Spring 2013

Practical Histopathology of Mouse Models of Human Disease

Week 9:

Histology versus Histopathology

& principles of Immunohistochemistry
HISTOLOGY:  
THE MICROSCOPIC STUDY OF BIOLOGICAL MATERIAL

PATHOLOGY: THE STUDY OF DISEASE and THE MORPHOLOGIC CHANGES THAT OCCUR IN

INJURY,  DEATH,

REPAIR, ADAPTATION:

ACCUMULATIONS, ATROPHY, HYPERTROPHY,

HYPERPLASIA, METAPLASIA

INFLAMMATION

NEOPLASIA
HYPERPLASIA: An increase in the number of cells in an organ or tissue, which may then have an increased volume.

Physiologic hyperplasia: Proliferation of mammary glandular epithelium at pregnancy, compensatory hyperplasia of the liver after partial hepatectomy.

HYPERTROPHY: An increase in size of cells and thus an increase in the size of the organ.

eg: physiologic hypertrophy of uterus during pregnancy, hypertrophy of the cardiac muscle in hypertension or valvular disease, hypertrophy of skeletal muscles due to heavy exercise.

ATROPHY: a shrinkage in the size of the cells due to:
- a decreased work load (when a limb is immobilized in a plaster cast)
- loss of innervation
- diminished blood supply
- loss of endocrine stimulation
- aging
Pt. with malabsorption with atrophy of small intestinal villi
Hemorrhage in mouse lungs is obvious if well inflated

Are these tumor nodules?
CELL INJURY: Due to: oxygen deprivation—
- ischemic (no blood flow),
- mechanical trauma (burns),
- chemical agents (acetaminophen),
- infectious agents,
- immunologic reactions,
- genetic defects, nutritional imbalances etc.

INTRACELLULAR ACCUMULATIONS: fatty change of liver cells in alcoholism or obesity, glycogen deposits in diabetes, accumulation of pigments like iron after hemorrhage

METAPLASIA: (one cell type is replaced by another cell type: cigarette smoking induced change of bronchial epithelial cells to squamous, Barrett's esophagitis--where the squamous epithelium of the esophagus is replaced by columnar epithelium)
Normal human liver

Cirrhosis (fibrosis) of liver

Fatty liver with H&E and Oil Red O on frozen sections

Liver fibrosis paraffin sections
H&E Trichrome
Normal

Pathology: obstruction of small intestine, due to tumor of lymph nodes

Pathology: Liver necrosis
H&E of chronic passive congestion of liver with centrilobular necrosis of hepatocytes
CELL DEATH:
- necrosis (occurs from the progressive degradative action of enzymes on the lethally injured cells)
- Large areas of tissue with cell death

apoptosis:
- Programmed destruction of cells during embryogenesis
- Hormone dependent involution in the adult
- Cell deletion in proliferating cell populations, immune cells, tumors, etc.
Pathology: inflammation of eyelids
Cell types that infiltrate injured tissue

Blood smear stained with Giemsa
INFLAMMATION AND REPAIR

Is a protective response, where the goal is to rid the body of the initial cause of injury and the consequences

ACUTE: relatively short duration. There is an alteration of blood vessels such that there is an exudation of fluid and plasma proteins, with an emigration of leukocytes, predominantly neutrophils, into the focus of injury.

CHRONIC: is of longer duration and is associated with the accumulation of lymphocytes and macrophages and allowing the repair process to occur, using angiogenesis and/or fibrosis.
Histology of pneumonia
Histology of normal lung parenchyma as compared to a section from a patient who died from long standing chronic Asthma.

http://pathhsw5m54.ucsf.edu/introduction.html
Neoplasm = “new growth”
Exceeds growth of normal tissue
Uncoordinated with growth of normal tissue
Continue to proliferate despite regulatory controls

Tumor = swelling.
Benign tumor -- no infiltration into surrounding tissue.
Malignant tumor = cancer

Cancer is the common term for all malignant tumors. Cancer derives from the Latin term crab presumably because it “adheres to any part that it seizes in an obstinate manner like the crab”
Carcinoma with mitosis
Multiple polyposis colon: a polyp is an outpouching of mucosal hyperplastic cells into the lumen
Benign versus Malignant Neoplasm

**Benign:**
- Remains localized
- Will not spread to other sites
- Can be surgically removed
- Clinical behavior innocent
- Patient will survive

**Epithelial**

**Non-epithelial**
- Connective tissue
- Fibers
- Cartilage / Bone
- Adipose (fat) tissue
- Blood, Lymphoid tissue
- Muscle
- Nervous tissue

**Malignant Neoplasms: Carcinoma or Sarcoma**
- invade basement membranes to infiltrate and destroy adjacent structures
- can metastasize (spread to distant places)
- may cause death
Lesions In the intestine: Benign and malignant
Dysplasia and in-situ carcinoma has not invaded basement membrane
Robbins and Kumar textbook of Pathology description of the process of malignant progression and metastasis.

- Clonal expansion, growth, diversification, angiogenesis
- Metastatic subclone
- Adhesion to and invasion of basement membrane
- Passage through extracellular matrix
- Intravasation
- Interaction with host lymphoid cells
- Tumor cell embolus
- Adhesion to basement membrane
- Extravasation
- Metastatic deposit
- Angiogenesis
- Growth
Bronchogenic carcinoma
Metastasis from a malignant neoplasm
SARCOMAS: Malignant tumors of supporting tissue

- chondrosarcomas—cartilage
- osteosarcomas—bone
- hemangiosarcomas—blood vessel
- gliomas (astrocytoma, glioblastoma)
- lymphomas
- melanomas
- rhabdomyosarcomas
- leiomyosarcomas
- fibrosarcomas
- seminoma, teratoma, etc.
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Principles of Immunohistochemistry
Processing of tissue:

- Isolate cells for culture
- Freeze for protein, lipid, sugar, DNA/RNA etc. extracts
- Process into paraffin blocks
- Process for EM
  - Fix
  - Dehydrate
  - Infiltrate with xylene
  - Infiltrate with hot paraffin wax
  - Make blocks for sections
  - Store at room temperature

Freeze for histology/histochemistry/immunohistochemistry
- Dry ice in 2-methyl butane
- OCT in plastic mold

Frozen or paraffin tissue can then be sectioned for histology: 3-30 micron sections.
Effect of different fixatives on preserving epitopes in frozen sections

If the tissue is paraffin embedded, some mouse monoclonals do not recognize the epitope, in spite of using retrieval techniques.
Effect of fixation on masking of epitopes
Tertiary reagent is used usually labeled with:

- DAB, AEC, red, SG, VIP
- Blue, Red (also fluoresces)

fluoresceinated compounds or with an enzyme

Remove endogenous binding sites in tissue, (biotin, HRP, collagen)

Tissue section: Frozen or deParaffinized
Panels showing H&E of Liver endothelial markers and a macrophage marker
FROZEN SECTION OF MOUSE SUBCUTANEOUS XENOTRANSPLANT

Review of H&E section helps with analysis of angiogenesis, using anti CD31
Immunofluorescence with anti CD31 to detect blood vessels in a tumor mass and deconvolution microscopy
CD3 for all T lymphocytes

CD19/20 for B lymphocytes

CD 68 for macrophages
Negative and Positive controls are essential
If the tissue is **frozen**

Unfixed:
   - Positive feature: antigens are unaltered
   - Negative feature: sections may fall off slide during staining

**Acetone fixed:**
   - precipitates proteins onto cell surface---may extract lipids
   - is needed for many of the "CD" antibodies

**Paraformaldehyde fixed:**
   - needs to be freshly made, or frozen soon after
   - is preferred over using 10% buffered formalin

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**Tissue section on glass slide:** Frozen
If the tissue is **paraffin** embedded,

--deparaffinize (remove the infiltrated paraffin wax, by using organic solvents)

--the section then needs to be rehydrated, by sequential immersion in graded alcohols (100%, 70%, 50% and then PBS)

--the deparaffinized section may need to be treated to expose buried antigenic epitopes with either proteases or by heating in low pH citrate buffer, or high pH EDTA buffer

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Tissue section: Paraffin embedded
Design controls for secondary and/or tertiary reagents

A. Reagent controls include:
   1. Slide that receives PBS alone
   2. Slide that receives BSA alone
   3. IgG control at the same concentration as the test antibody
   4. Another antibody, with the same isotype as the test antibody, not specific to the epitope recognized by test antibody
   5. Positive control reagent, same species as primary being tested

B. Tissue / or cells controls include:
   1. Tissue or cells not expected to be positive
   2. Tissue or cells expected to be positive
   3. Blocking reagent to delete positive reaction, to demonstrate specific binding by the test antibody
When testing a new antibody, one needs to know:

**Species of origin of the primary antibody:** mouse, rabbit, rat, hamster, chicken, goat, sheep, horse........

In order to:
--- design what cells or tissues will be used as positive and negative controls
--- design secondary and tertiary detecting reagents
--- design reagents to block non-specific binding

**Primary antibodies may be**
- Polyclonal
  (rabbit, sheep, goat, chicken)
- Or **Monoclonal**
  (rat, mouse)

**Tissue section:** Frozen or Paraffin embedded

- Unfixed or Fixed-
  Acetone, Parafomaldehyde, .

- Deparaffinize

- Antigen retrieval
- No antigen retrieval
Block Non-Specific Binding sites in tissues, because of the large variety of cells present

--- Block non-specific binding to extra cellular matrix components, usually use bovine serum albumin

If using HRP conjugates Block endogenous peroxidases in RBCs present in all tissues

If using alkaline phosphatase conjugates, endogenous alkaline phosphatase in tissues will contribute to annoying background binding eg: within sections of frozen intestine, Bone marrow, placenta. This may be removed using heat or 0.1M glycine

--- if using biotinylated secondary reagents Block endogenous biotin
There is endogenous biotin in most tissues other than spleen or thymus

--- Treat one set with block to prevent binding of first reagent
At each step of the immuno assay, if using new reagents, one needs to determine the optimum working dilution.

Unbound antibody washed off before application of secondary reagent.

Primary antibody: maybe used, already labeled with fluoresceinated compounds or with an enzyme label.

Block nonspecific binding sites before adding primary antibody / reagent.

Tissue section: Frozen or deParaffinized.
Unbound reagent is washed off before application of next reagent

Secondary reagent:
- may be used already labelled with fluoresceinated compounds or with an enzyme label
- OR it may be conjugated with Biotin, digoxigenin, etc.

Dilute secondary reagent with normal serum of species being tested to block nonspecific binding of secondary

Tissue section: Frozen or deParaffinized
Tertiary reagent is used usually labeled with:

- DAB, AEC, red, SG, VIP
- Blue, Red (also fluoresces)
- HRP, Alk.Phos
- or with an enzyme
- AMCA
- CY2, FITC
- PE, CY3
- fluoresceinated compounds

Remove endogenous binding sites in tissue to prevent nonspecific binding.

Tissue section: Frozen or deParaffinized

Wash off unbound tertiary before adding substrate or before mounting to view or before further amplification.
Using double stain methods to determine if two antibodies recognize the same epitope on cells in a tissue section

Need to use lower dilutions of antibodies when doing a double stain to compensate for additive effect.

May use antibodies sequentially and detect each with different fluoresceinated tags so that if the same epitope is recognized a new color will be visualized.

The second antibody may bind to the same epitope within cells that are less abundant.

One antibody may bind with higher affinity or recognize an epitope within cells that are more abundant.

Tissue section: Frozen or deParaffinized.
Apoptotic cells, detected using the TUNEL assay, shows FITC positive nuclei. Double labeling with CD4 shows that some of the TUNEL positive cells are of the CD4 cell lineage.
Co-localization and detection of similar epitopes on the same tissue section, using fluorescent markers
Frozen sections of mouse thymus with overlay

Where the application of AbB was followed by Mac1

In this overlay, where application of Mac1 was followed by AbB, one cannot visualize AbB because Mac1 is so abundant.
An example of further Amplification to detect low abundance epitopes in tissue

Incubation with more labeled streptavidin then detects the additionally generated biotin, thus enhancing detection levels by a factor of 100-1000

Biotinyl tyramide uses the HRP enzyme to deposit many biotin molecules that “cover” the antigen antibody complexes already formed over the epitope on the tissue section

Tissue section: Frozen or deParaffinized

Wash off unbound before developing or mounting
THANK YOU

Did you learn something interesting and informative?

Please mail the all important Evaluations (anonymous) to me at mail code 0687