Diagnostic Value of EAAT-1 and Kir7.1 for Distinguishing Endolymphatic Sac Tumors From Choroid Plexus Tumors

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Abstract

The endolymphatic sac tumor (ELST) is a low-grade carcinoma originating in the ear. These extremely rare tumors are capable of invading the cerebellopontine angle and might be mistaken for choroid plexus tumors (CPTs) in this region. Currently, these tumors are distinguished by conventional morphologic and immunohistochemical studies for S-100, cytokeratin, and GFAP expression, but all markers are variably expressed by both tumors. Therefore, we examined new promising markers such as EAAT-1 and Kir7.1 in 4 ELSTs and 35 CPTs located in the fourth ventricle or at the cerebellopontine angle in adults. Immunohistochemical expression of Kir7.1 was found in 30 (100%) of 30 and EAAT-1 in 32 (91%) of 35 CPTs tested and was absent in all ELSTs. Expression of GFAP was found in 16 (55%) and S-100 in 29 (100%) of 29 CPTs tested, but both markers were also expressed in 2 of 4 ELSTs examined. Specificity and sensitivity of Kir7.1 (both 100%) and EAAT-1 (100% and 91%, respectively) were superior to the values for S-100 (50% and 100%, respectively) and GFAP (50% and 55%, respectively) for distinguishing CPT from ELST.

Endolymphatic sac tumors (ELSTs) are low-grade carcinomas originating from the intraosseous portion of the endolymphatic sac epithelium. These tumors usually do not metastasize but invade the petrous part of the temporal bone and might protrude into the brain at the cerebellopontine angle. These rare tumors are neoplasms of adults and are associated with von Hippel-Lindau disease.1 Consistent with the tumor location, approximately one third of affected patients have vertigo, hearing loss, and tinnitus. Tumor growth in the posterior fossa might produce cerebellar symptoms and facial nerve palsy.

Histologic examination shows that ELST consists of papillary to glandular epithelia of low proliferative activity. Especially papillary formations with a single row of cuboidal epithelial cells resemble choroid plexus papillomas. Similarly, they express cytokeratins and glial fibrillary acidic protein (GFAP). Unless clear portions with dilated glands with colloid-like inclusions are present, a confident diagnosis of ELST might be difficult.2 Choroid plexus tumors (CPTs) might show atypical features such as an increased mitotic count and elevated proliferation activity, further blurring diagnostic distinction from ELSTs, which, in turn, compared with other papillary carcinomas, have low proliferative activity.

Studies have demonstrated that the inward-rectifier potassium channel Kir7.1 is present in normal choroid plexus epithelia4 and CPTs5 but absent in papillary ependymomas. Another marker of diagnostic potential is the excitatory amino acid transporter EAAT-1, which is expressed in CPT but absent in normal adult choroid plexus.6 Gliarial cells, predominantly astrocytes, contribute to the regulation of extracellular...
glutamate concentrations via reuptake of glutamate through glutamate transporters. The expression of GFAP and EAAT-1 in neoplastic choroid plexus epithelium results from glial maturation, a specialization of primitive neuroepithelial precursor cells, and is usually reactivated in reactive glial tissue and activated microglia in the central nervous system.

We studied the immunohistochemical expression of Kir7.1 and EAAT-1 in CPTs and ELSTs to determine their usefulness as markers in the diagnosis and analysis of these diseases and compared their expression with previously established markers GFAP and S-100. Our results indicate that EAAT-1 and Kir7.1 are highly useful for diagnosing and for distinguishing CPT from ELST.

Materials and Methods

Case Selection

In total, paraffin-embedded samples of 4 surgically removed ELSTs (from 3 men and 1 woman; mean age, 33 years; range, 25-56 years) were selected according to their location in the cerebellopontine angle. In addition, 75 CPTs originating in other regions than the fourth ventricle or cerebellopontine angle were excluded in the first round. Because ELST is a neoplasm of adults, all pediatric CPTs were also excluded in the next step. Finally, all choroid plexus carcinomas were also excluded because ELSTs are low-grade adenocarcinomas and have no malignant histologic appearance.

Among the total 35 remaining cases, 25 were classified as choroid plexus papilloma, World Health Organization (WHO) grade I (from 14 men; mean age, 43 years; range, 27-67 years; and 11 women; mean age, 45 years; range, 24-74 years) and 10 as atypical choroid plexus papilloma, WHO grade II (from 7 men; mean age, 55 years; range, 44-68 years; and 3 women; mean age, 53 years; range, 23-74 years) according to the latest WHO classification. All CPTs were screened for nuclear presence of BAF47/INI-1 to exclude Kir7.1+ atypical teratoid/rhabdoid tumor.11 All samples except 1 external case were retrieved from the archives of the Department of Neuropathology, University Clinic Tübingen, Tübingen, Germany.

Immunohistochemical Analysis

Samples were immunostained for Kir7.1 expression (polyclonal rabbit, dilution 1:6,00012), mouse monoclonal EAAT-1 (dilution 1:100; Novocastra, Newcastle upon Tyne, England), polyclonal GFAP (dilution 1:500; DakoCytomation, Glostrup, Denmark), polyclonal S-100 (dilution 1:2,500; DakoCytomation), and MIB-1 (dilution 1:100; DakoCytomation) using an automated immunohistochemical slide staining system (BenchMark, Ventana Medical Systems, Tucson, AZ). The automated standard protocol is based on an indirect biotin-avidin system that uses a universal biotinylated immunoglobulin secondary antibody and diaminobenzidine as substrate. The sections were counterstained with hematoxylin.

Case Evaluation

Because Kir7.1 expression was completely present or absent in all tumor cells, Kir7.1 was defined as positive if more than 1% of the neoplastic cells exhibited staining; otherwise, tumors were considered negative. The number of positive cells for cytoplasmic GFAP and S-100 were quantified as follows: 0, absent; 1, fewer than 1% positive cells; 2, 1% to 10% positive cells; 3, 11% to 20% positive cells; and 4, more than 20% positive cells, using a previously established scoring system.6 Membranous EAAT-1 and nuclear MIB-1 immunoreactivity was determined as the percentage index of positive cells in the complete tumor area analyzed. For GFAP and S-100, cytoplasmic staining was analyzed; for Kir7.1 and EAAT-1, only membranous staining was analyzed because these are membrane-based proteins.

Contingency analysis followed by Pearson χ² testing was used to compare distribution of positive and negative tumors between CPT WHO tumor grades using the JMP statistical tool (SAS, Cary, NC). Photomicrographs of selected cases were generated on an Olympus BX50 microscope (Olympus, Center Valley, PA) using the ProgResC10+ digital camera system (JenOptik, Jena, Germany) and mounted together with Corel Draw X3 (Corel, Ottawa, Canada).

Results

Of 4 ELST cases, 2 were GFAP+, and the other 2 were S-100-. Expression of Kir7.1 and EAAT-1 was completely absent in all ELSTs. The MIB-1 index in ELSTs ranged from 2% to 5% (mean, 3%).

In CPTs, membranous expression of Kir7.1 was present in 30 (100%) of 30 tumors; EAAT-1 was noted in 32 (91%) of 35 tumors; S-100 was found in 29 (100%) of 29 tumors; and GFAP was observed in 16 (55%) of 29 tumors. For detailed immunohistochemical results in choroid plexus papillomas, see Table I. All 3 negative EAAT-1 cases (staining index, 0%) had a diagnosis of WHO grade I choroid plexus papilloma. GFAP– tumors (score 0) were found in WHO grade I (10/24 [42%]) and WHO grade II (5/9 [56%]) CPTs. Expression of EAAT-1 (P = .28) and GFAP (P = .45) did not significantly differ between grade I and grade II CPTs.

Expression of GFAP had a specificity of 50% and a sensitivity of 55% (positive predictive value [PPV], 89%; negative predictive value [NPV], 13%) for distinguishing between CPT and ELST. Expression of S-100 had also a specificity of 50% but a sensitivity of 100% (PPV, 93%; NPV, 100%).
**Image 1** H&E and immunohistochemical stains of an endolymphatic sac tumor (ELST, left) that extended to the right cerebellopontine angle in a 27-year-old man and a choroid plexus papilloma (CPT), World Health Organization grade I (right), located in the left cerebellopontine angle in a 64-year-old woman. Both exhibit a predominant papillary growth pattern (H&E) and express S-100, but they lack glial fibrillary acidic protein (GFAP). In contrast with strong membranous excitatory amino acid transporter (EAAT)-1 and Kir7.1 expression in CPT, expression of these markers was absent in ELST (all images x100).
Expression of EAAT-1 had a specificity of 100% and a sensitivity of 91% (PPV, 100%; NPV, 57%), and expression of Kir7.1 had a specificity and a sensitivity of 100% (PPV and NPV, each 100%).

Discussion

Among the large number of differential diagnoses of tumors located in the cerebellopontine angle (reviewed by Bonneville et al13), the distinction between CPTs and papillary ependymomas is usually not difficult in histologic specimens when ependymal rosettes are present. In complicated cases, molecular data might be helpful for establishing the diagnosis.14 In contrast, CPT and the rare ELST are epithelial tumors of low mitotic and proliferative activity. Both tumors exhibit papillary formations of a single-layered cuboidal ependymal epithelium and, thus, might be misdiagnosed, especially when glandular formations, more typically of ELST, are absent.15

Current guidelines recommend immunohistochemical studies for cytokeratin and GFAP to secure the diagnosis of ELST.2 However, the diagnostic value of both markers is limited because cytokeratin is usually also expressed by CPTs, and GFAP is not consistently found in CPTs.16,17 Microarray-based and immunohistochemical studies have identified new markers such as Kir7.1, EAAT-1, and stanniocalcin as highly suitable markers for identifying CPTs. Unfortunately, these studies did not include ELST as a possible differential diagnosis, and, furthermore, the CPT samples consisted mainly of CPTs of the lateral ventricles.5,17

Because stanniocalcin antibodies for diagnostic purposes are no longer available, we focused our current analysis on Kir7.1 and EAAT-1. We found both markers to be absent in ELSTs, while being expressed in the vast majority of CPTs. Our results indicate that Kir7.1 and EAAT-1 are highly specific (100%), and Kir7.1 is slightly more sensitive than EAAT-1 (100% and 91%, respectively). Both markers are clearly superior to S-100 and GFAP in differentiating between CPTs and ELSTs. A limitation of our study is the low number of ELST analyzed, but one has to keep in mind that ELSTs are extremely rare, with approximately 100 cases reported to date,2 and the number of ELSTs found in the cerebellopontine angle (thus truly suggesting the differential diagnosis of CPTs) is even more limited.

Regular screening of papillary tumors in the cerebellopontine angle with Kir7.1 and/or EAAT-1 might aid pathologists in recognizing tumors that do not belong to the category of CPT. However EAAT-1 expression is not restricted to plexus-epithelial tumors, but is also widely expressed in gliomas (including ependymomas, another potential differential diagnosis to CPT)18 and is absent in epithelial and mesenchymal neoplasms. Kir7.1 was also initially considered a plexus-specific potassium transporter, but recent data also indicate that Kir7.1 at least is also focally expressed in some papillary tumors of the pineal region19 and atypical teratoid/rhabdoid tumors,11 probably owing to plexus-epithelial dedifferentiation of these tumors. Because Kir7.1 expression has also been reported in normal human epithelial tissues such as intestinal and thyroid cells and kidney,12,20 it could be possible that tumors deriving from these tissues might also be immunoreactive for Kir7.1. However, in 2 series examining 45 and 77 cerebral metastases of carcinomas with different primary sites, all brain metastases analyzed so far lacked expression of Kir7.1 and EAAT-1,17 respectively.

Kir7.1 and EAAT-1 are suitable diagnostic tools to distinguish ELST from CPT and are superior to GFAP and S-100. In cases with presumed CPT at the cerebellopontine angle, negative immunoreactivity of Kir7.1 and/or EAAT-1 should prompt pathologists to consider other differential diagnoses.

* Kir7.1 is considered positive or negative. GFAP and S-100 were scored semiquantitatively (0, absent; 1, <1% positive cells; 2, 1%-10%; 3, 11%-20%; 4, >20% positive cells).

Table 1

<table>
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<tr>
<th>Diagnosis</th>
<th>No./Total</th>
<th>Kir7.1+</th>
<th>Mean (Range)</th>
<th>Mean (Range)</th>
<th>Mean (Range)</th>
<th>Mean (Range)</th>
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<tr>
<td></td>
<td>Samples</td>
<td>EAAT-1 (%)</td>
<td>MIB-1 (%)</td>
<td>GFAP Score</td>
<td>S-100 Score</td>
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<tr>
<td>Choroid plexus papilloma, WHO I</td>
<td>25/25</td>
<td>19 (0-50)</td>
<td>1 (0.1-3)</td>
<td>0.8 (0-3)</td>
<td>3.4 (0-4)</td>
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<tr>
<td>Atypical choroid plexus papilloma,</td>
<td>10/10</td>
<td>10 (1-50)</td>
<td>6 (0.3-10)</td>
<td>0.5 (0-2)</td>
<td>2.9 (0-4)</td>
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<td>WHO II</td>
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<tr>
<td>Endolymphatic sac tumor</td>
<td>0/4</td>
<td>0</td>
<td>3 (2-5)</td>
<td>0.8 (0-2)</td>
<td>0.5 (0-1)</td>
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</table>

GFAP, glial fibrillary acidic protein; EAAT, excitatory amino acid transporter; WHO, World Health Organization.

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References


