primary melanocytic lesions		
Malignant melanoma	8/10	80%
Tumors of uncertain histogenesis		
Haemangioblastoma (WHO grade I)	7/10	70%
Lymphomas and Hemopoietic Neoplasms	0/12	0%
Malignant lymphomas	0/10	0%
Plasmocytoma	0/2	0%
Metastatic tumors	2/11	18.2%
Small cell cancer of the lung	2/2	100%
Non-neoplastic lesions	0/56	0%
Focal epilepsies (Ammon's horn sclerosis)	0/15	0%
Inflammation	0/11	0%
Gliosis	0/30	0%

The cellular expression of MAP2 is precisely regulated during brain development as well as in mature nervous tissue [10, 16, 17]. In contrast to adult brain and spinal cord, in which MAP2A, MAP2B and MAP2C are confined to neurons, oligodendroglial precursor cells transiently produce an alternatively spliced isoform including exon 13 (MAP2E) [20]. This isoform is likely to occur also in astroglial precursors [4]. Major advantages using MAP2 immunoreactivity for the histopathological diagnosis, thus, comprise:(1) restricted physiological expression of high and low molecular weight isoforms within neuronal cell lineages of the adult nervous tissue, which is helpful for the differentiation of neoplastically transformed glial cells from reactive and preexisting glial cell populations in surgical specimen; (2) the propensity of MAP2 immunoreactivity to morphologically delineate cellular characteristics, i.e., shape of cell bodies and branching pattern of processes, which is helpful for pattern recognition in specific tumor entities; and (3) phenotypic and genotypic similarities between neoplastically transformed and immature glial precursor cells expressing the MAP2E isoform, which suggest an origin of neoplastic glial cells from immature precursor cells rather than from differentiated astrocytes and oligodendrocytes in the human brain [4].

This surveillance and the presence of various MAP2 isoforms in neuroepithelial neoplasms render the MAP2 epitope useful for the neuropathological diagnosis of brain tumors. However, some inconsistencies have been described for the distribution of MAP2 within human central nervous system neoplasms [4, 21]. To characterize MAP2 expression within the broad spectrum of neuroepithelial tumors versus non-neoplastic brain lesions, we report about an extensive series of histological specimens obtained from two large neuropathology centers in Germany.

Materials and methods

Surgical and biopsy specimens from 960 patients with neuroepithelial tumors (n=888), meningeal/mesenchymal tumors (n=29), tumors of uncertain origin (n=10), metastatic tumors and lymphomas (n=33), or non-neoplastic lesions (n=56) were retrieved from the archives of the Department of Neuropathology at the University of Bonn and University of Erlangen (Table 1). This series includes neoplasms submitted for review to the German Brain Tumor Reference Center (at the Department of Neuropathology, University of Bonn). Each specimen was classified according to World Health Organization guidelines [12] using hematoxylin and eosin staining as well as a panel of immunohistochemical reactions (see below and Fig. 1).

Immunohistochemistry

A systematic examination of tumor specimens was performed using a panel of immunohistochemical markers. This panel was adapted to the respective tumor entities and included: gliomas (MAP2, GFAP, p53, Ki67, CD34); neuronal tumors (MAP2, GFAP, p53, Ki67, Syn, NFP, CD34); embryonal tumors (MAP2, GFAP, p53, Ki67, Syn, NFP, S-100); meningiomas/mesenchymal tumors (MAP2, Ki67, Vim, EMA, S-100); primary melanocytic lesions (MAP2, S-100, Vim, Ki67); lymphomas (MAP2, CD20, CD3, Ki67); metastases (MAP2, Lu5); non-neoplastic lesions (MAP2, GFAP, CD68, Ki67).

The following immunohistochemical markers were used: glial fibrillary acidic protein (GFAP; clone GF2, DAKO), p53 (DAKO), Ki67 epitope (Mib1; Dianova), S100 (Swant, Bellinzona, Switzerland), panepithelial antigen (Lu5; DAKO), synaptophysin (SY38; DAKO), neurofilament protein (NFP; clone 2F11, DAKO), stem cell epitope CD34 (QBend10; Immunotech), vimentin (V9; DAKO), epithelial membrane antigen (DAKO), CD68 (DAKO). For detection of the microtubule-associated protein MAP2, we used the monoclonal antibody clone C (supplied by Dr. Riederer). Its specificity in human brain tumor samples has been extensively studied by in situ hybridization and Western blotting experiments [4]. Commercially available antibodies reacting with either high or low molecular weight isoforms of MAP2 were available from Sigma-Aldrich (clone HM-2^{10}) and from Chemicon (MAB364).

All biopsy samples were fixed overnight in 4% formalin and routinely processed into liquid paraffin. Sections were cut at 4 µm with a microtome (Microm, Heidelberg), stretched in hot water (60°C) and mounted on slides coated with 3-aminopropyltriethoxysilane (DAKO). The slides were air-dried in an incubator at 37°C overnight. The immunocytochemical staining was performed using the avidin-biotin method (Vector Labs, Burlingame, CA) and 3,3′-diaminobenzidine as chromogen. Microwave pretreatment was used for all immunohistochemical reactions with the exception of synaptophysin. Sections were analyzed by four observers (S.M., R.B., O.D.W., and I.B.) and the histopathological grading was based on the revised WHO classification system 2000 [12].

Results

For the purpose of our study, we examined MAP2 immunoreactivity in 1,019 surgical brain specimens using a monoclonal antibody directed against MAP2A, MAP2B and MAP2C isoforms (clone C). Previous studies demonstrated the specificity of this immunoreaction in paraffin-embedded histological sections as well as by Western blotting and in situ hybridization [4]. In the following description of cellular morphologies and pattern recognition, we will refer to this staining as MAP2.

MAP2 staining patterns in glio-neuronal neoplasms

The MAP2 epitope is a well-established marker for ganglion cells in the central nervous system, spinal cord, autonomous and vegetative nervous system. This also applies to glio-neuronal tumors, in which neuronal profiles were consistently immunolabeled with antibodies directed against the MAP2 epitope. In contrast, the glial component was virtually devoid of MAP2 staining. All central neurocytomas (WHO grade II) examined in this study (n=12) showed MAP2-immunoreactive neuronal processes and neuropil islands (Fig. 2C). The distinct neuronal cell component in dysembryoplastic neuroepithelial tumors (DNTs), i.e., floating neurons (Fig. 2B), were also labeled. Virtually no MAP2 expression was detectable in the neoplastic glial component (Fig. 2A, B). Only one DNT in our series expressed MAP2 in the oligodendroglia-like small cell population. Notwithstanding, the precise distinction of any MAP2-immunoreactive cell as being of glial or neuronal origin is difficult to obtain in some instances. Further characterization will, therefore, be manda-

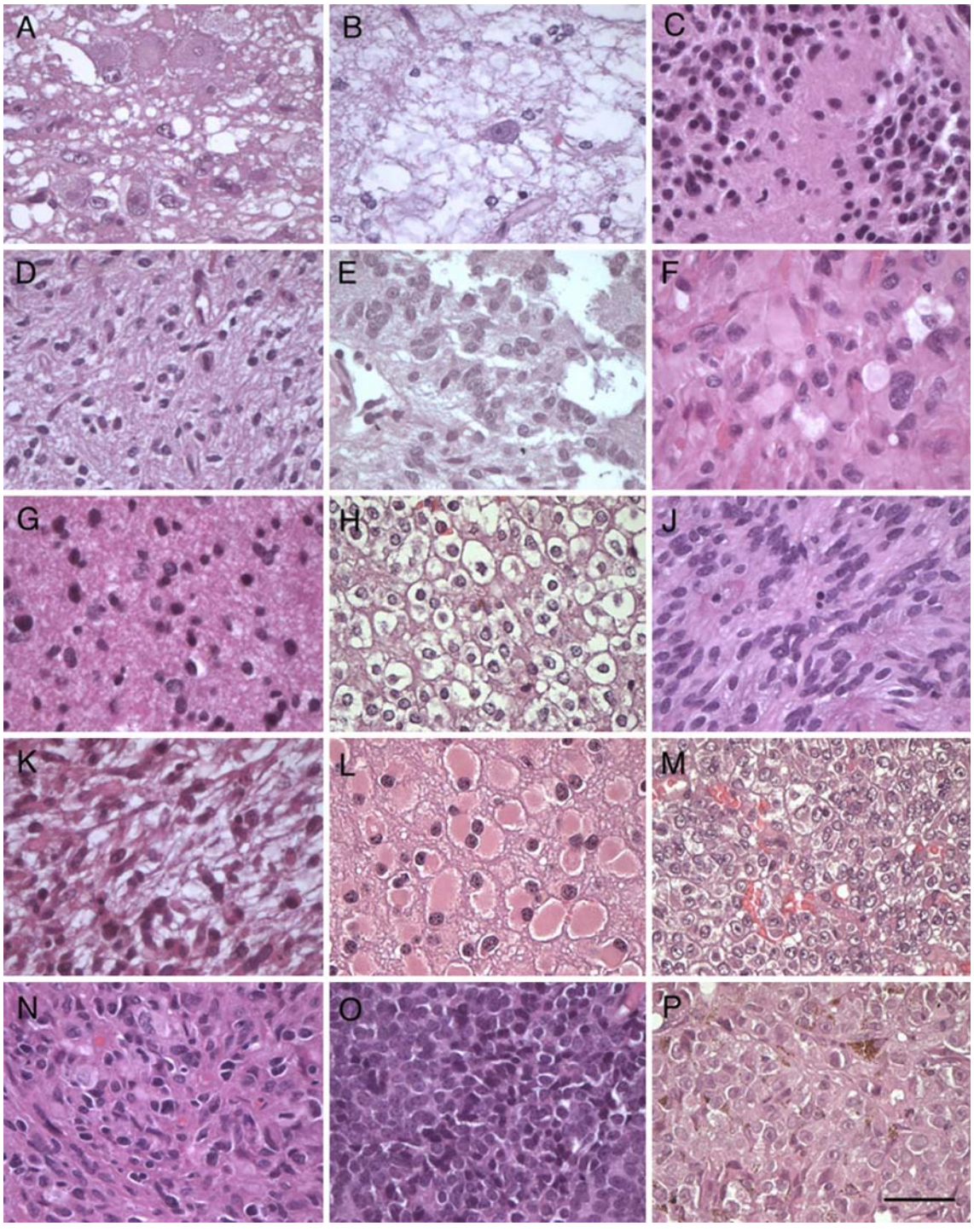


Fig. 1 Hematoxylin and eosin staining of major tumor entities included in this study. **A–C** Mixed glio-neuronal and neuronal tumors: **A** ganglioglioma (WHO grade I), **B** dysembryoplastic neuroepithelial tumor (*DNT*; WHO grade I), **C** central neurocytoma (WHO grade II). **D–F** Low-grade astrocytic tumors: **D** pilocytic astrocytoma (WHO grade I), **E** atypical pilocytic astrocytoma, pilomyxoid features in other parts of the tumor specimen (analogous to WHO grade II), **F** pleomorphic xanthoastrocytoma (WHO grade II). **G–J** Diffuse, low-grade gliomas: **G** diffuse astrocytoma (WHO grade II), **H** oligodendroglioma (WHO grade II), **J** ependymoma (WHO grade II). **K–M** Anaplastic gliomas: **K** anaplastic astrocytoma with process forming cell population in this area (WHO grade III), **L** anaplastic astrocytoma (in this ocular field, gemistocytic differentiation is prominent; WHO grade III), **M** anaplastic oligodendroglioma (WHO grade III). **N–P** Highly malignant neoplasms: **N** glioblastoma multiforme (WHO grade IV), **O** medulloblastoma (WHO grade IV), **P** cerebral metastasis of a melanoma. *Bar* in **P** (also for all other panels in Figs. 1 and 2) 50 µm

tory to unequivocally assign MAP2 expression in this tumor category.

MAP2 staining patterns in glial neoplasms

Samples of oligodendrogliomas (n=111) and mixed gliomas (n=126) were examined immunohistochemically. In 99.6% of these tumors, prominent MAP2 staining was observed in the majority of neoplastic cells. We failed to detect this immunoreaction in only a single case (Table 1). In particular, an oligodendroglial cell morphology with a predominant round, process-sparse architecture was easily recognized using MAP2 immunohistochemistry (Fig. 2H). Other cell elements displayed pseudounipolar morphologies or extended multiple processes; however, these latter morphologies were less dominantly observed in "pure" oligodendrogliomas compared to astrocytic gliomas (Fig. 2G, K). In addition, minigemistocytes were also found to be labeled. A consistent immunohistochemical MAP2 pattern was not detected in 428 tumor samples of astrocytic lineage. In these cases, the number and morphology of MAP2-positive tumor cells showed considerable variability and most cellular elements had significant ramifying cytoplasmic processes (Fig. 2G). Gemistocytes were weakly immunolabeled in many instances (Fig. 2L). Thus, significant differences can be described between astrocytic and oligodendroglial tumors using MAP2 immunohistochemistry. Distinctive features were also recognized in other glioma variants (see below).

Pilocytic astrocytomas (WHO grade I) were found to harbor MAP2 positive bi- or multipolar cellular elements. In our experience, these profiles appeared morphologically distinct from those described for diffuse gliomas: (1) in pilocytic astrocytomas, MAP2 expression was found only in a subpopulation of tumor cells, and (2) cellular profiles played often delicate processes and ramifications (Fig. 2D). In addition, a tumor variant with marked MAP2-positive cell clusters was identified (Fig. 2E). This subtype shared some histological features with the pilomyxoid variant of pilocytic astrocytomas and further studies are warranted to better characterize a potential association.

In contrast to astrocytomas and oligodendrogliomas, the majority of ependymal tumors were not labeled with antibodies against the MAP2 epitope (Fig. 2J). In our series of 59 ependymomas WHO grade II and III, only 31% revealed solitary MAP2-immunoreactive cells and these were not associated with the characteristic architectural feature of the tumor, i.e., ependymal rosettes or pseudorosettes.

Malignant glial tumors (n=396) were consistently immunoreactive for the MAP2 epitope (91%), including anaplastic astrocytomas, anaplastic oligodendrogliomas and glioblastomas. However, characteristic morphological features of the cellular architecture, as described for their low-grade counterparts, were often blurred due to an increased staining of the tumor matrix (Fig. 2N).

MAP2 staining patterns in embryonal neoplasms

MAP2 immunoreactivity was consistently recognized in embryonal brain tumors, i.e., medulloblastomas (Fig. 2O), supratentorial primitive neuroectodermal tumors (PNETs), or atypical teratoid/rhabdoid tumors (Table 1). This finding was in contrast to a more circumscribed expression of synaptophysin and neurofilament protein in these tumors. These neuronal proteins appear to be associated with an advanced stage of neuronal differentiation.

MAP2 staining patterns in meningeal/mesenchymal neoplasms

None of 10 meningiomas (WHO grade I or II) or other mesenchymal tumors included in this study (i.e., chondrosarcomas) were found to express MAP2 in histological specimens. On the other hand, approximately 50% of anaplastic meningiomas (WHO grade III) revealed MAP2 immunoreactivity. This finding has to be taken into consideration for the differential diagnosis of malignant tumor phenotypes.

MAP2 staining patterns in hemangioblastomas

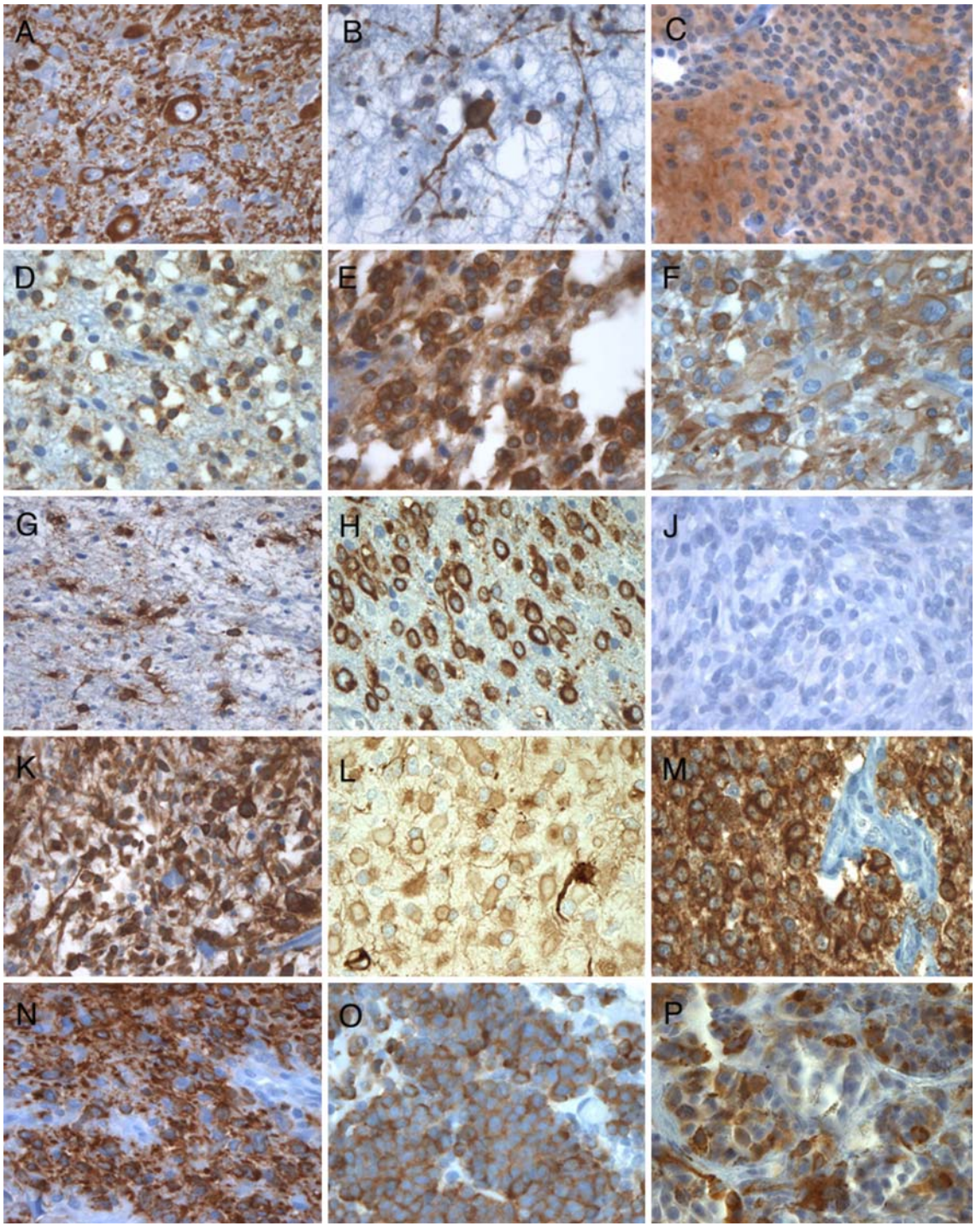
Seven out of 10 capillary hemangioblastomas (WHO grade I) showed patchy immunoreactivity for MAP2 within the population of vacuolated stromal cells. This distinct pattern had to be separated from neuronal profiles of cerebellar parenchyma, which was often entrapped at the tumor border. Tumors associated with von-Hippel-Lindau disease also revealed MAP2-immunoreactive tumor cell populations.

MAP2 staining patterns in metastatic neoplasms

Non-neuroectodermal neoplasms and metastatic tumors usually lack MAP2-immunoreactive neoplastic cell elements. Few exceptions have to be considered. Melanocytic neoplasms harbored immunoreactive cell populations to a variable extent (Fig. 2P). Furthermore, small cell carcinomas of the lung were immunoreactive for the MAP2 epitope. This may reflect the neural crest origin of these specific tumor entities.

MAP2 staining patterns in non-neoplastic, reactive lesions of the brain

MAP2 is abundantly expressed in neurons of the central nervous system, spinal cord and in ganglion cells of the autonomous and vegetative nervous system. We have studied reactive glia cells under various pathological conditions, including focal epilepsies, ischemia and encephalitis. None of the reactive astrocytic cell populations were found to be MAP2 immunoreactive. Lack of MAP2 ex-



pression in normal or reactive glia is very helpful in identifying neoplastic cell infiltrates in the subcortical white matter. This can be appreciated already in small stereotactic biopsies. However, tumor cells invading the neocortex were less specifically recognized due to the abundant MAP2 expression of neuropil and neuronal profiles.

Discussion

A total of 826 out of 960 tumors (86%) showed immunoreactivity for the neuronal marker protein MAP2 within its major neoplastic cell population. A positive MAP2 reaction was observed in the vast majority of glial tumors (90.4%), whereas only a minority of mesenchymal (meningeal) neoplasms and virtually no carcinoma metastases or lymphomas expressed MAP2. The lack of glial MAP2

◀ **Fig. 2** MAP2 Immunohistochemistry in tumors of the nervous system. All images represent corresponding areas of the tumor specimen depicted in Fig. 1. **A** Ganglioglioma (WHO grade I). MAP2 immunoreactivity is confined to highly differentiated neuronal cell elements and to the neuropil matrix. Neoplastic glial cells are not labeled with MAP2 antibodies. **B** DNT (WHO grade I). The small cell, "oligodendroglia-like" component of this tumor entity consistently lacks MAP2 immunoreactivity, whereas floating neurons and their neuronal processes are clearly demarcated. **C** Central neurocytoma (WHO grade II). MAP2 immunoreactivity is detectable in the neuronal component, preferentially in neuropil islands. **D** Pilocytic astrocytoma (WHO grade I) with MAP2 labeling of small, neoplastic glial cells and their delicate cytoplasmic branches. **E** Atypical pilocytic astrocytoma (analogous to WHO grade II). Multipolar tumor cells with clustering and strong MAP2 expression were observed in this variant. **F** Pleomorphic xanthoastrocytoma (WHO grade II). Weak to moderate reactivity for MAP2 was noted in the majority of the neoplastic, pleomorphic glial cells. **G** Diffuse astrocytoma (WHO grade II). Some tumor cells stain with antibodies directed against MAP2. In contrast to tumor cells of oligodendroglial lineage, astrocytomas exhibit bi- or multipolar processes. **H** Oligodendroglioma (WHO grade II). The characteristic MAP2 immunoreactivity pattern with prominent perinuclear cytoplasmic labeling of small, rounded tumor cells with only few, unipolar processes. **J** Ependymoma (WHO grade II). Ependymomas usually lack MAP2 staining. **K** Anaplastic astrocytoma (WHO grade III). This tumor shows abundant MAP2-immunoreactive elements. Labeled tumor cells exhibit processes of various morphologies. **L** Anaplastic astrocytoma (WHO grade III) with a prominent gemistocytic phenotype (see Fig. 1L). Gemistocytic glioma cells are also immunoreactive for MAP2, although with weak intensities. However, intermingled tumor cells resembling the characteristic pattern described for oligodendroglioma are detected in many astroglial neoplasms. Thus, MAP2C pattern recognition requires careful analysis in high-grade gliomas. **M** Anaplastic oligodendroglioma (WHO grade III). MAP2 immunostaining is present in round tumor cells and within the tumor matrix. **N** Glioblastoma multiforme (WHO grade IV). The majority of the tumor cells with various morphologies are MAP2 positive. **O** Medulloblastoma (WHO grade IV). Cytoplasmic MAP2 immunoreactivity (lacking prominent process formation) are detected in the majority of embryonal tumors. **P** Melanoma metastasis. Tumor cells are immunoreactive for MAP2, although to a variable extend

expression in non-tumorous lesions of the brain can be helpful in recognizing and discriminating neoplastic cells from reactive astrocytes. In addition to the abundance of MAP2 in neuroepithelial tumors, which has previously been reported in several independent studies [4, 14, 21], MAP2 immunoreactivity revealed characteristic cellular morphologies and is a valuable parameter for pattern recognition in low-grade gliomas.

Histopathological pattern recognition serves as an important diagnostic tool to identify neoplastically transformed cell populations within a complex tumor phenotype [11]. Our current understanding of glioma cell development is very much influenced by lineage determination into astrocytic, oligodendrocytic and ependymal progenies. Characteristic morphological profiles of these particular cell types relate to process formation with fibrillary or process-sparse phenotypes, cytoplasmic properties, i.e., clear cell vs gemistocytic morphologies, as well as growth patterns and rhythmic architectures. The immunohistochemical detection of GFAP protein is routinely used for the diagnosis of astrocytomas, ependymomas and glioblastomas. However, GFAP does not reliably differentiate between neoplastic and non-transformed glial cells. With the observation of a consistent expression of MAP2, the panel of immunohistochemical markers suitable for pattern recognition in neuroepithelial tumors could be extended. Recent studies confirmed the expression of a novel embryonic isoform of the MAP2 protein in neoplastically transformed glial cells [4, 20, 21]. The protein, which has now been recognized as MAP2E [21], contains exon 13, which is spliced in adult MAP2 isoforms, i.e., MAP2A–D. However, MAP2A–C are abundantly expressed in differentiated neurons, whereas MAP2E is restricted to glial precursors and glial neoplasms [8, 20]. It can be hypothesized, that this isoform is related to process formation and cell migration, the latter being a characteristic feature of both cell populations, i.e., glial precursors and neoplastically transformed glioma cells.

The differential diagnosis of low-grade gliomas includes astrocytic gliomas, oligodendrogliomas, mixed gliomas, ependymomas, and a number of glio-neuronal tumors, such as DNT, gangliogliomas and central neurocytomas. Many histopathological studies were carried out to identify specific features discriminating the tumor entity and biological behavior. Whereas ultrastructural studies have been very helpful in identifying features of ependymal and neuronal cell lineages, these extensive/expensive techniques have not reached routine application in most laboratories. Recent advances in molecular neuropathology may provide valuable new tools. Oligodendrogliomas with allelic loss of chromosome 1p and 19q appear to respond favorably to polychemotherapy [13, 15], atypical teratoid and rhabdoid tumors can be differentiated from PNETs by mutations of the INI1 gene [2]. For routine diagnostics, immunohistochemical reactions still remain mandatory. However, most markers recognized for neuroepithelial tumors during the last decade are not tumor cell specific. GFAP is not only expressed by neoplastic astrocytes but also in minigemistocytic and glio-fibrillary oligodendroglioma cells, as well as reactive non-neoplastic astrocytes. Earlier studies have addressed the potential for neuronal cell differentiation in oligodendrogliomas. Indeed, few publications have identified neuronal marker proteins in oligodendrogliomas, such as neurofilament or MAP2 [9, 14]. However, we interpret MAP2 expression in these tumors not as an indicator for a neuronal phenotype but rather as a marker for glial progenitors. This relationship was recently confirmed by studies of the MAP2E isoform, which is transiently expressed in oligodendroglial precursors of the developing human and murine brain as well as in various glial tumors [21].

The data obtained in our present study render MAP2 immunoreactivity as a useful parameter for the differential diagnosis of glial neoplasms. Major findings include the following:

1. Oligodendrogliomas (with their characteristic honeycomb appearance) are consistently immunolabeled with MAP2 antibodies (Table 1); most tumor cells show a process-sparse morphology (Fig. 2H).
2. Astrocytomas, either diffuse or pilocytic, display MAP2-immunoreactive tumor cells with bipolar or multipolar ramification patterns, suitable for differentiating both biological entities (Fig. 2).

3. Ependymomas contain only a minor fraction of MAP2-immunoreactive cells (Table 1, Fig. 2J).
4. Benign glio-neuronal tumors do not express MAP2 within the glial component (Fig. 2A–C).
5. The pattern and extent of MAP2-immunoreactive cells tend to become more variable in malignant gliomas of WHO grade III or IV (Fig. 2K–O).

However, some exceptions have to be noted. In our experience, many oligodendrogliomas show areas with MAP2-positive process-bearing cells. It is difficult to exclude an admixture of astrocytic tumor cells in these cases; GFAP immunoreactivity remain very helpful in these circumstances. The morphological variability of MAP2-immunoreactive cells in pilocytic astrocytomas appears remarkable. A distinct subtype of pilocytic astrocytomas exhibiting small cell clusters was extensively immunoreactive for MAP2 and lacked prominent cell processes. These cases may refer to the pilomyxoid variant of pilocytic astrocytomas, but this notion has to be confirmed in further studies. In addition, we have recently identified a diffuse astrocytoma subtype lacking MAP2 immunoreactivity [5]. These tumors were consistently associated with long-term epilepsy, had a very benign clinical course and survival periods beyond 10 years without recurrence [18]. Ependymal tumors may contain occasional MAP2-positive neoplastic cells, either close to rosettes or dispersed within the tumor matrix, but these elements always constitute a minority of tumor cells. Glio-neuronal tumors display a strongly MAP2-immunoreactive neuronal component, which can always be identified. A small subset of these tumor specimens also reveals a cellular phenotype with small cytoplasm and extensive process arborization. We have not yet confirmed the neuronal nature of this cell population. However, the majority of glial cells, which are considered to represent the neoplastic cell component, do not express MAP2. Diffusely infiltrating MAP2-immunoreactive glioma cells can be recognized in small biopsy samples obtained from white matter. This feature facilitates the identification of infiltrating glioma cells. To our knowledge, the only exception are oligodendroglial progenitors in multiple sclerosis plaques [19] or in HIV encephalitis [8].

A recent study of MAP2E immunoreactivity in 81 adult and 42 pediatric brain tumors described a positive reaction in ependymomas (n=4) and DNT (n=1) [21]. Whereas our significantly larger series did not reveal prominent labeling of neoplastic glial cell elements in ependymomas and DNT, MAP2E appeared to be expressed in these cases. A potential explanation is that the embryonic isoform shows a distribution distinct from MAP2A–C. However, further studies from different laboratories should extend these findings, and also help to clarify the cellular nature and origin of MAP2-immunoreactive cells in low-grade neuroepithelial tumors.

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