Pathology and The Pathology of Neoplasia

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Department of Pathology

Outline
What is Pathology?
What is a Pathology Department?
The pathologist’s “tools”
Pathology Definitions and Concepts
How do we approach a pathology specimen?
A General Classification of Neoplasia.
The ugly histologic face of cancer (recognizing malignancy).
Grading and Staging Malignancy.
The Changing World of Pathology
Pathology and Research

Pathology

“...branch of medicine which treats (studies) of the essential nature of disease, especially of the structural and functional changes in tissues and organs of the body which cause or are caused by disease.”
   - Dorland’s Medical Dictionary 24th Ed.
“...something abnormal- the anatomic and physiologic deviations from the normal that constitute disease or characterize a particular disease.”
   - Webster’s Seventh New Collegiate Dictionary
A Department of Pathology

Patient Care
ANATOMIC PATHOLOGY
CLINICAL PATHOLOGY

Experimental (Research)
TISSUE PROCUREMENT
TRANSLATIONAL
BENCH

Anatomic Pathology

- Surgical Pathology
  - Frozen Section (Intra-operative consultations)
  - Biopsy and Resection specimens

- Cytopathology
  - Pap smears (Gynecologic specimens)
  - Fine needle aspirates, washes, brushes

- Autopsy
Clinical Pathology
- Hematology
- Chemistry
- Microbiology
- Blood Bank (Transfusion Medicine)
- Tissue Typing (Transplant Pathology)
- Molecular Diagnostics
- Flow Cytometry
- Cytogenetics

Anatomic Pathology in Cancer
- Patient has signs or symptoms of cancer
- Clinician/Radiologist performs biopsy
- Pathologist diagnoses cancer on biopsy
- Clinician performs resection/excision
- Pathologist stages and evaluates cancer
- Molecular Characteristics of Tumor Evaluated for use in "Personalized Medicine"

The Pathologist’s Tools
- The “Grossing Station”
- Fixation (usually with Formalin-37% formaldehyde)
  - Preserve tissue, enhance detail for microscopy
- Tissue Processing (usually overnight)
  - Remove tissue water gradually, replace with paraffin wax
- Paraffin Embed and cut tissue sections
- Hematoxylin and Eosin Stain
- The Light Microscope
Gross Examination

Cassettes and Tissue Sections

Tissue Processor

1. Dehydrate Tissue
   • Progressive series of alcohols removes water
2. Clear Ethanol with xylene
3. Replace with paraffin
Tissue Embedded in Liquid Paraffin

Sections Cut (~ 5μm) by microtome

Hematoxylin and Eosin (the H&E)

The pink and purple foundation of pathology

Hematoxylin
- Purple/blue stain (BASOPHILIC)
- Stains acidic materials
- Stains nuclear contents

Eosin
- Pink stain (EOSINOPHILIC)
- Stains most cytoplasmic contents
Ancillary Studies
- Flow cytometry
- Immunohistochemical stains
- Cytochemical stains
- Cytogenetics
- Electron microscopy
Immunohistochemistry (IHC)

IHC: what is it good for?

- Identify or confirm the source of a tumor
  - CK7, CK20, other markers "specific" to tumor type

- Increase the sensitivity of tumor detection
  - e.g., find individual metastatic melanoma cells in a lymph node using Melan-A

- Predict tumor behavior/prognosis
  - proliferative index (Ki-67)

- Identify potential therapeutic targets
  - Estrogen receptor – Tamoxifen (breast cancer)
**Immunohistochemistry**

**DISADVANTAGES**

- **FALSE Negative**
  - Antigen - not present in tissue, degraded
  - Antibody - too dilute, expired, wrong antibody

- **FALSE Positive**
  - Cross reactive or non-specific antibody binding
  - Endogenous peroxidase or avidin/biotin binding
  - Entrapped normal cells
  - Tumor uptake of normal cell antigens
Definitions and Concepts

Definitions

• TUMOR
  Swelling.

• CANCER
  “crab”, tumor with fatal course and association with formation of secondary tumors (metastasis)

Definitions

• EPITHELIUM (epithelial) → when malignant it is called CARCINOMA
  Derived from ectoderm or endoderm: the protective, absorptive and secretory lining of the body both externally (e.g. skin), and internally (e.g. lining of colon, pancreatic glands)

  Common types of epithelium are: squamous and columnar/glandular
Definitions

- **MESENCHYME** (mesenchymal) → when malignant it is called **SARCOMA**

  Largely derived from mesoderm: structural and functional elements of the body- “connective tissue” (cartilage, bone, collagen, nerve, blood vessels, etc...)

Definitions

- **NEOPLASIA**
  Any new or abnormal growth (typically implies a clonal population of cells).

- **NEOPLASM**
  Mass of new tissue which persists and grows independently of its surrounding structures and which has no physiologic use
Definitions

- **METAPLASIA**
  A change in tissue cells to a form not normal for that tissue, usually reversible and protective.

- **HYPERPLASIA**
  An increase in the NUMBER of cells, while maintaining normal tissue architecture.

Images taken from WWW: “The GastroLab Endoscopy Archive”

Metaplasia (Barrett’s Esophagus)
INVASION

The penetration of neoplastic epithelial cells through the basement membrane.

Also used less specifically to refer to cells (epithelial or mesenchymal) penetrating any adjacent existing structure.

DIFFERENTIATION

Degree to which a neoplasm recapitulates normal tissue in all its characteristics (often assessed as a GRADE)
Well Differentiated Lung Adenocarcinoma (Bronchioloalveolar Carcinoma 200x)

Poorly differentiated lung adenocarcinoma (200x)

Definitions

- **ATYPIA**
  The condition of being irregular, refers generally to abnormalities of histologic appearance - not always neoplastic in nature (can be “reactive”, as in setting of radiation therapy or inflammation)

- **ANAPLASIA**
  A loss of normal differentiation and form.
**Definitions**

- **DYSPLASIA**
  
  Abnormality of maturation and development, manifesting as architectural, organizational and cytologic atypia

  MILD → MODERATE → SEVERE
  (SEVERE usually is Carcinoma in-situ)

  DYSPLASIA IS ALWAYS ATYPICAL, ATYPIA IS NOT ALWAYS DYSPLASTIC!

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**Colonoscopy**

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**Colonic Adenoma (Dysplasia 100x)**

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Colonic Adenoma (Dysplasia 200x)

Definitions

- **PLEOMORPHISM**
  Variation in cellular size and shape

- **MALIGNANT**
  A neoplasm with invasive and metastatic capacity.

Pulmonary Neuroendocrine Carcinoma

- Carcinoid (400x)
  LOW GRADE, MINIMAL TO NO ATYPIA
  MONOMORPHIC

- Small Cell Carcinoma (400x)
  HIGH GRADE, SEVERE ATYPIA
  PLEOMORPHIC
BEWARE: GROSS PHOTOS AHEAD

Approaching a pathologic specimen.

GROSS APPEARANCE
- "dissect" and describe the specimen
- Take carefully selected portions of tissue for subsequent examination under the microscope

MICROSCOPIC APPEARANCE
- Routine Hematoxylin and Eosin stained slides
- Special histologic techniques (stains, immunohistochemistry)
**Gross Specimen...**

Hepatocellular Carcinoma

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**Another Gross Specimen**

Adenocarcinoma of colon

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**Approaching a pathologic specimen**

- Is there pathology present?
- What is the general nature of the pathology?
  - Infectious, neoplastic, inflammatory/autoimmune
- If it IS neoplastic, what parameters must be collected?
  - Benign or malignant?, tumor type, grade, stage, resection margin status
General Types of Neoplasia

- **EPITHELIAL** (eg. Carcinoma)
  - Generally derived from ectoderm or endoderm
- **MESENCHYMAL** (eg. Sarcoma)
  - Generally derived from mesoderm (neural tissue is an exception → it's ectodermal)
- **HEMATOPOEITIC** (eg. Lymphoma)
  - Generally derived from mesoderm
- **MIXED** (eg. Carcinosarcoma)
- **OTHER** (eg. Germ cell tumor)

EPITHELIAL TUMORS

- adenoma
- papilloma
- carcinoma
- etc

Carcinoma

- Malignant tumor of epithelium

- Comes in different types:
  - Squamous cell carcinoma
  - Adenocarcinoma (glandular)
  - Neuroendocrine carcinoma

- Often preceded by a pre-invasive lesion:
  - severe dysplasia (carcinoma in-situ)
Adenocarcinoma of Lung

Normal Bronchial Epithelium (400x)

Bronchial Squamous Metaplasia (200x)

Severe Bronchial Squamous Dysplasia/Carcinoma in-situ (200x)

Invasive Squamous Cell Carcinoma (400x)
Carcinoma

- One hallmark of an epithelial neoplasm is cellular expression of cytokeratin (CK) intermediate filaments.

- Cytokeratin expression profile can also help identify the primary site/source of a carcinoma (immunohistochemistry).
  - CK 7, CK 20, CK 5/6

MESENCHYMAL TUMORS

- OMA
- SARCOMA

Mesenchymal tumors

- Tumors of connective/mesenchymal tissue

Named according to their putative origin and differentiation:

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Malignant</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle</td>
<td>leiomyosarcoma</td>
<td>leiomyoma</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>rhabdomyosarcoma</td>
<td>rhabdomyoma</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>angiosarcoma</td>
<td>angioma</td>
</tr>
<tr>
<td>Cartilage</td>
<td>chondrosarcoma</td>
<td>chondroma</td>
</tr>
<tr>
<td>Bone</td>
<td>osteosarcoma</td>
<td>osteoma</td>
</tr>
<tr>
<td>Adipose (fat)</td>
<td>liposarcoma</td>
<td>lipoma</td>
</tr>
</tbody>
</table>
Mesenchymal Tumors

- Usually express the intermediate filament vimentin, and NOT cytokeratins
- May also express markers of differentiation:
  - Smooth Muscle → smooth muscle actin
  - Nerve → S100
  - Vascular → Cluster of Differentiation 31 (CD31)

HEMATOPOETIC NEOPLASIA

- leukemia
- lymphoma
Hematopoetic Neoplasia
- Derived from bone marrow cells (red, white)

Lymphoma (cells usually involve lymph node or other lymphoid tissue like spleen)
- T-cell (CD3+, CD20-)
- B-cell (CD20+, CD3-)
- NK cell (CD56+)

Leukemia (cells circulate in blood)
- Myeloid
- Lymphoid

MALT Lymphoma of Lung (40 x)
MALT Lymphoma (400 x)
So how do we know if a bunch of cells is benign or malignant?

**Recognizing Malignancy**

1. Architecture and Behavior
2. Proliferation (mitotic rate, antigenic markers)
3. Necrosis
4. Cytology (nuclear and cytoplasmic)

**Recognizing Malignancy**

- Architecture and behavior
  - How closely does tumor resemble normal tissue?
  - What is the contour of the tumor?
  - Is there invasion of adjacent tissue?
  - Is there invasion of blood vessels or nerves?
  - Does it have a capsule?
Mammogram of Ductal Carcinoma

BENIGN
- Organized
- Well circumscribed
- No infiltration of adjacent tissue.
- Normal stroma

MALIGNANT
- Disorganized
- Poorly circumscribed
- Infiltrates adjacent fat.
- Desmoplastic stroma.
Recognizing Malignancy

- **Proliferative Rate**
  - What is the mitotic count? (number of mitoses per 10 high-power 400x microscopic fields)
  - Are mitoses normal in form?
  - What is the percentage of cells expressing markers of proliferation? (Ki-67/Mib-1)

- **Necrosis**
  - Is there tumor necrosis? (function of rapid uncontrolled proliferation, loss of cell cycle control)

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**Hepatocellular Carcinoma with Necrosis**

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**Hepatocellular Carcinoma with Mitosis**

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Recognizing Malignancy

- Cytology (a function of rapid proliferative rate and abnormal, active cellular ‘machinery’)
  - What is the character of the nuclear chromatin?
  - Are there abnormal nucleoli present?
  - What is the contour of the nuclear membrane?
  - What is the nucleus:cytoplasmic ratio? (N:C ratio)
  - Is the cytoplasm mature?

Benign or malignant nuclei (400x)?

<table>
<thead>
<tr>
<th>#1</th>
<th>#2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALIGNANT</td>
<td>BENIGN</td>
</tr>
</tbody>
</table>

GRADING AND STAGING
MALIGNANCY

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Grading Malignancy

- Grading is based on cytologic and/or architectural characteristics of the tumor.
- Attempts to predict future behavior of a tumor.
  - High, Severe or Poor essentially means Bad behavior.
- Generic systems for many tumors:
  - Well → Moderate → Poor → Undifferentiated
  - Severe → Moderate → Mild
  - High → Low

Some tumor types have specific, well validated grading systems:

- Prostate → Gleason Grade
  - Primarily based on tumor architecture
- Breast → Nottingham Grade
  - Tubule formation, Nuclear features, Mitotic rate
- Endometrium → FIGO Grade
  - Architecture with nuclear feature modifier

Gleason Grading of Prostate Carcinoma

Figure Taken from Epstein J. et al. Am J Surg Pathol 29(9): 1228 (2005).
Cancer Staging

- Staging is a precise method for establishing the type and extent of tumor at a specific point in time during the course of the disease. Staging is critical to the modern management of cancer because it allows the clinical team to:
  1. Plan treatment
  2. Estimate prognosis
  3. Group similar patients to evaluate results on treatment protocol studies
  4. Facilitate information exchange between institutions
  5. Otherwise contribute to the study of cancer

Staging

- Performed according to guidelines published by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC or Union Internationale Contre le Cancer)

- Stage is NOT the same thing as grade

- The staging system for each tumor type is different (see example for melanoma)

Staging Melanoma

- Breslow Depth (3.1 mm)
- Ulceration
**Staging Melanoma**

**Tis**  Melanoma in-situ  
**T1**  Melanoma measuring 1.0 mm or less  
   a) no ulceration, mitosis <1/mm²  
   b) ulceration or mitosis >=1/mm²  
**T2**  Melanoma measuring 1.01 – 2.0 mm  
   a) no ulceration  
   b) ulceration  
**T3**  Melanoma measuring 2.01 – 4.0 mm  
   a) no ulceration  
   b) ulceration  
**T4**  Melanoma measuring >4.01 mm  
   a) no ulceration  
   b) ulceration

**Staging Melanoma**

**NX**  Lymph nodes not assessed  
**N1**  One lymph node positive  
**N2**  Two or three nodes positive OR  
   In-transit/Satellite metastasis  
**N3**  Four or more nodes positive OR  
   In-transit/Satellite metastasis AND nodes positive  
**M0**  No distant metastasis  
**M1**  Distant metastasis

**AJCC Stage Groups**

**Melanoma**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Tis</th>
<th>N0</th>
<th>M0</th>
</tr>
</thead>
<tbody>
<tr>
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<td>M0</td>
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<td>IB</td>
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<td>N0</td>
<td>M0</td>
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<tr>
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<td>T2a</td>
<td>N0</td>
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<td></td>
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<td>M0</td>
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<tr>
<td>III</td>
<td>any T</td>
<td>any N+</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>any T</td>
<td>any N+</td>
<td>M1</td>
</tr>
</tbody>
</table>
Melanoma Survival by AJCC Stage

Figure taken from Melanoma Molecular Map Project (www.mmmp.org)

ESOPHAGUS
BREAST
National Cancer Institute SEER Data 1998-2001

And finally...
ADVANCES IN PATHOLOGY
AND PATHOLOGY IN RESEARCH
The Changing World of Pathology

Traditional histopathology merges with molecular and genetic evaluation:
- Molecular or genetic classification of cancer.
- Identify primary origin of metastasis

Personalized therapy
- Use biopsy tissue to plan best treatment
- OmniSeq multigene platform

2008 WHO Classification of myeloid neoplasms and acute leukemia

Acute myeloid leukemia and related neoplasms
Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- APL with t(15;17)(q22;q12); PML-RARA
- AML with t(9;11)(p22;q23); MLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RB1-RUN1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- Provisional entity: AML with mutated NPM1
- Provisional entity: AML with mutated CEBPA

Molecular Classification

Should we group tumors by genotype or “target” rather than conventional histology or origin site?

BRAF mutated tumors:
- Melanoma
- Colorectal adenocarcinoma
- Lung adenocarcinoma
- Papillary thyroid carcinoma
Melanocytic Tumor of Uncertain Malignant Potential - MELTUMP

- Lesions in which traditional histological criteria may be insufficient to fully predict biology

- Example:
  - Spitz Nevus
  - Atypical Spitz Nevus
  - Atypical Spitz Tumor \( \leftarrow \) MELTUMP
  - Spitzoid melanoma

How can we improve our diagnosis?

- Immunohistochemistry – sometimes helpful
- Comparative genomic hybridization
- Fluorescence in-situ hybridization
- Proprietary molecular tests – controversial
CGH

- Conventional melanoma
  - Genomic instability
  - Most have multiple chromosome copy number gains or losses

- Spitz nevi
  - Very rare to have multiple abnormalities
  - Some single abnormalities (gain 11p, gain 7q)
  - BAP1 mutation, HRAS mutation, kinase fusions

“Melanocytic tumor of uncertain malignant potential”

**Risk Assessment for Atypical Spitzoid Melanocytic Neoplasms Using FISH to Identify Chromosomal Copy Number Aberrations**

*Prunei Ojvand, MD*; *Richard T. Schreiber, MD*; *Dawson Xu, MD*; *Phil D. Goodwin, MD*; *Elliott F. Eisner, MD*; *Roxie M. Abraham, MD*; *Douglas Zelina, MD*; *Vikas G. Pinto, MD*; *Paul**

*Philip E. Solis*, MD; *Reynold L. Barr*, MD; *Montana Cooper*, MA

*Prudei Tran*, MD; *Jean Auster*, MD; *Hong Liu*, PhD

*Sharonne Sizerman*, PhD and *Karin Bazer*, MD

Spitz Tumor assessment by FISH

- **HIGH RISK** – Progression beyond SLN
  - 9p21 homozygous deletion (CDKN2A gene/p16)

- **INTERMEDIATE RISK**
  - 6p25 gain (RREB1 gene)
  - 11q13 gain (CCND1 gene)

- **LOW**
  - 6q23 deletion (MYB gene)
  - No abnormalities by FISH
The Future of Pathology

- Traditional histopathology merges with molecular and genetic evaluation:
  - Molecular or genetic classification of cancer.
  - Identify primary origin of metastasis
- Personalized therapy
  - Use biopsy tissue to plan best treatment
  - OmniSeq multigene platform

Lung Adenocarcinoma: Molecular targets

Based on data from:
Ding et al., Nature 455:1069, 2008
And modified from a figure developed by:
Dr. F. Hirsch, University of Colorado

the EGFR Pathway

Basic Algorithm for Handling Lung Cancer Specimens

Tumor Biopsy

Small Cell

Non-small cell

Advanced Disease

Surgical Candidate

Histologic Lymph node

Squamous
Non-squamous

Stains to further classify

Squamous Biomarkers?

Treatment

Non-squamous

EGFR
K-ras
EML4/ALK

Personalized Treatment

Personalized Medicine

OmniSeq Comprehensive™

Next generation molecular sequencing of DNA and RNA
144 cancer associated “actionable” genes such as ALK, EGFR, PTEN, KRAS, NRAS, BRAF, etc
Detects mutations, translocations/fusions, copy number changes, etc.

RPCI Center for Personalized Medicine

Personalized medicine offers glimpse into the future
OmniSeq Panel

Pathology and Research

- Initiate both basic and translational research
- Tissue procurement and specimen archiving
- Bridge between clinic and research bench
  - Analyze human/animal experimental histology
  - Build and evaluate tissue based experiments
    - Example: tissue microarray
  - Provide clinical perspective to scientific groups

Pathology Resource Network (PRN)
LIMS: Biospecimens

A tally of primary lung adenocarcinomas with genomic DNA banked at RPCI.

Tumor Procurement

Procurement events from one random Friday's surgical schedule...

Tissue may be distributed FRESH to labs or flash frozen for long term banking. Procurement group provides QA evaluation of samples.

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Grading and Staging Malignancy
The Changing World of Pathology
Pathology and Research

The End

feel free to contact me with questions or comments:
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