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SHORT ORIGINAL COMMUNICATION

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Sensitivity and specificity of epithelial membrane antigen staining patterns in ependymomas

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Abstract Pattern and extent of epithelial membrane antigen (EMA) immunoreactivity in ependymomas as compared to other glial tumors have only been investigated in small series. To determine sensitivity and specificity of EMA staining, 54 ependymomas were evaluated in comparison to 54 glioblastomas, 43 fibrillary astrocytomas and 21 oligodendrogliomas. Distinct punctate intracytoplasmic EMA immunoreactivity was observed in 48/54 ependymomas (89%), whereas ring-like EMA staining was observed in 17/54 ependymomas (31%). Apart from the absence in most myxopapillary ependymomas, neither staining pattern was related to tumor grade or localization. Dot-like EMA immunoreactivity was less frequently observed in glioblastomas [32/54 (59%), $P<0.05$ vs ependymomas], fibrillary astrocytomas [10/43 (23%), $P<0.001$ vs ependymomas] and oligodendrogliomas [2/21 (10%), $P<0.001$ vs ependymomas], whereas ring-like EMA staining was absent. Sensitivity and specificity of punctate EMA staining for the diagnosis of ependymoma as compared to other glial tumors were determined: A finding of 5 EMA dots/high-power field was associated with a sensitivity of 72% and a specificity of 81%. The presence of ring-like EMA positive structures was less sensitive (32%), but highly specific (100%). To conclude, distinct punctate and ring-like EMA staining might serve as sensitive and specific markers of ependymal differentiation in glial tumors and, thus, may aid the diagnosis of ependymoma.

Keywords Ependymoma · Epithelial membrane antigen · Immunohistochemistry · Diagnosis

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Introduction

The diagnosis of ependymoma is usually straightforward in glial tumors with abundant formation of perivascular pseudorosettes or ependymal rosettes. However, diagnostic difficulties may be encountered when ependymal differentiation is less obvious. Epithelial membrane antigen (EMA), a highly glycosylated transmembrane protein, is a diagnostic marker for epithelial differentiation also expressed by normal ependymal cells [3, 12]. In ependymomas, punctate EMA immunoreactivity reflecting intracytoplasmic lumina, as well as ring-like intracytoplasmic or luminal EMA expression have been described [1, 2, 3, 4, 7, 8, 11, 13]. However, the extent of ependymal EMA staining patterns as compared to other glial tumors has only been investigated in small series [2, 11]. Moreover, sensitivity and specificity of EMA staining patterns, which is a prerequisite for routine diagnostic use, have not been determined. Therefore, the aim of the present study was to investigate pattern and extent of EMA immunoreactivity in ependymomas as compared to other glial tumors.

Materials and methods

Formalin-fixed and paraffin-embedded specimens of 54 ependymomas (34 male patients, 20 female; mean age 38 years, range 1–82 years) as well as 54 glioblastomas, 43 fibrillary astrocytomas (WHO grade II), 15 anaplastic oligodendrogliomas (WHO grade III) and 6 oligodendrogliomas (WHO grade II) were investigated. All tumors were diagnosed and graded according to WHO criteria. Sections, 2 µm thick, were stained using a monoclonal antibody against EMA (clone E29, 1:400, Dako) and the streptavidin-biotin method on an automated staining system (TechMate, Dako). Dot-like EMA immunoreactivity was quantified by counting at least five non-overlapping high-power fields (HPF, ×400 magnification, area of visual field). Statistical analysis was done by chi-square test or one-way analysis of variance by ranks (Kruskal-Wallis-ANOVA) followed by Mann-Whitney U-test for post hoc comparisons using the Statistica software package. A P value of less than 0.05 was considered significant.

Results

As summarized in Table 1, 31/54 ependymomas (57%) were of spinal localization, 16/54 (30%) were localized in the posterior fossa and 7/54 (13%) supratentorially. The diagnostic criteria for anaplastic ependymoma were met by 13 tumors (24%). Among the 35 grade II tumors, there were 2 papillary ependymomas, 2 tancytic ependymomas and 1 clear cell ependymoma. Myxopapillary ependymomas (n=6) accounted for 19% of spinal tumors.

Table 1 Localization and tumor grade of 54 ependymomas

Localization	Grade III	Grade II	Grade I
Supratentorial	5	2	0
Posterior fossa	7	9	0
Spinal	1	24	6
Total	13	35	6

EMA immunoreactivity was present in 48/54 ependymomas (89%). Distinct dot-like intracytoplasmic EMA immunoreactivity was observed in the majority of tumors (48/54, Fig. 1a). Additionally, intracytoplasmic or luminal ring-like EMA staining was observed in 17/54 tumors (31%, Fig. 1b). EMA immunoreactivity was also found on luminal surfaces of ependymal linings (4/54). Apart from their absence in most myxopapillary ependymomas, neither staining pattern was related to tumor grade or localization. Moreover, quantification of dot-like EMA immunoreactivity did not reveal differences between grade II and grade III ependymomas [median (quartiles): 18 (5–51) vs 25 (9–71) dots/HPF].

EMA immunoreactivity was less frequently observed in glioblastomas (32/54, P<0.05 vs ependymomas), fibrillary astrocytomas (10/43, P<0.001 vs ependymomas) and oligodendrogliomas (2/21, P<0.001 vs ependymomas). In astrocytic tumors, EMA staining was mostly restricted to diffuse staining of pleomorphic cells, sometimes with patchy perinuclear accentuation (Fig. 1c). Distinct dot-like intra-

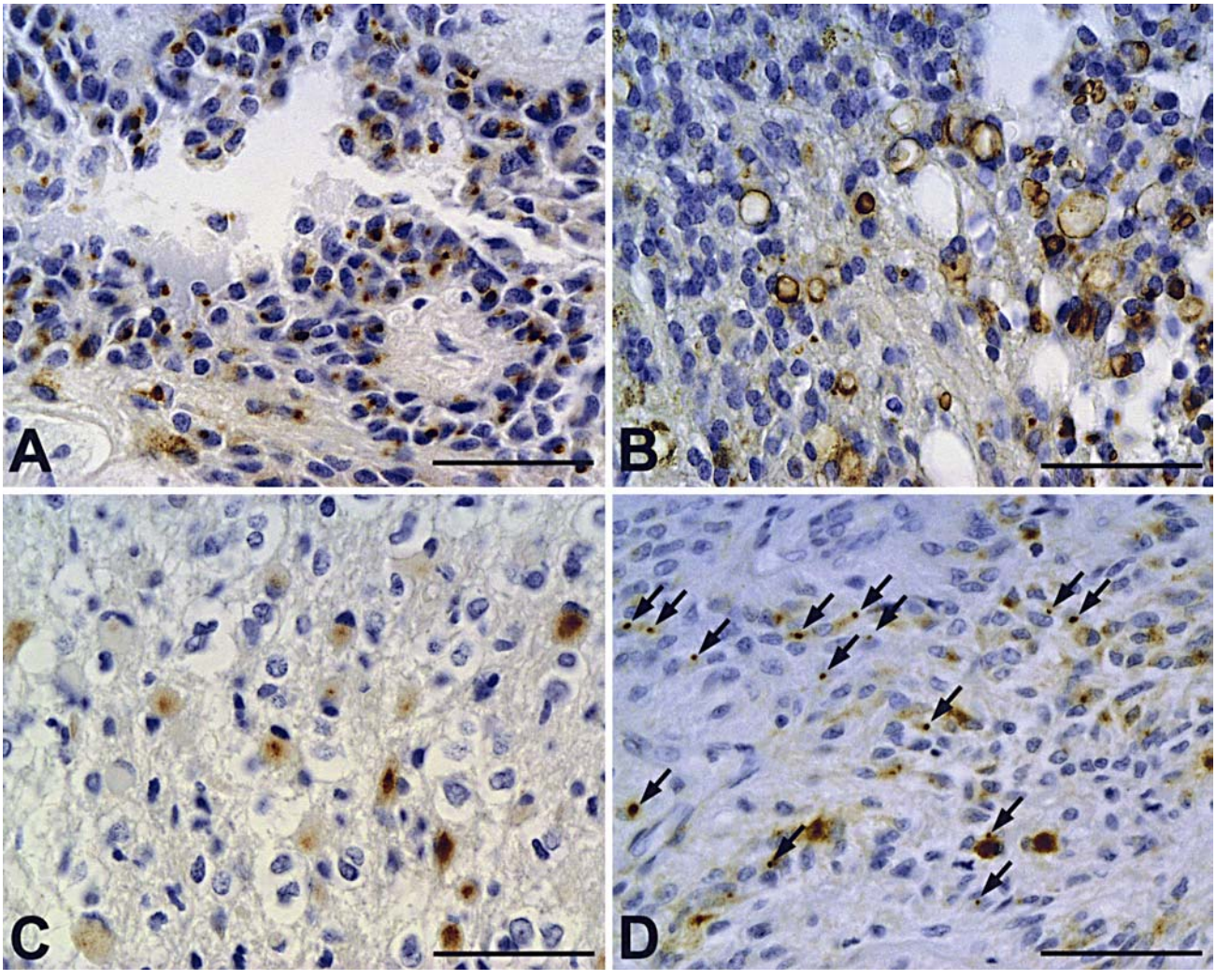

Fig. 1 EMA staining patterns in ependymomas and astrocytic tumors. Distinct punctate intracytoplasmic immunoreactivity (**A**) and ring-like staining (**B**) in ependymomas as compared to diffuse staining of pleomorphic cells, sometimes with patchy perinuclear accentuation in a fibrillary astrocytoma (**C**) and a glioblastoma (**D**). Note the additional presence of distinct punctate intracytoplasmic immunoreactivity in the glioblastoma (*arrows*). Sections counterstained with hematoxylin (*EMA* epithelial membrane antigen). *Bars* 100 µm

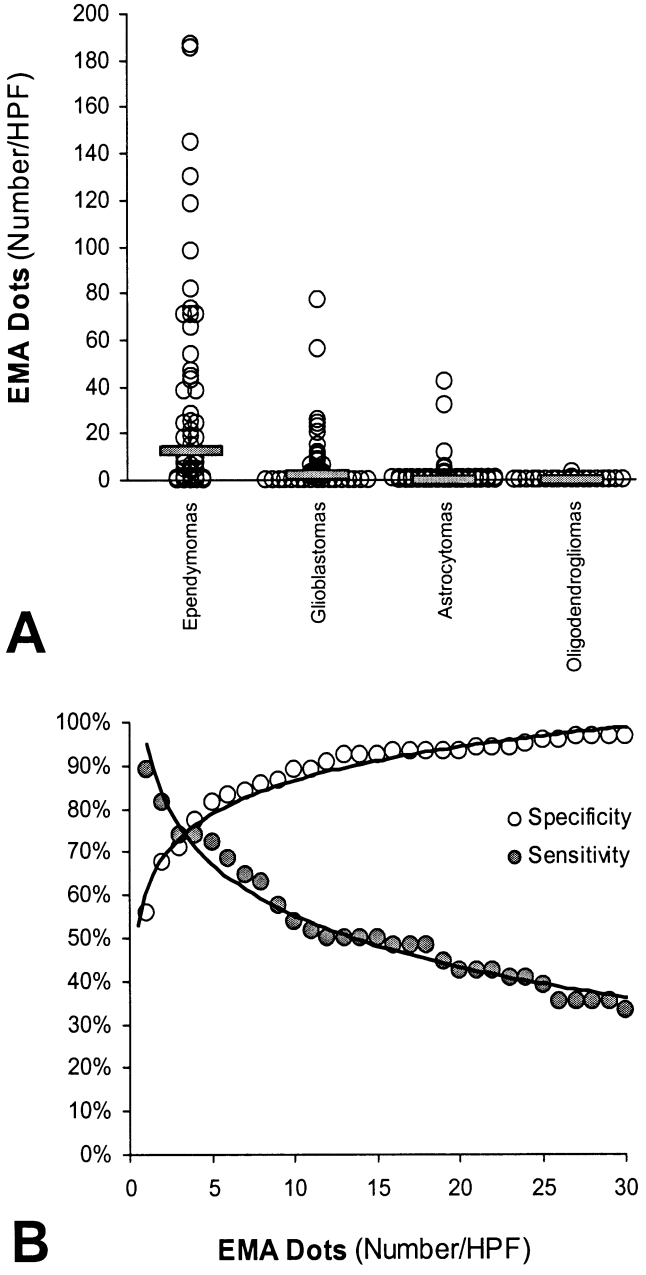

Fig. 2 **A** Extent of punctate dot-like EMA staining in ependymomas (n=54) as compared to glioblastomas (n=54), astrocytomas (n=43) and oligodendrogliomas (n=21). Individual data (*circles*) and medians (*bars*) are presented. **B** Sensitivity and specificity of punctate dot-like EMA staining for the diagnosis of ependymoma as compared to other glial tumors according to the number of EMA dots/HPF (*HPF* high-power field)

cytoplasmic staining was rarely noted in astrocytomas, but occasionally observed in glioblastomas (Fig. 1d). The number of EMA dots/HPF was significantly lower in glioblastomas [median (quartiles): 2 (0–7), P<0.001], astrocytomas [0 (0–0), P<0.001] and oligodendrogliomas [0 (0–0), p<0.001] as compared to ependymomas (Fig. 2a). Ring-like EMA staining was not encountered in any of the astrocytic or oligodendroglial tumors. Sensitivity and specificity of punctate EMA staining for the diagnosis of ependymoma as compared to other glial tumors were determined according to the number of EMA dots/HPF. As shown in Fig. 2b, a finding of 5 EMA dots/HPF was associated with a sensitivity of 72% and specificity of 81%. The presence of ring-like EMA positive structures was less sensitive for the diagnosis of ependymoma (32%) but highly specific (100%).

Discussion

In the present series, the majority of 54 ependymomas displayed EMA immunoreactivity, a finding that is mainly due to the presence of dot-like punctate intracytoplasmic staining, the „EMA dot". The proportion of ependymomas displaying this staining pattern, which probably reflects formation of intracytoplasmic microlumina, is higher than previously reported [2, 3, 12, 13]. In all of these studies the monoclonal antibody E29 has been employed. Therefore, even though differences in methodology cannot be entirely ruled out, it seems most likely that the extent of dot-like EMA immunoreactivity might be underestimated unless several HPF are scrutinized. In contrast to previous findings of increased [8] or decreased [1, 12, 13] EMA staining in anaplastic ependymoma, no association between presence or extent of EMA immunoreactivity and grade of malignancy was noted in the present study. The occasional presence of distinct dot-like EMA immunoreactivity in high-grade astrocytic tumors in addition to diffuse staining with granular accentuation [2, 5] has not been reported previously and might reflect aberrant ependymal differentiation. Even though this study focuses on the sensitivity and specificity of dot-like EMA staining for the diagnosis of ependymoma as compared to other glial tumors, it has to be kept in mind that EMA staining has been described in a variety of other tumors, e.g., meningioma, chordoid glioma of the third ventricle, rhabdoid tumor or chordoma, albeit as membranous or diffuse staining patterns [6, 9, 10].

To conclude, punctate and ring-like EMA staining might serve as sensitive and specific markers of ependymal differentiation in glial tumors, and thus may aid the diagnosis of ependymoma.

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References

1. Cruz-Sanchez FF, Rossi ML, Esiri MM, Reading M (1988) Epithelial membrane antigen expression in ependymomas. Neuropathol Appl Neurobiol 14:197–205
2. Cruz-Sanchez FF, Garcia-Bachs M, Rossi ML, Rodriguez-Prados S, Ferrer I, Coakham HB, Ferreres JC, Figols J, Palacin A (1992) Epithelial differentiation in gliomas, meningiomas and choroid plexus papillomas. Virchows Arch [B] 62:25–34

3. Figarella-Branger D, Gambarelli D, Dollo C, Devictor B, Perez-Castillo AM, Genitori L, Lena G, Choux M, Pellissier JF (1991) Infratentorial ependymomas of childhood. Correlation between histological features, immunohistological phenotype, silver nucleolar organizer region staining values and post-operative survival in 16 cases. Acta Neuropathol 82:208–216
4. Hirato J, Nakazato Y, Iijima M, Yokoo H, Sasaki A, Yokota M, Ono N, Hirato M, Inoue H (1997) An unusual variant of ependymoma with extensive tumor cell vacuolization. Acta Neuropathol 93:310–316
5. Hitchcock E, Morris CS (1987) Cross reactivity of anti-epithelial membrane antigen monoclonal for reactive and neoplastic glial cells. J Neurooncol 4:345–352
6. Ho DM, Hsu CY, Wong TT, Ting LT, Chiang H (2000) Atypical teratoid/rhabdoid tumor of the central nervous system: a comparative study with primitive neuroectodermal tumor/medulloblastoma. Acta Neuropathol 99:482–488
7. Ho KL, Caccamo DV, Garcia JH (1994) Intracytoplasmic lumina in ependymomas: an ultrastructural study. Ultrastruct Pathol 18:371–380
8. Kaneko Y, Takeshita I, Matsushima T, Iwaki T, Tashima T, Fukui M (1990) Immunohistochemical study of ependymal neoplasms: histological subtypes and glial and epithelial characteristics. Virchows Arch [A] 417:97–103
9. Miettinen M (1984) Chordoma. Antibodies to epithelial membrane antigen and carcinoembryonic antigen in differential diagnosis. Arch Pathol Lab Med 108:891–892
10. Reifenberger G, Weber T, Weber RG, Wolter M, Brandis A, Kuchelmeister K, Pilz P, Reusche E, Lichter P, Wiestler OD (1999) Chordoid glioma of the third ventricle: immunohistochemical and molecular genetic characterization of a novel tumor entity. Brain Pathol 9:617–626
11. Takeuchi H, Kubota T, Sato K, Llena JF, Hirano A (2002) Epithelial differentiation and proliferative potential in spinal ependymomas. J Neurooncol 58:13–19
12. Uematsu Y, Rojas-Corona RR, Llena JF, Hirano A (1989) Distribution of epithelial membrane antigen in normal and neoplastic human ependyma. Acta Neuropathol 78:325–328
13. Vege KD, Giannini C, Scheithauer BW (2000) The immunophenotype of ependymomas. Appl Immunohistochem Mol Morphol 8:25–31