Introduction to Neoplasia and Immunohistochemistry

Philippe Labelle, DVM, DACVP
Antech Diagnostics
12th Biannual William Magrane Basic Science Course in Veterinary and Comparative Ophthalmology
General Considerations

- Epithelial cells
- Mesenchymal cells
  - Spindle cells
  - Round/leukocytic cells
Epithelial Neoplasms

- Glands
- Nests
- Trabeculae
- Cords

- Cuboidal, columnar or polygonal
- Distinct borders
- Mature cells have abundant cytoplasm; Basal cells have less cytoplasm
- Neuroepithelium often forms packets and usually has features similar to epithelium (not from surface ectoderm)
Epithelial Neoplasms
Epithelial Neoplasms
Mesenchymal-Spindle Neoplasms

- Spindle
- Streams
- Bundles
- Whorls
- Indistinct borders
- Scant cytoplasm
Mesenchymal-Spindle Neoplasms
Mesenchymal-Spindle Neoplasms
Round Cell/Leukocytic Neoplasms

• Round

• Sheets

• “round cell” is use both for a category of cells (leukocytes) as well as a descriptor of cell shape

• Histiocytic sarcoma is a common exception. The cells may be round, spindle or polygonal/epithelioid, often in combination
Round Cell/Leukocytic Neoplasms

Lymphoma                Plasmacytoma
Mast cell tumor          Histiocytoma
Ocular Neoplasia

• The rules are similar to other organs
• There are many exceptions

• Iris and ciliary epithelium (neuroectoderm) share some but not all features of epithelial (ectoderm) cells
• Lens epithelium takes on a mesenchymal phenotype
• Melanocytic neoplasia does not neatly fit in any category
Ocular Neoplasia
Immunohistochemistry

• Polyclonal and monoclonal
• Fixation
• Antigen retrieval
• Indirect method
• Detection

Immunohistochemistry

3-Amino-9-ethylcarbazole (AEC)

3,3′diaminobenzidine tetrachloride (DAB)
Immunohistochemistry

- Read as positive vs negative
- Distribution
- Localization
- Intensity
- Percentage or absolute number of cells
Immunohistochemistry

MNF116     Factor VIII
Immunohistochemistry

Lymphoma
CD79a
CD3
CD79a
Neg
Immunochemistry

Factor VIII
Immunohistochemistry

Melanoma Dx panel

CD3

CD204
Immunohistochemistry
Immunohistochemistry

CD79a
Immunohistochemistry

Ki-67
Immunohistochemistry

• Common antibodies

• Cytokeratin, vimentin, melanocytic markers (melan-A, PNL-2, TRP1-2), muscle markers (SMA, actin, desmin), neuro/neuroendocrine markers (S100, NSE, GFAP, SYN, PGP9.5), round/leukocytic markers (CD45, CD18, CD3, CD20/CD79a, Pax5, CD204, CD117)
Table 2. Previously Reported Diagnostic Immunohistochemistry of Primary Ocular Neoplasms in Dogs

<table>
<thead>
<tr>
<th>Neoplasm (No. of Cases)</th>
<th>Positive Immunoreactivity(^a) (No. of Cases)</th>
<th>Negative Stains (No. of Cases)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbal hemangiosarcoma (1)</td>
<td>CD31</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Corneal squamous cell carcinoma (1)</td>
<td>Pan-CK, E-cadherin</td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>Iridociliary epithelial tumors (20)</td>
<td>Vimentin (18/20), NSE (8/8), Pan-CK (4/23), desmin (3/11), S100 (10/20), GFAP (1/20)</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Iridociliary adenoma (14)</td>
<td>Pan-CK (9/14), CK20 (7/14)</td>
<td>CK7 (0/14)</td>
<td>33</td>
</tr>
<tr>
<td>Iridociliary adenocarcinoma (8)</td>
<td>Pan-CK (6/8), vimentin (1/1), NSE (1/1), S100 (1/1)</td>
<td>CK7 (0/7), CK20 (0/7)</td>
<td>33, 78</td>
</tr>
<tr>
<td>Medulloepithelioma (8)</td>
<td>Pan-CK (1/7), GFAP (1/1)</td>
<td>CK7 (0/7), CK20 (0/7)</td>
<td>33, 69</td>
</tr>
<tr>
<td>Malignant teratoid medulloepithelioma (1)</td>
<td>Vimentin, CD99</td>
<td>CK, NSE, TNF, GFAP, synaptophysin</td>
<td>1</td>
</tr>
<tr>
<td>Uveal schwannoma/ peripheral nerve sheath tumor (14)</td>
<td>Vimentin (11/11), S100 (8/8), GFAP (9/13), PGP9.5 (7/9), laminin (2/9)</td>
<td>SMA (0/7), desmin (0/7), Melan-A (0/7), MITF (0/7), TNF (0/1)</td>
<td>64, 79</td>
</tr>
<tr>
<td>Uveal malignant melanoma (1)</td>
<td>Melan-A</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Ocular melanosis (14)</td>
<td>Vimentin (8/8), HMB45 (8/8), MITF (8/8)</td>
<td>SMA (0/8), S100 (0/8), Melan-A (0/14), chromogranin A/B (0/8), PGP9.5 (0/8), synaptophysin (0/8), CK (0/8), CD45 (0/8)</td>
<td>54, 72</td>
</tr>
<tr>
<td>Mixed germ cell tumor (1)</td>
<td>Pan-CK</td>
<td></td>
<td>51</td>
</tr>
<tr>
<td>Myxoid leiomyoma of the iris (1)</td>
<td>Desmin</td>
<td>S100</td>
<td>4</td>
</tr>
<tr>
<td>Retinoblastoma (1)</td>
<td>GFAP</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>Neuroepithelial tumor of the retina (1)</td>
<td>Vimentin, synaptophysin</td>
<td>CK, GFAP, NSE, TNF, S100, CD56, CD99</td>
<td>29</td>
</tr>
<tr>
<td>Retinal-optic nerve glioma/ astrocytoma (20)</td>
<td>Vimentin (1/1), NSE (1/1), S100 (1/1), GFAP (18/20)</td>
<td>TNF (0/1), Melan-A (0/1)</td>
<td>9, 43, 48</td>
</tr>
<tr>
<td>Orbital meningoia (14)</td>
<td>Vimentin (14/14), S100 (14/14), NSE (4/4), GFAP (1/4)</td>
<td>CK (0/14)</td>
<td>39, 47, 53</td>
</tr>
</tbody>
</table>

CK, cytokeratin; NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; SMA, α-smooth muscle actin; MITF, microphthalmia transcription factor; TNF, triple neurofilament.

\(^{a}\)Positive immunoreaction in at least some neoplasms.

P. Labelle et al. Vet Pathol 2011;49:860-869
Immunohistochemistry

• Cytokeratin
• Intermediate filament
• Normal eye: Corneal epithelium, RPE

• Neoplasms: SCC, some iridociliary neoplasms, metastatic CA
Immunohistochemistry

• Vimentin
• Intermediate filament
• Normal eye: almost everything

• Neoplasms: melanocytic, iridociliary, astrocytoma, meningioma, metastatic sarcomas, lymphoma, histiocytic sarcoma
Immunohistochemistry

- Melanocytic markers (melan-A, PNL-2, TRP-1, TRP-2)
- Targets in melanogenesis
- Normal eye: melanocytes, corneal endothelium, neuroepithelium, tapetum, meninges
- Neoplasms: Melanocytic, iridociliary?, metastatic?
Immunohistochemistry

- Muscle markers (SMA, actin, desmin)
- Intermediate (desmin) or microfilaments
- Normal eye: constrictor, dilator, ciliary muscles; choroidal stromal cells, lens epithelium, neuroepithelium, others

- Neoplasms: leiomyoma/sarcoma, iridociliary, others?
Immunohistochemistry

- Neuron/neuroendocrine markers (S100, NSE, GFAP, SYN, PGP9.5)
- Normal eye: Neuroepithelium, retina, uveal stromal cells, muscles, lens epithelium
- Neoplasms: Iridociliary, astrocytoma, melanocytic
Immunohistochemistry

• Round/leukocytic markers (CD45, CD18, CD3, CD20/CD79a, Pax5, CD204, CD117)
• Cluster of differentiation
• Normal eye: Leukocytes
• CD45 panleukocytic, CD18 panleukocytic, CD3 T-cell, CD20/CD79a/Pax5 B-cell, CD204 histiocytic (interstitial), CD117 (mast cell and other cells)

• Neoplasms: Lymphoma, histiocytic, etc
Immunohistochemistry

- Melanocytic
  - Vim
  - +/- Melan-A/PNL-2/TRP1-2
  - Neuro

- Iridociliary
  - Vim
  - +/- CK
  - +/- Melan-A/PNL-2/TRP1-2?
  - Neuro
Immunohistochemistry

- Melanocytic
  - Vim
  - +/- Melan-A/PNL-2/TRP1-2
  - Neuro
- Lymphoma
  - Vim
  - CD45 +/- CD18
  - CD3 or CD20/CD79a/pax5
Immunohistochemistry

- Lymphoma
  - Vim
  - CD18
  - CD3 or CD20/CD79a/pax5

- Round cell PTS
  - +/- Vim
  - CD18?
  - CD3 AND/or CD20/CD79a/pax5
Immunohistochemistry

<table>
<thead>
<tr>
<th>Imm</th>
<th>Melanocytic</th>
<th>Iridociliary</th>
<th>Lymphoma</th>
<th>Histiocytic sarcoma</th>
<th>Metastatic CA</th>
<th>Metastatic SA</th>
<th>Schwannoma</th>
<th>Astrocytoma</th>
<th>Meningioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ck</td>
<td></td>
<td>+/-</td>
<td></td>
<td>X</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vim</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>+/-</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Melanocytic</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuro</td>
<td>X</td>
<td>X</td>
<td></td>
<td>+/-</td>
<td>+/-</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cd45/CD18</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD204</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>