American Society of Cytopathology
Core Curriculum in Molecular Biology
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Chapter 1

Molecular Basis of Cancer
*Molecular Oncology*

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What Are Tumors?

Tumors are the result of a disease process in which a single cell proliferates abnormally, resulting in an accumulation of progeny cells.

What Is Cancer?

Cancer is a collection of diseases characterized by the uncontrolled growth and spread of abnormal cells.

American Cancer Society
www.cancer.org
## Comparison of Benign and Malignant Neoplasms

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gross appearance</strong></td>
<td><strong>Gross appearance</strong></td>
</tr>
<tr>
<td>• Smooth margins, encapsulated</td>
<td>• Rough margins, no capsule</td>
</tr>
<tr>
<td>• Center resilient, soft, viable</td>
<td>• Firm center, focal necrosis</td>
</tr>
<tr>
<td><strong>Microscopic pattern</strong></td>
<td><strong>Microscopic pattern</strong></td>
</tr>
<tr>
<td>• Resembles normal, well-</td>
<td>• Poorly differentiated</td>
</tr>
<tr>
<td>• Poorly differentiated</td>
<td>• Invasive to adjacent tissues</td>
</tr>
<tr>
<td>• Intact basement membrane</td>
<td>• Disrupted basement membranes</td>
</tr>
<tr>
<td>• Cells and nuclei are normal</td>
<td>• Cells and nuclei are large/irregular</td>
</tr>
<tr>
<td>size/shape</td>
<td></td>
</tr>
</tbody>
</table>

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## Comparison of Benign and Malignant Neoplasms (cont.)

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Slow growth rate</td>
<td>• Rapid growth rate</td>
</tr>
<tr>
<td>• No metastatic disease</td>
<td>• Metastases are common</td>
</tr>
<tr>
<td>• Produces local effects</td>
<td>• Can cause local and/or distant pathophysiological effects</td>
</tr>
</tbody>
</table>

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Cancer Is a Genetic Disease

• Cancer is a disease of genes or gene regulation that begins in a single cell and results in the loss of control of cell growth.

• Any cell type can become malignant.

• Carcinogenesis can result from acquired and/or inherited genetic abnormalities.
Cancer: A Group of Multifactorial/Polygenic Diseases

- Environmental
  - Chemical
  - Radiation (UV)
- Infectious
  - Viruses (EBV, HPV)
- Hereditary
  - Germline genetic defects
Cancer Pathogenesis: A Multi-step Process

Normal epithelial cell → Malignant transformation → Tumorigenesis → Carcinoma

Multi-step Cancer Induction with Chemicals Occurs in Definable Stages

Initiation → Promotion → Progression
Clonal Basis of Cancer Development

• Tumors arise from a single ancestral cell that has accumulated critical initiating mutations (genetic changes)
• In some cases, these mutations (genetic changes) are present in all cells (genetic predisposition).
• Multiple mutations are needed for tumor formation.
• The number of mutations is independent of the tumor type.
Carcinogenesis Is a Multistage Process

Chemical radiation virus

- Defects in cellular differentiation
- Defects in growth control
- Resistance to cytotoxicity

Genetic change

Selective clonal expansion

Genetic change

Normal Cell

Initiated Cell

Preneoplastic Lesion

Malignant Tumor

- Activation of Protooncogenes
- Inactivation of Tumor Suppressor Genes

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Oncology Markers

• Used to assess genetic mutations for an individual patient’s tumor (a.k.a. tumor profiling)
• Helps guide clinicians in selecting the most appropriate therapeutic regimen
• Development of molecular pharmacogenetics is result in new prognostic indicators being used for the assessment of individual tumors
Review: Classification of Neoplasms

• Neoplasia: 2 broad groups
  – Solid tumors
    • Designated according to tissue of origin
      – Carcinoma (epithelial)
      – Sarcoma (bone, cartilage, muscle, blood vessels, fat)
      – Teratocarcinoma: multiple cell types
  – Hematological malignancies
    • Arise from white blood cells
      – Leukemia
      – Lymphoma
        » Hodgkin’s disease
        » Non – Hodgkin’s lymphoma
        » Plasma cell neoplasms
Cancer: Caused by Nonlethal Genetic Mutations Affecting Certain Genes.

- **Oncogenes**, as proto-oncogenes, normally promote cell division or cell survival.
  - Support cell survival by inhibiting apoptosis
  - **100 oncogenes identified in human genome**
  - Oncogene mutations are usually a gain of function
  - Results from amplification or translocation of DNA regions containing the genes
Cancer: Causes by Nonlethal Genetic Mutations Affecting Certain Genes.

- **Tumor suppressors**: genes normally arrest cell division
- Include factors controlling transcription or translation of genes
- Repairs DNA damage and promotes apoptosis
- Counteracts movement of cell from G1 to S or G2 to M phase of mitosis
  - G1 check point and G2 checkpoint
- More than 30 tumor suppressor genes currently identified
  - Tumor suppressor gene mutations are usually a loss of function
  - Results in inactivation tumor suppressor gene products
    - **Occur through deletions, translocations, or mutation of the gene**
Proto-oncogene and Tumor Suppressor Gene Products Regulate Cell Proliferation

Normal

- Proto-oncogene products
- Regulated by tumor suppressor genes
- Cell growth and proliferation

Cancer

- Loss or mutation of tumor suppressor genes
- Oncogenes
- Abnormal cell growth and proliferation
- Neoplasia
The Cell Cycle

- **G1**: Cell growth
- **S**: DNA synthesis and chromosome replication
- **G2**: Cell growth
- **M**: Mitosis and cytokinesis

This diagram illustrates the cell cycle, a continuous process that cells go through to grow and divide.
Molecular Detection of Disease

• Targets:
  – Tissue-specific markers (antigens, gene rearrangements)
  – Disease-specific markers (translocations, point mutations, polymorphisms in tumor suppressor or oncogenes)
  – Viruses

• Methods:
  – Hybridization, blotting (Southern Blot)
  – Standard PCR, RT-PCR, electrophoresis
  – Real-time PCR with gene or patient-specific probes
Gene and Chromosome Abnormalities Observed in Cancer

- Gene mutations (oncogenes, tumor suppressor genes)
- Chromosome structural abnormalities (translocations, deletions, insertions)
- Chromosome number abnormalities (aneuploidy, polysomy)
Ras Point Mutations

- Most common oncogene abnormality in human cancers
- ras genes code for membrane-associated proteins that trigger cell division
- Mutated ras proteins are stuck in the “ON” position
Molecular Abnormalities in Solid Tumors, \textit{K-ras}

- The Kirsten rat sarcoma viral oncogene (\textit{K-ras}) encodes a key component of cell signaling.
- Mutations in \textit{K-ras} are the most common oncogene mutations in cancer.
- \textit{K-ras} mutations are associated with tumor malignancy and may affect response to some therapies.
- \textit{K-ras} gene mutations are detected by PCR or direct sequencing.
K-ras Mutations

K-ras mutations

• Colorectal tumors: GI tumors with mutations in K-ras have been shown to be more likely to reoccur.

• The proto-oncogene recognized as mutated most commonly in endometrial carcinoma is K-ras (10-30%).
Cellular Proto-oncogenes Activated by Gene Amplification

**c-myc**
- Leukemias, breast, stomach, lung, and colon carcinomas, neuroblastomas, and glioblastomas

**c-K-ras**
- Lung, ovarian, and bladder carcinomas

**PRAD-1**
- Breast and squamous cell carcinomas

**mdm2**
- Sarcomas
Cellular Proto-oncogenes Activated by Chromosomal Translocation Producing Aberrant Protein Expression

**c-myc**
- Burkitt’s lymphoma and other B-cell lymphoma

**PRAD-1**
- Chronic B-cell leukemia

**bcl2**
- Follicular B-cell lymphoma
Molecular Abnormalities in Solid Tumors, HER2/neu

- The **HER2/neu** gene encodes one of a family of human epidermal growth-factor receptors (EFGR).
- This gene is frequently amplified in breast cancer cells, resulting in increased amounts of HER2 cell surface protein.
- HER2-expressing tumors are sensitive to **herceptin**, a monoclonal antibody therapy.
- HER2 protein is detected by immunohistochemistry (IHC).
- **HER2/neu** gene amplification is detected by fluorescence in situ hybridization (FISH), cytogenetics, and immunostaining.
Her2/neu

• 25% breast cancer patients have tumors with overexpressed Her2/neu gene
• Generally associated with a poor prognosis; patients eligible for herceptin treatment
• IHC for Her2/neu often difficult to discern due to weak positive signals or background staining
• Large percentage of tumors considered positive by IHC for Her 2 show no gene amplification; therefore, IHC produce false positive results
Differences in Interpretation: HER-2 Assessment by FISH and IHC

FISH

High amplification

Low amplification

Normal

IHC

3+

2+

1+

Negative/0
Her2/neu

- FISH testing may have a sensitivity and specificity of 96-100% for detecting over expression of the Her2/neu gene
- Enhances the opportunity for more effective therapeutic triage

* Available with Ventana (ISH) and Vysis, Inc. (PathVision)

Ventana Benchmark (ISH)
*Single Colormetric marker

Vysis (Pathvision)
*Dual Colormetric marker
Her 2 Neu

• PCR also available for testing of Her2/neu using Roche Light Cycler

• Chromogenic ISH assays for Her2/neu (for use with bright field microscopy) are currently under development and offer promising options to fluorescent microscopic interpretation
Tumor Suppressor Genes

Code for proteins that down-regulate cell division.

- Recessive acting/loss of function
- Expression inhibits cancer formation

Knudson’s two-hit hypothesis of cancer development: Both alleles are inactivated by mutation or deletion.
Tumor Suppressor Genes or Their Protein Products Are Inactivated in Cancer

• One gene copy is mutated, while the other is lost (allelic deletion or loss of heterozygosity).
  – Most mutations are acquired (somatic).
  – Some are inherited (Knudson’s hypothesis).
  – First hit (mutation) is inherited; second hit results from somatic mutation or loss (chromosomal deletion).
Tumor Suppressor Protein Functions

- Regulation of cell proliferation (cell cycle progression)
- Contact inhibition (cellular growth control)
- Signal transduction (cell membrane to nucleus)
- Gene expression (nuclear transcription factors)
- Cell cycle checkpoints ("guardians" of the genome)
- DNA repair ("caretakers" of the genome)
Tumor Suppressor: p53

• Located on human chromosome 17

• Represents the most commonly mutated gene among all human cancers.

• Normal (wild-type) p53 protein localizes to the nucleus and has a short half-life.
p53 (cont.)

- Mutation results in abnormal accumulation of protein in the nucleus.

- After DNA damage, p53 functions to delay the G1/S cell cycle transition, enabling DNA repair.

- Wild-type p53 protein induces apoptosis.
Inherited Breast Cancer Risk

- **BRCA1** and **BRCA2** are tumor suppressor genes encoding proteins that participate in DNA repair.
- Inherited mutations in BRCA1 or BRCA2 significantly increase risk of breast cancer at an early age.
- Most mutations are detected by direct sequencing of both genes.
Hereditary Nonpolyposis Colorectal Carcinoma

- Hereditary nonpolyposis colorectal carcinoma (HNPCC) accounts for about 5% of colon cancer.
  - Also known as Lynch Syndrome
- HNPCC is the most common form of hereditary colon cancer.
- HNPCC is associated with mutations in genes encoding components of the mismatch repair system (MMR)
Molecular Detection of Leukemia and Lymphoma

• Targets:
  – Antibodies, gene rearrangements, translocations, point mutations, polymorphisms, viruses

• Methods:
  – Hybridization, blotting
  – Standard PCR, RT-PCR, electrophoresis
  – Real-time PCR with gene or patient-specific probes
Gene Rearrangements (GR)

• Gene rearrangements are normal events that occur in lymphocytes.
• Antibody genes [immunoglobulin heavy chain genes, immunoglobulin light chain genes ($\kappa$, $\lambda$)] and T-cell receptor genes ($\alpha$, $\beta$, $\gamma$, $\delta$) rearrange.
• Rearrangement occurs independently in each cell.
Gene Rearrangements

Gene rearrangements may be used to detect leukemias and lymphomas arising from cells that have rearranged their immunoglobulin (Ig) or T cell receptor (TCR) genes.
Lymphomas and Kappa Lambda

- Detection of Kappa and Lambda light chain mRNA in plasma cells and B-lymphocytes
- Each immunoglobulin molecule contains either two
- Copies of Kappa or lambda light chains
  - K/L ratio 2:1 = Reactive Lymphoid Hyperplasia
  - K/L or L/K ratio 3:1 or greater: B cell Lymphoma *

Ventana Benchmark (ISH)
Lymphomas

• One of the oldest tests in molecular diagnostics utilizes the Southern Blot approach to search for gene rearrangements or translocations useful in the diagnosis of lymphomas
Urothelial Carcinoma

- 55,000 cases/year
- ~12,500 deaths
- >95% of the bladder and upper urinary tract cancers in this country are UC’s
- Squamous cell carcinomas of bladder: more common in Middle Eastern countries
Urothelial Carcinoma

• Cytology:
  – High sensitivity in detection of HGTCC
  – Greater difficulty in detecting LGTCC
    • Challenging to differentiate normal urothelial cells from low grade tumor cells

• Most bladder cancers reoccur
  – early recurrence often difficult to detect cytologically with cystoscopic specimens.
Urothelial Carcinoma: Testing Methods

- *Antigen based methods
- BTA-Stat, Immunocyt, NMP22, FDP
- Cytology
- Molecular genetic methods
- Telomerase, microsatellite analysis
- Flow cytometry/Digital Image Analysis
- Cytogenetic methods
- FISH (Vysis, Inc. (UroVision)) multi-color test that detects aneuploidy for chromosomes 3, 7, 9p21, and 17

- *not very effective in confirming the primary diagnosis of low-grade urothelial carcinoma
Urothelial Carcinoma: Testing Method (Vysis)

Control Slides (Separate)

DNA Probe Mixture
CEP 3, CEP 7, LSI 9p21, CEP 17

20X SSC

NP-40
Urovysion Intended Use

• Non-invasive method
• Monitoring of tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with bladder cancer.
FNA Applications

• Breast cancer:
  – Mutated p53 found in 50% of all human cancers, including up to 80% of breast cancer and colon cancer
  – The p53 protein normally suppresses cell growth, but when mutated, allows for uncontrolled cellular growth
  – PCR-based implications and current genomic microarrays with assess mutant p53
FNA Applications

- **Pancreatic cancer**: K-ras mutations are seen in 90% of pancreatic carcinomas and up to 80% of cholangiocarcinomas

- **Effusions**: FISH analysis for chromosomes 3, 8, 10, and 12 are being used to determine hyperdiploidy for equivocal or “atypical” cells
FNA Applications

K-ras mutations

• **Colorectal tumors**: GI tumors with mutations in K-ras have been shown to be more likely to reoccur.

• The proto-oncogene recognized as mutated most commonly in endometrial carcinoma is K-ras (10-30%).
IHC Versus Molecular Diagnostics

• ISH and FISH:
  – Greater sensitivity and specificity

• IHC:
  – Faster
  – Less expensive
  – Allows pathologist to assess target gene expression along with other visible landmarks in the slide

* Some labs use IHC as initial screening method, then confirm with ISH or FISH*
Summary

• Molecular testing analyzes tissue-specific and tumor-specific (mutation) targets.
• Genome, chromosome, and gene mutations are useful targets for diagnosis and detection of solid tumors.
• Immunohistochemical markers and FISH both have advantages and disadvantages when used separate. Using the two methods together increases sensitivity and specificity.