New Immunohistochemical Markers in the Evaluation of Primary Non-Glial Central Nervous System Tumors

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Houston, TX
Recent Advances

- Germinoma
- Hemangioblastoma
- Meningioma
- Solitary Fibrous Tumor/Hemangiopericytoma
- Hereditary Schwannoma
- Craniopharyngioma vs. Rathke’s cleft cyst
Germinoma

- Occurs predominantly in the midline
- Suprasellar and pineal regions
- Biphasic pattern – large cells with prominent nucleoli admixed with lymphocytes
- When lymphocytes predominate, immunohistochemical analysis may be critical for diagnosis
Germinoma

- Placental alkaline phosphatase, PLAP – well-established marker; **membranous** pattern of staining
- C-kit proto-oncogene (CD117)
  - Present on cell surface in almost all seminomas and dysgerminomas
  - Rarely expressed in other germ cell tumors
  - CD30 and C-kit in combination have been useful in separating embryonal components in gonadal tumors
Germinoma

- **OCT4** (POU5F1, OCT3 or OTF3) – Nuclear transcription factor expressed in germ cells
- Regulation of “pluripotent” potential in germ cells
- Necessary for stem cell formation
- Almost 100% immunoreactivity in seminoma and embryonal carcinomas of the testes (Jones et al, Am J Surg Path 2004) negative in other germ cell components
- **Nuclear** staining pattern
Germinoma

- Hattab et al (Am J Surg Path, 2005) examined intracranial germinomas and confirmed the findings of OCT4 and PLAP immunoreactivity patterns described in testicular seminomas and ovarian dysgerminomas.
- Significantly stronger staining for OCT4 than PLAP overall.
Hemangioblastoma

- WHO 2007 grade I neoplasm of “uncertain histogenesis”
- Composed of capillaries and stromal cells – cerebellum and spinal cord
- Sporadic or associated with Von Hippel Lindau
- Necessity to differentiate from metastatic renal cell carcinoma in those patients with VHL
- Jarrel et al (J Neurosurg 2006) reported 8% of patients with VHL had metastases (most commonly RCC) within a hemangioblastoma
Hemangioblastoma – Alpha Inhibin

- Produced in Sertoli cells of the testes and granulosa cells of the ovary normally
-Expressed in sex cord stromal tumors and adrenal cortical tumors

Hoang (Am J Surg Path 2003) demonstrated cytoplasmic immunoreactivity in stromal cells in hemangioblastoma and negative staining for renal cell carcinoma
Hemangioblastoma

Alpha-Inhibin
Expression of aquaporin 1 associated with cyst formation in hemangioblastoma (Chen et al, J Neuroonc 2006)
Hemangioblastoma – Aquaporin 1

- Weinbreck et al (Am J Surg Path, 2008) found that a panel including alpha-inhibin and aquaporin 1 were reliable positive markers for hemangioblastoma.
- **Membranous** pattern – aquaporin 1
- AE1/AE3 and CD10 were reliable markers of clear cell renal cell carcinoma (CCRCC).
- Combined use of AE1/AE3 and aquaporin 1 were reliable for the differentiation of hemangioblastoma and metastatic CCRCC.
Aquaporin 1 in hemangioblastoma

*Figure 3.* Photographs revealing representative immunohistochemical results of AQPI expression in hemangioblastomas and control brain specimens. (a) Typical histological characteristics of cystic hemangioblastoma with large vacuolated stromal cells and a rich capillary network (hematoxylin and eosin). (b) Significant upregulation of AQPI expression on membranes of stromal cells in cystic hemangioblastomas. No expression of AQPI was found on microvascular endothelial cells. Scale bars, 25 μm.
Figure 4. Photographs showing immunofluorescence in cystic hemangioblastomas stained with AQPI (green) and CD34 (red). In hemangioblastomas, AQPI was strongly expressed on membranes of stromal cells. Positive CD34 was detected on microvascular endothelial cells of hemangioblastomas. Scale bars, 50 μm.
Facts & Figures at TMC

- One of the world’s largest medical center complexes
  - 46 Institutions
  - 73,600 employees
  - 6,500 beds
  - 5.5M patient annually
  - 10,000+ Intl. patients
  - 13 Academic & RI’s
  - 10 Hospitals
  - 26,000,000 gross sf

- TMC Corporation
  - Owns land
  - Grants restricted deeds to institutions
  - Provides roads, utilities, parking garages
Meningioma

- Claudin-1
- PHH-3
- Secretory meningioma variant study
Meningioma – Claudin-1

- Tight junction-associated protein
- Identified in perineurial cells and soft tissue perineuriomas (Folpe et al Am J Surg Path, 2002)
- Bhattacharjee et al (AANP 2003) reported claudin-1 immunoreactivity in 85% of meningiomas with a punctate membranous pattern
- Hahn et al (AJCP 2006) found a similar pattern, but in a small percentage of cases, 53% studied
Expression of Claudin-1, a Recently Described Tight Junction-Associated Protein, Distinguishes Soft Tissue Perineurioma From Potential Mimics

Andrew L. Folpe, M.D., Steven D. Billings, M.D., Jesse K. McKenney, M.D., Shawn V. Walsh, M.D., Asma Nusrat, M.D., and Sharon W. Weiss, M.D.

Perineuriomas are rare benign soft tissue tumors having an immunophenotype paralleling the normal perineural cell [S-100 protein negative and epithelial membrane antigen (EMA) positive]. Because EMA expression in perineuriomas may be focal and/or faint, there is continued interest in the development of new markers of perineural differentiation. Perineurial cells differ from almost all other mesenchymal cell types by virtue of their formation of tight junctions. In the present study, we showed that claudin-1, a recently described tight junction protein, is expressed in perineuriomas but not in schwannomas. One of four schwannomas contained a subpopulation of perivascular, dendritic, claudin-1-positive cells of presumed perineurial lineage. This is the first study to document expression of claudin-1 in perineural cells and suggests a role for claudin-1 immunohistochemistry in the diagnosis of perineuriomas. Although claudin-1 should not replace EMA in the diagnosis of perineurioma, we think that it may play a valuable adjunctive role in difficult cases. In particular, claudin-1 is one of very limited number of EMA immunoreactive benign soft tissue tumors.
Phospho-Histone H3 (pHH3)
A Mitotic Figure Immunostain
Phospho-Histone H3
Phospho-Histone H3 (pHH3)
Phospho-Histone H3 (pHH3)
Phosphohistone H3 (pHH3)

The Mitosis-Specific Antibody Anti-Phosphohistone-H3 (PHH3) Facilitates Rapid Reliable Grading of Meningiomas According to WHO 2000 Criteria

Teresa Ribalta, MD, PhD, * Ian E. McCutcheon, MD, ‡ Kenneth D. Aldape, MD, PhD, † Janet M. Bruner, MD, † and Gregory N. Fuller, MD, PhD †

**Anatonic Pathology / PHH3 and Mitotic Index in Meningiomas**

**Prognostic Significance of the Mitotic Index Using the Mitosis Marker Anti-Phosphohistone H3 in Meningiomas**

Yoo-Jin Kim, MD,¹ Ralf Ketter, MD,² Wolf-Ingo Steudel, MD,² and Wolfgang Feiden, MD¹

**Key Words:** Meningioma grading; Recurrence; Phosphohistone H3; PHH3; Mitosis; Ki-67

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<table>
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<tr>
<th></th>
<th>No. of Cases</th>
<th>H&amp;E MI</th>
<th>PHH3 MI</th>
<th>Ki-67 LI</th>
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<tr>
<td><strong>WHO grade</strong></td>
<td></td>
<td></td>
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<tr>
<td>Benign (grade I)</td>
<td>215</td>
<td>0 (0-3)</td>
<td>1 (0-14)</td>
<td>2 (0-13.6)</td>
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<td>Atypical (grade II)</td>
<td>45</td>
<td>4 (0-15)</td>
<td>5 (0-31)</td>
<td>6.3 (0.6-20.6)</td>
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<tr>
<td>Anaplastic (grade III)</td>
<td>5</td>
<td>21 (20-38)</td>
<td>34 (30-50)</td>
<td>6 (3-34.3)</td>
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<td><strong>Brain invasion</strong></td>
<td>14</td>
<td>1.5 (0-38)</td>
<td>3 (0-50)</td>
<td>3.5 (0.8-34.3)</td>
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<tr>
<td>Recurrent vs nonrecurrent</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>33</td>
<td>2 (0-31)</td>
<td>4 (0-41)</td>
<td>3.2 (0.3-25.5)</td>
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<tr>
<td>Nonrecurrent</td>
<td>232</td>
<td>0 (0-38)</td>
<td>1 (0-50)</td>
<td>2.5 (0-34.3)</td>
</tr>
</tbody>
</table>

H&E MI, mitotic figure counts in 10 high-power fields in the area of highest mitotic activity, assessed in H&E-stained slides; Ki-67 LI, Ki-67 labeling index, which is the percentage of immunolabeled nuclei determined in 5 high-power fields in the areas of highest labeling density; PHH3 MI, mitotic figure counts in phosphohistone H3 in the same way as in H&E-stained sections; WHO, World Health Organization.

* Data are given as median (range).
<table>
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<th>Outcome</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
<th>( P^* )</th>
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<tr>
<td>Age</td>
<td>1.03 (1.00-1.06)†</td>
<td>.0415</td>
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<td>Extent of resection (subtotal resection)</td>
<td>2.19 (0.97-4.91)</td>
<td>.0589</td>
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<tr>
<td>H&amp;E MI</td>
<td>1.08 (1.03-1.14) ‡</td>
<td>.0007</td>
</tr>
<tr>
<td>PHH3 MI</td>
<td>1.07 (1.06-1.10) ‡</td>
<td>.0004</td>
</tr>
<tr>
<td>Ki-67 LI</td>
<td>1.03 (0.96-1.10) §</td>
<td>.3645</td>
</tr>
</tbody>
</table>

H&E MI, mitotic index assessed in H&E-stained slides; LI, labeling index; PHH3 MI, mitotic index assessed in anti–phosphohistone H3–immunolabeled specimens.

* By the log-rank test.
† Hazard ratio per year of patient age.
‡ Hazard ratio per mitosis.
§ Hazard ratio per percentage in the Ki-67 LI.
Phosphohistone H3 expression has much stronger prognostic value than classical prognosticators in invasive lymph node-negative breast cancer patients less than 55 years of age

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Validating the prognostic value of proliferation measured by Phosphohistone H3 (PPH3) in invasive lymph node-negative breast cancer patients less than 71 years of age

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Einar Gudlaugsson · Jan Klos · Kjell H. Kjellevold ·
Håvard Søiland · Jan P. A. Baak

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Abstract We validated and compared the prognostic value of the proliferation marker phosphohistone H3 (PPH3) with classical variables in 241 T1-2N0M0 breast cancer patients less than 71 years old with long-term follow-up (median 117 months) and without adjuvant treatment. PPH3 was measured by automated digital image analysis. Thirty-seven patients (15%) developed distant metastases and 29 (12%) died. The previously established PPH3 prognostic threshold H3 < 13 (n = 157; 65% of all cases) vs. ≥ 13 (n = 84; 35% of all cases) was the strongest prognostic threshold exceeding all other characteristics, with 10-year recurrence-free survival of distant metastases of 96 and 64%, respectively (P = < 0.0001, hazard ratio = 7.8, 95% confidence interval = 3.4–17.9). PPH3 is robust as it showed high inter-observer reproducibility and was prognostic over wide range of thresholds around 13 and is the strongest prognostic variable in invasive node-negative breast cancer patients less than 71 years old.

Introduction

Lymph node-negative breast cancer has a relatively good prognosis (10–30% mortality). The typical 15-year survival improvement with adjuvant systemic chemotherapy (AST) is 35% relative and 10% absolute, which is less substantial than in lymph node-positive patients [1]. Discomfort, costs and the serious side effects of AST must be balanced against the relatively good prognosis and moderately favorable prognostic treatment effect in the node-negative subgroup of patients. Accurate and reliable prognostic markers could be valuable in decision-making regarding AST or not. The proliferation factor mitotic activity index (MAI) has been shown to be the strongest prognostic and predictive factor in lymph node-negative patients less than 71 years of age [2–5]. Notably, most genes constituting prognostic gene signatures are linked to proliferation [6, 7]. Moreover, the MAI is inexpensive, easy to use, and highly
Meningioma, General

- Cytokeratin expression depends on meningioma subtype
  - Focal positivity except in microcystic and anaplastic tumors
  - CK18 positivity commonly present in all types
  - Negativity overall for CK20 (Mieettinen and Paetau, 2002)
Secretory Meningioma

- CK immunoreactivity is unique, confined to cells adjacent to secretory material ("pseudopsammomoma bodies") – CK18, also CK7, CK8 and CK19
- CEA immunoreactivity well described in the secretory droplets
- Recent review of 6 cases (Caffo et al, J of Clin Neuroscience, May 2008) examining extracellular matrix proteins laminin, fibronectin and type IV collagen found staining for all three proteins in 2 of the 6 cases, and mild to absent immunoreactivity in the remaining 4 cases
The Methodist Hospital is recognized as one of the leading hospitals in the United States. A legacy of medical milestones and proven quality has attracted patients, healthcare professionals and institutional collaborations from around the world for more than 80 years.
Solitary Fibrous Tumor to Hemangiopericytoma – A Continuum?

- Panel of immunostains including EMA, CD99, BCL-2 and CD34 for distinguishing meningioma from SFT and HPC
- SFT and HPC having overlapping histologic features
- SFT and HPC remain separate entities in CNS as opposed to soft tissues in which this diagnosis has merged and HPC is a “pattern”
SFT/HPC Immunohistochemical staining patterns

- BCL-2 and CD34 are diffuse and strongly positive in SFT; weak and focal in HPC
- CD34 is strongly positive not only in vessels, but in the cytoplasm in SFT
  - Usually diffuse, with less staining in HPC
- CD99 often shows a similar pattern to CD34, but may be negative
- EMA is often negative, distinguishing these tumors from meningiomas, but may have focal positive staining areas
Cellular SFT/HPC – Reticulin Stain
Cellular SFT/HPC – BCL-2
Cellular SFT/HPC     EMA

200 μm
Cellular SFT/HPC- CD34
Cellular SFT/HPC – CD99
Cellular SFT/HPC – MIB-1
San Jacinto Methodist Hospital
- 268 Licensed Beds
- 14,241 Inpatients
- 56,903 ER Visits

Methodist Sugar Land Hospital
- 54 Licensed Beds
- (expanding to 300 in 2008)
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Methodist Willowbrook Hospital
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West Houston Methodist Hospital
- West Houston, co-located with new TCH
- 200 beds
- Spring 2010
Hereditary Schwannoma

- *INI-1/SMARCB1* protein recently implicated in pathogenesis of schwannoma in a family with familial schwannomatosis
- Mutations of *SMARCB1* gene identified
- Mosaic loss of expression of INI1
RESEARCH ARTICLE

Immunohistochemical Analysis Supports a Role for INI1/SMARCB1 in Hereditary Forms of Schwannomas, but Not in Solitary, Sporadic Schwannomas

Sushama Patil1; Arie Perry1; Mia MacCollin2; Shumin Dong3; Rebecca A. Betensky4; Tu-Hsueh Yeh5,6; David H. Gutmann5; Anat O. Stemmer-Rachamimov2,7

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2 Department of Neurology, 6 Molecular Neuro Oncology Laboratory, 7 Division of Neuropathology, Massachusetts General Hospital, Boston, Mass.
3 Department of Biostatistics, Harvard School of Public Health, Boston, Mass.
4 Department of Neurology, Chang Gung Memorial Hospital and University, Taipei, Taiwan.
INI-1 staining (Panel B) in hereditary schwannoma versus the diffuse positivity in a solitary, sporadic tumor (Panel A).
Sporadic Schwannoma
RAPID COMMUNICATION

Evidence of a Four-Hit Mechanism Involving SMARCB1 and NF2 in Schwannomatosis-Associated Schwannomas

Roberta Sestini,¹ Costanza Bacci,¹ Aldesia Provenzano,¹ Maurizio Genuardi,¹,² and Laura Papi¹∗

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Communicated by Georgia Chenević-Trèich

Schwannomatosis is characterized by the onset of multiple intracranial, spinal, or peripheral schwannomas, without involvement of the vestibular nerve, which is instead pathognomonic of neurofibromatosis type 2 (NF2). Recently, a schwannomatosis family with a germline mutation of the SMARCB1 gene on chromosome 22 has been described. We report on the molecular analysis of the SMARCB1 and NF2 genes in a series of 21 patients with schwannomatosis and in eight schwannomatosis-associated tumors from four different patients. A novel germline SMARCB1 mutation was found in one patient; inactivating somatic mutations of NF2, associated with loss of heterozygosity (LOH) of 22q, were found in two schwannomas of this patient. This is the second report of a germline SMARCB1 mutation in patients affected by schwannomatosis and the first report of SMARCB1 mutations associated with somatic NF2 mutations in schwannomatosis-associated tumors. The latter observation suggests that a four-hit mechanism involving the SMARCB1 and NF2 genes may be implicated in schwannomatosis-related tumorigenesis. Hum Mutat 29(2), 227–231, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: Schwannomatosis; SMARCB1; NF2; INI1; SNF5
TMH Outpatient Facility
Project Scope
24 Floor tower
TPC $ 331 M
760,000 GSF
occupied space
846,000 GSF
parking – 1370
spaces
Craniopharyngioma vs. Rathke Cleft Cyst

- Sellar/suprasellar masses with differing clinical outcomes
- Purely cystic craniopharyngiomas vs. Rathke cleft cysts with squamous metaplasia and without significant ciliated epithelium may be extremely challenging
- Craniopharyngioma may also rarely demonstrate ciliated epithelium, compounding an already difficult problem
Beta-catenin

- Adamantinomatous craniopharyngiomas have been found to have an activating mutation in **exon 3** of the *beta-catenin* gene in approximately 90% of cases examined (Buslei et al, Acta Neuropathologica, 2005).
- Pattern of staining is inhomogeneous and may be clustered in “whorls”.
- Whorl-like immunoreactivity is associated with positive staining for CK8 (CAM5.2) and CK18.
Beta-catenin

- Rathke cleft cyst (RCC) and papillary craniopharyngioma immunoreactivity is exclusively **membranous**
- Distinctive staining patterns may be useful in separating these entities, especially in small surgical specimens
- NOT useful for distinguishing papillary craniopharyngioma from RCC with squamous metaplasia
Adamantinomatous Craniopharyngioma

The Differential Diagnosis of Central Nervous System Tumors
A Critical Examination of Some Recent Immunohistochemical Applications

Mark A. Felton MD  Marc K. Rosenblum MD

Arch Pathol Lab Med—Vol 132, March 2008

Immunohistochemistry in CNS Tumors—Edgar & Rosenblum

Strong immunoreactivity for beta catenin in a “whorl”
Beta – catenin – negative staining of “wet” keratin
CAM 5.2 (CK8)
CK5-6
Beta-Catenin membranous staining pattern in epithelium of papillary craniopharyngioma
Papillary Craniopharyngioma

CK7
Papillary Craniopharyngioma

CK7
Rathke’s cleft cyst
Rathke’s Cyst – Beta-catenin

Membranous pattern as in papillary CP
Rathke’s Cyst

CK7
Utilizing the Differences

Craniopharyngioma, Adamantinomatous Type

“Whorl-like” positivity for beta-catenin

Superficial staining for CK7, diffuse staining for CK5/6

Rathke’s Cyst

Membranous pattern of staining for beta-catenin as in Papillary craniopharyngioma

Similar patterns of staining as for adamantinomatous craniopharyngioma for CK7 and CK5/6 (CAM 5.2)
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