An Introduction To Descriptive Immunohistochemistry

Davis-Thompson Foundation

2016 Descriptive Veterinary Pathology Course

Immunohistochemistry: What is it?

“the identification of a tissue constituent in situ by means of a specific antigen-antibody interaction where the antibody has been tagged with a visible label” (Immunohistochemical Methods and Protocols, p. 3)

IHC Resources

- The Journal of Histotechnology
- Applied Immunohistochemistry and Molecular Morphology
- DAKO, Serotec and other industry publications
- Lectures
- Internet
- Veterinary Pathology
IHC Identification Targets

- Cell Structural Components
- Infectious Agents
- Enzymes and Hormones
- Cytokines, chemokines and receptors
- Extracellular Components

Specimens

- Tissue sections, formalin-fixed or frozen
- Cell smears, suspensions, cytopsins
- Cells or tissue sections for electron microscopy

Controls

- Necessary to make a valid interpretation of staining
- Helpful in troubleshooting
- Reagent controls and Tissue controls
Tissue controls

- Positive control tissue contains the antigen of interest; negative tissue does not
- Internal controls are structures in the tissue that can stain either positive or negative

Trouble Spots

- IHC results are subject to more interpretation than other assays
- Antibody reactivity differs among species
- Fixation plays an important role:
  - Different susceptibility of antigens
  - Variable antigen retrieval
  - Time of fixation and fixation variable
  - Geographic differences, esp. altitude

Slide Interpretation

- Examine controls; determine whether staining run is valid
- Note any internal positive or negative controls
- Determine specific staining – state cell type, location in cell, intensity and distribution
**Best Practices**
- Always read the IHC together with the H&E
- Always interpret IHC with proper controls
- Use antibody panels rather than single tests
- Know your antibodies
- Don’t base your diagnosis on the absence of staining only
- IHC is a complimentary tool to H&E light microscopic diagnosis

**The Key to Description**
- Quantitation of staining (negative, light, medium, heavy)
- Distribution of positivity (cytoplasmic, nuclear, cell membrane, etc.) Is the location appropriate for the antibody?
- Presence or absence of an internal control

**IHC Description**
- Tissue
- Concise H&E description
- For each IHC panel:
  - Evaluate external control
  - Evaluate internal control
  - Distribution, intensity and location of staining
  - Morphologic diagnosis
Intermediate Filaments

- Cytokeratin
- Vimentin
- Desmin
- Glial fibrillary acidic protein (GFAP)/Nestin
- Neural fibrillary protein (NFP)

Intermediate Filaments – What are they?

- Component of the cytoskeleton
- Have specific distributions in tissues and cells
- Possess a nucleic acid binding function
- May modulate calcium influx into the cell and nuclear transcription
Limitations of Intermediate Filaments

- They are sensitive to formalin fixation and standard tissue processing
- There is a possibility of cross-reaction
- There may be co-expression of two filaments in a single cell or tissue

Cytokeratin

- At least 20 different keratins
- Divided into 2 groups:
  - type I or acidic
  - type II or basic
- Many subtypes are expressed in normal and neoplastic epithelium
- Keratins can also be grouped by molecular weight

Cytokeratins

<table>
<thead>
<tr>
<th>Type (basic)</th>
<th>Distribution</th>
<th>Gene (family)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK4</td>
<td>Keratinized squamous epithelium</td>
<td>CK1a</td>
</tr>
<tr>
<td>CK5</td>
<td>Cornea</td>
<td>CK1a</td>
</tr>
<tr>
<td>CK19</td>
<td>Nonkeratinized epithelium</td>
<td>CK14</td>
</tr>
<tr>
<td>CK6</td>
<td>Basal cells of glandular and squamous epithelium, myoepithelium, mesothelium</td>
<td>CK4, CK7</td>
</tr>
<tr>
<td>CK1, CK8</td>
<td>Simple epithelium, superficial squamous epithelium</td>
<td>CK1a</td>
</tr>
<tr>
<td></td>
<td>Simple epithelium of GI tract, Merkel cells, urethra</td>
<td>CK1a</td>
</tr>
</tbody>
</table>
Cytokeratin

- Low Molecular Weight are in all epithelia, except squamous
- High Molecular Weight is located in squamous epithelia

Keratin Expression

- Normal epithelium
- Benign epithelial cutaneous tumors
- Carcinomas
- Mesothelium and mesothelioma
- Thymoma

Keratin Expression

- Chordoma
- Nonseminomatous germ cell tumors
- Synovial cell sarcoma
- Carcinosarcoma
- Carcinoids
- Meningioma, rarely
Respiratory epithelium, horse

Complex adenoma, cat

Colonic Adenocarcinoma, dog
Ocular neoplasm, cat

Ciliary body adenocarcinoma

Vimentin

- Most ubiquitous intermediate filament in the body
- First intermediate filament expressed embryologically
- Replaced by the intermediate filament specific for the cell type during development, but retained in mesenchymal cells
Vimentin Expression

- Myocytes
- Endothelial cells
- Fibroblasts
- Adipocytes
- Chondrocytes
- Melanocytes
- Macrophages
- Lymphocytes
- Sertoli cells
- Ovarian granulosa cells

Vimentin Expression

- Mesenchymal neoplasms
- Melanoma
- Mesothelioma
- Thymoma
- Synovial sarcoma
- Lymphoma (Lymphosarcoma)
- Transmissible venereal tumor
- Primitive neuroendocrine tumor

Splenic lymphoma, dog
Complex mammary gland adenoma, dog

Dual Expression of Intermediate Filaments

Tibiotarsal joint, dog
Vimentin

Keratin

DX: Mesothelioma

Desmin

- Primarily used to determine a possible myogenic origin for sarcomas
- Expressed in embryonic and adult skeletal, smooth and cardiac muscle
- Is NOT expressed in myoepithelium
Desmin Expression

- Most muscle tumors:
  - Rhabdomyoma
  - Rhabdomyosarcoma
  - Leiomyoma
  - Leiomyosarcoma
  - Juvenile rhabdomyosarcoma

Desmin

- May be expressed in the following neoplasms with divergent phenotypes:
  - Primitive neuroectodermal tumor
  - Epithelioid sarcomas
  - Rhabdoid-type tumors

Gingiva, dog
Neural Fibrillary Protein (NFP)

- Major structural component of neurons and their processes
- Largely specific for cells of the neural crest and neuroectoderm
- Usually expressed in neural tumors with variable expression in neuroendocrine tumors

NFP Expression

- Neuromas
- Ganglioneuromas
- Neuroblastoma
- Ganglioneuroblastoma
- Carcinoid
- Medulloblastoma
- Esthesioneuroblastoma
- Teratoma
- Pheochromocytoma
Cerebral mass, dog

NFP Expression

Ganglioneuroblastoma

Glial Fibrillar Acidic Protein (GFAP)

- The major protein of astrocytes
- Also present in developing and neoplastic ependymal cells and oligodendrocytes
- Poorly differentiated (high grade) astrocytomas usually do not express GFAP
**Mass, subcutis, cat**

**GFAP**

**Peripheral nerve sheath tumor**

**Nestin**

- The main intermediate filament of immature astrocytes
- Used as a marker for astrocyte progenitors and neural stem cells
- Stains medium to high grade forms of astrocytoma vs. GFAP
Lysozyme

- Used as a macrophage marker

- In dogs it is expressed in:
  - Monocytes, neutrophils
  - Serous cells in mucosa-associated exocrine glands
  - Renal proximal tubular epithelium

Other Macrophage Markers

- CD 68: nonhuman primates, cat, dog
- Mac 387: nonhuman primate, cat
- CD18: dog

Kidney, dog
**Multifocal vimentin + with a positive internal control**

**Lysozyme**

**Histiocytic sarcoma**

**Factor VIII Related Antigen**

- 99% von Willebrand factor

- Expressed in endothelial cells, megakaryocytes and platelets

- Useful in staining poorly-differentiated hemangiosarcomas (sometimes)
Cutaneous neoplasm, dog

Factor VIII Antigen

Hemangiosarcoma

Platelet Endothelial Cell Adhesion Molecule (PECAM) (CD31)

• Also known as CD 31
• Expressed in endothelial cells, platelets and by some macrophages
• May be used in conjunction with Factor VIII to identify vascular origin
Thyroid Gland

- **Thyroglobulin** expressed in normal follicular epithelium and neoplasms arising from it
- **Thyroid transcription factor-1 (TTF-1)** expressed in follicular epithelium
- **Calcitonin** expressed in parafollicular C cells and neoplasms arising from them
- **Chromogranin** expressed in C cells

Neoplasm, neck, dog

Thyroglobulin

Thyroid follicular carcinoma
Thyroid mass, baboon

Calcitonin

Medullary carcinoma

S-100 Protein

- Primarily expressed by glial cells, but also expressed by:
  - Adipocytes
  - Chondrocytes
  - Melanocytes
  - Interdigitating reticular cells in LN
Neoplasms that express S-100

- Schwannomas
- Neurofibromas
- Astrocytomas
- Oligodendrogliomas
- Nerve sheath tumors
- Chordomas
- Chondrosarcoma
- Melanoma
- Liposarcoma
- Synovial sarcoma

Subcutaneous mass, cat

S-100 Protein

Peripheral nerve sheath tumor
Neuron Specific Enolase (NSE)

- Not very specific
- Normally expressed in: neurons, nerve fibers, neuroendocrine cells
- Expressed in the following neoplasms:
  - Neuroneuroendocrine tumors
  - Schwannomas
  - Melanomas
  - Astrocytomas
  - Gangliomas
  - Glioblastomas
  - Meningiomas
  - Gastrointestinal stromal tumors (GISTS)
- Must use in conjunction with other markers

Synaptophysin and Chromogranin

- Used to demonstrate neuroendocrine origin
- Very sensitive to formalin fixation

Synaptophysin

Pancreatic islet cell, dog
Chromogranin

Adrenal medulla, dog

Pharyngeal neoplasm, dog

Synaptophysin

Neuroendocrine carcinoma
**Muscle Markers**

- Actin or muscle specific actin
- Smooth muscle actin
- Myoglobin

**Actin**

- Expressed by skeletal, smooth and cardiac muscle
- May also be expressed by actin-containing fibroblasts
- Used in conjunction with desmin to confirm myogenic origin

**Smooth Muscle Actin**

- Specifically expressed by smooth muscle cells
- May also stain actin-containing fibroblasts (myofibroblasts)
- Used to differentiate skeletal vs. smooth muscle origin
Myoglobin

- Expressed by normal skeletal and cardiac muscle
- Expressed late in cell differentiation
- Used to differentiate skeletal vs. smooth muscle origin

Spindle Cell Neoplasm

- Neural crest origin
  - GFAP
  - Melan-A, HMB-45, PNL2
  - Melanoma
  - Factor VIII or CD31
  - Vascular origin
    - Actin, desmin
    - Myoglobin
    - Smooth muscle
    - Skeletal or cardiac muscle

Neoplasm thoracic wall, rat
Diagnosis?

**Rhabdomyosarcoma**

Gastric submucosa, dog
Diffuse vimentin immunoreactivity

Diffuse desmin immunoreactivity

Diffuse actin immunoreactivity
Diffuse smooth muscle actin immuno-reactivity

Negative staining for CD117

Negative staining for GFAP
DIAGNOSIS?

Leiomyosarcoma

Myoepithelial Markers

- Calponin
- Maspin
- p63

Mammary neoplasm, cat
Leukocyte and Platelet Markers

- **CD 1a**: dendritic and Langerhans cells; some thymocytes
- **CD 3**: pan T cell marker
- **CD 18**: neutrophils, mast cells, macrophages
- **CD 45RA**: pan B lymphocyte
- **CD 56**: NK cells

Leukocyte and Platelet Markers

- **CD 68**: histiocytes and monocytes
- **CD 79a**: B lymphocytes, plasma cells
- **BLA 36**: B lymphocyte
- **Lambda/kappa light chains**: plasma cells
- **CD 117 (c-kit)**: mast cells, Gastrointestinal stromal tumors (GIST)
Round cell neoplasm

- CD79a, BLA, CD45RA, CD20
- Kappa, lambda light chains
- Plasma cell tumor
- Lysozyme, CD68, MAC 387
- Histiocytic
- CD 117

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Duodenal mass, dog
*Vet. Path.*, 39:5, pp.557-64
Mast cell tryptase

Dx: Mast Cell Tumor

Cutaneous neoplasm, dog

CD 3

Dx: Epitheliotropic lymphoma
Mass on the lip, dog

CD 3
Interpretation?

CD 79a
DX: B-cell Lymphoma
Diagnosis of Melanomas

- Have many different histologic appearances
- Many are vimentin and S-100 +
- Melan A has been used as a specific marker
- in cutaneous and oral melanomas

Oral neoplasm, dog

Melan A

**DX: Malignant Melanoma**
Infectious Disease Diagnosis

- IHC can be used to identify bacteria, fungi, viruses, protozoa, and algae
- Some cross-reactivity with some of the antibodies for bacteria and protozoa
- Provides permanent preparation with the organism
- Identifies infected cells/tissues

MERS-Coronavirus; Lung

Lassa, brain
Example

Microscopic Description (19 years):

- Normal urothelial hyperplasia
- Desquamative cystitis

Histology:

- Low-power view: thickened urothelial lining
- High-power view: hyperplastic urothelial cells

Self-Evaluation

- Haired skin, dog
Haired skin: Peripheral Nerve Sheath tumor

Self-exam - Tissue from a dog

Tissue from a dog
Tissue from a dog

Desmin

Self-evaluation #2 - Tissue from a dog

Morphologic Diagnosis:

- Mucous membrane: rhabdomyosarcoma