Tumor Genetics

MGL-12
May 13th 2014
Cancer Genetics

- Types of Genetic Alterations in Cancer
- Evidence that Mutations Cause Cancer
- Multistage Model of Carcinogenesis
- Oncogenes, Tumor Suppressor Genes,
CELL GROWTH

Cancer is a Disease of the Cell Cycle

- Cells in the body are "programmed" to:
  - To develop
  - To grow
  - To differentiate
  - To die
  in response to a complex system of biochemical signals.

- Cancer results from the emergence of a clone of cells freed of these developmental programming constraints and capable of inappropriate proliferation.
Response to the Environmental Signals

**SIGNS**
- Growth Factors
- Steroids
- Cell to Cell Interaction

**RESPONSE**
- Differentiation
- Growth and Death
- Mitosis
Control of the Cell Cycle

Mechanisms for controlling progress through the cell cycle:

- Transitions between different phases of the cell cycle ($G_1$, $S$, $G_2$, and $M$) are regulated at checkpoints.
- Checkpoints
- Length of Telomeres
- Chemical Signals from within and outside the cell
Cell Cycle Checkpoints

- G1: cytoplasm doubles
- S: chromosomes replicate
- G2: assembly of components for division
- Mitosis
  - P: cytokinesis
  - M: Spindle Assembly Checkpoint
  - A: Apoptosis Checkpoint
  - T: DNA Damage Checkpoints

DNA Damage Checkpoints

Spindle Assembly Checkpoint
Length of Telomeres

Telomeres are structures at the ends of chromosomes that shorten with each cell division. After 50 divisions, the shortened length of telomeres causes mitosis to stop.
Chemical Signals that Control the Cell Cycle

1. Cyclin and Kinase
   - proteins that initiate mitosis
   - requires buildup of cyclin to pair with kinase

2. Hormones
   - chemical signals from specialized glands that stimulate mitosis

3. Growth Factors
   - chemical factors produced locally that stimulate mitosis
Cyclins and CDKs

- Important checkpoint proteins are the **cyclins** and the **cyclin-dependent kinases (CDKs)**; complexes formed between cyclins and CDKs cause the cell cycle to advance.

- The CDKs **phosphorylate** target proteins but are inactive unless they are associated with a cyclin protein.

- Cell cycling requires the alternate **formation and degradation** of cyclin/CDK complexes.
Signaling Molecules

- Growth Factors
- Receptors for Growth Factors and Hormones
- Intracellular Signal Transducers
- Nuclear Transcription Factors
- Cell-Cycle Control Proteins
Types of Growth Factors for Differentiation

Examples:

I. Growth factors
- PDGF/sis

II. Growth factor receptors
- EGF / erb B
- PDGF

III. Transducer
- Protein kinases / e.g., src
- ras proteins / e.g., H-ras

IV. Nuclear transcription factors
- e.g., jun
- fos
- myc
- myb
- N-myc
- p53
- RB
Cancer

- Cancers arise when critical genes are mutated, causing unregulated proliferation of cells.
- These rapidly dividing cells pile up on top of each other to form a tumor.
- When cells detach from the tumor and invade surrounding tissues, the tumor is **malignant** and may form secondary tumors at other locations in a process called **metastasis**.
- A tumor whose cells do not invade surrounding tissues is **benign**.

*Cancer Cell Do Not Grow Faster Than Normal Cells
Rather, Their Growth is Just Uncontrolled*
How do we define cancer?

Cancer is a group of disorders that causes cells to escape normal controls on cell division

- Cancer cells divide more frequently
- Cancer cells are not inhibited by contact with other cells and can form tumors
- Cancer cells can invade other tissues, a process called metastasis
Cancer: General Etiology and Pathogenesis

- **Etiologic agents:**
  - Environmental (chemical, physical, and biological)
  - Hereditary (familial cancer syndromes)

- **General mechanisms:**
  - Acquired capabilities: *(Self-maintained replication, longer survival, genetic instability, neoangiogenesis, invasion and metastasis)*
  - Activation of oncogenes, inactivation of TSG, non-effective DNA repair
  - Caretaker and gatekeeper pathways
## THE CAUSES OF GENOMIC CHANGES IN CANCER: Somatic Changes

<table>
<thead>
<tr>
<th>Cause</th>
<th>Damage</th>
<th>Cancer Risk</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>UV</td>
<td>Skin Ca, Melanoma</td>
<td>P53 (CC-TT)</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>Thyroid Ca., Leucemia</td>
<td>Translokalation</td>
</tr>
<tr>
<td>Chemical</td>
<td>Benzopren</td>
<td>Lung Ca.</td>
<td>p53 (G-T)</td>
</tr>
<tr>
<td></td>
<td>Aflatoxin</td>
<td>Liver Ca.</td>
<td>p53 (249 G-T)</td>
</tr>
<tr>
<td></td>
<td>Oxidative Stress</td>
<td>Geriatric Ca</td>
<td>P53 (C-T)</td>
</tr>
<tr>
<td>Biological</td>
<td>HBV</td>
<td>Liver Ca.</td>
<td>Virus DNA Integration</td>
</tr>
</tbody>
</table>

- **UV:** Ultraviolet radiation causes skin cancer and melanoma.
- **Radiation:** Thyroid cancer and leukemia can be caused by radiation.
- **Benzopren:** Lung cancer can be caused by benzopren.
- **Aflatoxin:** Liver cancer can be caused by aflatoxin.
- **Oxidative Stress:** Geriatric cancer can be caused by oxidative stress.
- **HBV:** Hepatitis B virus (HBV) can cause liver cancer.
Exogenous Sequences

- Tumor viruses
  - contribute genes resulting in abnormal cell growth
- Cervical cancer
  - HPV (human papilloma viruses)
- Burkitt’s lymphoma
  - EBV (Epstein-Barr virus)
- Hepatocellular carcinoma
  - hepatitis viruses
Genetic Mechanisms of Tumors

- Gene deletions / amplifications
- Mutations
  - Insertional
  - Point Mutations
- Genetic Instability
  - Microsatellite Instability (MSI)
  - Chromosomal Instability (CIN)
Evidence that Mutations Cause Cancer

- Most carcinogens are mutagens
  - *Not all mutagens are human carcinogens*

- Some cancers segregate in families
  - *Genes cloned, mutations lead to cancer in animals*

- Oncogenes and Tumor Suppressor Genes
  - *Found in human tumors, enhance growth*

- Chromosomal instability

- Defects in DNA repair increase probability of cancer
Cancer Arises From Gene Mutations

**Germline mutations**
- Present in egg or sperm
- Are heritable
- Cause cancer family syndromes

**Somatic mutations**
- Occur in nongermline tissues
- Are nonheritable

Mutation in egg or sperm ➔ All cells affected in offspring ➔ Somatic mutation (eg, breast)
Types of Genetic Alterations in Cancer

- Subtle alterations
- Chromosome number changes
- Chromosomal translocation
- Amplifications

Each type represents one of the mutations a cell can accumulate during its progression to malignancy
Subtle Alterations

- Small deletions
- Insertions
- Single base pair substitutions
  (Point mutations)
Three classes of error lead to aneuploidy in tumor cells
Changes in Chromosome Number and Structure Are Often Associated with Cancer

- Random translocations: breast, colon, prostate (common epithelial tumors)
- Non-random translocations: leukemia, lymphoma (reciprocal translocation between chromosome 9 and 22 causes chronic myelogenous leukemia)
Chromosomal Rearrangements: Burkitt’s Lymphoma

- Burkitt’s lymphoma is associated with reciprocal translocations involving chromosome 8 and a chromosome carrying an immunoglobulin gene (2, 14, or 22).

- The translocations juxtapose \textit{c-myc} to the genes for the immunoglobulin genes, causing overexpression of \textit{c-myc} in B cells.

- The \textit{c-myc} gene encodes a transcription factor that activates genes for cell division.
A Reciprocal Translocation Involved in Burkitt’s Lymphoma

- 8p21.1

Normal chromosomes

Translocation chromosome

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# MOST FREQUENT CLONAL CHROMOSOME ABNORMALITIES IN HEMATOLOGIC MALIGNANCIES

<table>
<thead>
<tr>
<th>DIAGNOSIS:</th>
<th>ABNORMALITY:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, ALL</td>
<td>t(9;22)(q34;q11.2)</td>
</tr>
<tr>
<td>AML</td>
<td>t(8;21)(q22;q22)</td>
</tr>
<tr>
<td>APL</td>
<td>t(15;17)(q22;q12~21)</td>
</tr>
<tr>
<td>AML with EO</td>
<td>inv(16)(p13q22)</td>
</tr>
<tr>
<td>MDS /AML</td>
<td>5q-, -7, 7q-, +8, 20q-</td>
</tr>
<tr>
<td>CLL</td>
<td>del(13q), +12</td>
</tr>
<tr>
<td>ALL</td>
<td>t(1;19)(q23;p13)</td>
</tr>
<tr>
<td></td>
<td>t(4;11)(q21;q23)</td>
</tr>
<tr>
<td>Burkitt’s Lymphoma</td>
<td>t(8;14)(q24;q32)</td>
</tr>
<tr>
<td>Follicular Lymphoma</td>
<td>t(14;18)(q32;q21)</td>
</tr>
</tbody>
</table>
## Chromosomal Rearrangements or Translocations

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Translocation</th>
<th>Proto-oncogene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Burkitt lymphoma</strong></td>
<td>t(8;14)</td>
<td>c-myc&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>t(8;22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(2;8)</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic myelogenous leukemia</strong></td>
<td>t(9;22)</td>
<td>bcr-abl&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Acute lymphocytic leukemia</strong></td>
<td>t(9;22)</td>
<td>bcr-abl&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>c-myc is translocated to the IgG locus, which results in its activated expression
<sup>2</sup>bcr-abl fusion protein is produced, which results in a constitutively active abl kinase
### Examples of Chromosomal Regions That Show Loss of Heterozygosity in Tumors

<table>
<thead>
<tr>
<th>Chromosome Region</th>
<th>Disorder(s)</th>
<th>Associated TSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>lq</td>
<td>Breast carcinoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>3p</td>
<td>Small-cell lung carcinoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>5q</td>
<td>Familial polyposis coli; colorectal carcinoma</td>
<td>MCC</td>
</tr>
<tr>
<td>11p</td>
<td>Wilms tumor; rhabdomyosarcoma</td>
<td>WTI</td>
</tr>
<tr>
<td>13q</td>
<td>Retinoblastoma; breast carcinoma; osteosarcomas</td>
<td>RB 1</td>
</tr>
<tr>
<td>17p</td>
<td>Colorectal carcinoma; breast cancer</td>
<td>TP53</td>
</tr>
<tr>
<td>18q</td>
<td>Colorectal carcinoma</td>
<td>DCC</td>
</tr>
<tr>
<td>22</td>
<td>Neurofibromatosis, type 2</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Mechanisms Leading to Loss of Heterozygosity

Normal allele
• Mutant allele

Loss of normal allele

Chromosome loss
Deletion
Unbalanced translocation
Loss and reduplication
Mitotic recombination
Point mutation
Conclusion: People heterozygous for a tumor-suppressor gene are predisposed to cancer.
Amplifications

- Seen only in cancer cells
  - 5 to 100-fold multiplication of a small region of a chromosome
- “Amplicons”
  - contain one or more genes that enhance
Checkpoints in Tumor Cells

- In tumor cells, cell cycle checkpoints are often deregulated due to genetic defects in the machinery that \textit{alternately} raises and lowers the abundance of the cyclin/CDK complexes.

- \textbf{These mutations may be:}
  - in the genes encoding the cyclins or CDKs,
  - in genes encoding the proteins that respond to specific cyclin/CDK complexes
  - in genes encoding proteins that regulate the abundance of these complexes.
Oncogenes and Tumor-Suppressor Genes

- **Oncogene**: dominant-acting stimulatory genes that cause cancer

- **Proto-oncogenes**: responsible for basic cellular functions in normal cells; when mutated, they become oncogenes.

- **Tumor-Suppressor Genes**: Inhibit cancer and recessive acting; when mutated, normal cells become cancerous.
Cancers develop through an accumulation of somatic (not a single) mutations in proto-oncogenes and tumor suppressor genes.

1. Growth Factors
2. TM GF R (TKase)
3. Integral MR
4. Ras GTPase
5. Cytoplasmic Oncogenes
6. Nuclear Oncogene
Multiple Mutations in Cancer

- Most malignant tumors cannot be attributed to mutation of a single gene.
- Tumor formation, growth, and metastasis depend on the accumulation of mutations in several different genes.
- The genetic pathways to cancer are diverse and complex.
- Changes in DNA Methylation Are Often Associated with Cancer
Tumors Are Clonal Expansions

1. A cell is predisposed to proliferate at an abnormally high rate.
2. A second mutation causes the cell to divide rapidly.
3. After a third mutation, the cell undergoes structural changes.
4. A fourth mutation causes the cell to divide uncontrollably and invade other tissues.
Environmental vs. Hereditary Cancer

Cancer Etiology

- Environmental: 85
- Hereditary: 15
Familial Clustering of Cancer

- Epidemiological studies show an increased relative risk of cancer in individuals with a family history of cancer

- This is probably due to a mixture of rare highly penetrant genes, commoner lower penetrance genes and environmental effects
Inherited Cancer Genes

- Neurofibromatosis type-1
- P53 gene
- Familial Polyposis Gene (APC)
- Hereditary nonpolyposis colon cancer (HNCC)
- Breast Cancer Genes (BRCA1, BRCA2)
- P16 Familial Melanoma
- RET proto-oncogene and multiple endocrine neoplasia
SOLID TUMORS SARCOMAS

- In addition to leukemias and lymphomas, some sarcomas also have specific chromosomal abnormalities.

- One example is $t(11;22)$ seen in Ewing’s sarcoma in which the DNA binding domain of a transcription factor FLI1 is fused with the transactivation domain of EWSR1 gene.
RECURRENT ABNORMALITIES
EPITHELIAL TUMORS

• Small cell ca of the lung del(3)(p14-p24)
• Wilm’s tumor del(11)(p13)
• Breast Her-2/neu amplification
• Mostly multiple abnormalities
Ovarian Cancer

- 2 cytogenetic pathways
  1: +7, +8q and +12
  2: 6q- and 1q-

- karyotypic evolution

- CGH reveal multiple changes in the malignant and fewer changes in borderline tumors
Uterine leiomyomas and leiomyosarcomas

- Benign tumors such as leiomyomas also show recurrent chromosomal abnormalities such as t(12;14) and deletion of 7q
- 40% of leiomyomas show abnormal karyotypes
- Leiomyosarcomas show complex chromosomal rearrangements

**LOSS**

- 13q (59%)
- 10q (59%)
- 2q (35%)
- 16q (29%)

**GAIN**

- 5p (35%)
- 6p amplification
- 17p amplification
The Familial Polyposis Gene (A PC),

• The familial polyposis gene (APC), which strikingly predisposes to colon cancer, was ultimately identified by mutations in patients.
• The inherited gene is also involved in the great majority of sporadic cases of colon polyps and colon cancer.
• This tumor suppressor gene has been shown to function as a major regulator of the Wnt pathway, a signaling system that is well characterized both biochemically and developmentally.
Morphological and Molecular Changes in Adenoma and Carcinoma Sequence

NORMAL COLON

- Mucosa
  - Submucosa
  - Muscularis propria

MUCOSA AT RISK

- Germline (inherited) or somatic (acquired) mutations of cancer suppressor genes ("first hit")
  - APC at 5q21
    - Mismatch repair genes, e.g., MSH2 at 2p22

ADENOMAS

- Methylation abnormalities
  - Inactivation of normal alleles ("second hit")
  - APC
    - β-catenin
    - MSH2
  - Protooncogene
    - K-ras at 12p12
  - Homozygous loss of additional cancer suppressor genes
    - p53 at 17p13
    - LOH at 18q21
  - Additional mutations
    - Gross chromosomal alterations

CARCINOMA

- Many genes

Chromosome Alteration Gene

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>5q</th>
<th>Mutation</th>
<th>APC</th>
<th>12p</th>
<th>Mutation</th>
<th>K-ras oncogene</th>
<th>18q</th>
<th>Loss</th>
<th>DCC</th>
<th>17p</th>
<th>Loss</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal colon epithelium</td>
<td>Proliferating epithelium</td>
<td>Benign adenoma</td>
<td>Intermediate adenoma</td>
<td>Late adenoma with villi</td>
<td>Cancerous adenoma</td>
<td>Colon cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cancer and repair: HNPCC

- Normal epithelium
- Adenoma
- Carcinoma
- Metastasis

Mismatch repair defects

Accumulation of mutations in multiple genes

Oncogenes: gain of function) = increased proliferation, etc.

Tumor suppressors: (loss of function)
Pathway to Androgen-Independent Prostate Cancer

Pathway to androgen-independent prostate cancer

- Inactivation of HPC1 tumor suppressor gene
- Silencing of TP53 tumor suppressor gene by hypermethylation
- Inactivation of various tumor suppressor genes (e.g., RB)
- Inactivation of CDH1 tumor suppressor gene
- Inactivation of TP53 tumor suppressor gene
- Inactivation of KAI1 metastasis suppressor gene
- Overexpression of BCL-2 oncogene
- Alteration in androgen receptor

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Retinoblastoma

- Deletions 13q14 or mutations of the RB1 gene
- Cell cycle regulatory protein that inhibits G1 to S phase transition
- 80% de novo mutations
- High rate of loss of heterozygosity in tumor tissue
The Development of Hereditary Cancer

2 normal genes → 1 damaged gene → 2 damaged genes → Tumor develops

In hereditary cancer, one damaged gene is inherited

1 damaged gene → 1 normal gene → 2 damaged genes → Tumor develops
### Nonheritable vs Heritable Retinoblastoma

<table>
<thead>
<tr>
<th>Feature</th>
<th>Nonheritable</th>
<th>Heritable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>Unilateral</td>
<td>Usually bilateral</td>
</tr>
<tr>
<td>Family history</td>
<td>None</td>
<td>20% of cases</td>
</tr>
<tr>
<td>Average age at dx</td>
<td>~2 years</td>
<td>&lt;1 year</td>
</tr>
<tr>
<td>Increased risk of second primaries</td>
<td>No</td>
<td>Osteosarcoma, other sarcomas, melanoma, others</td>
</tr>
</tbody>
</table>

- Affects 1 in 20,000 live-born infants
- Males and Females equally affected
Breast Cancer
BRCA1 and BRCA2

• High (60-80%) lifetime risk of breast cancer, both genes.
• Increased ovarian cancer risk (BRCA1>BRCA2)
• Surveillance for both indicated; mammography, MRI, Ultrasound
• Consider prophylactic surgery
The Inherited Breast Cancer Genes: BRCA 1 and BRCA2

- Mutations in BRCA 1 and BRCA2 are responsible for a large proportion of inherited breast cancer cases.
- These mutations usually result in a truncated protein product and loss of function.
- The protein products of both of these genes interact with RAD51, a DNA repair protein.
BRCA1-Linked Hereditary Breast and Ovarian Cancer

- Breast, dx 45, d. 89
- Ovary, dx 59
- Breast, dx 59
- Breast, dx 36
- 36

Noncarrier
BRCA1-mutation carrier
Affected with cancer
How much of breast cancer is hereditary?

Breast Cancer
- Sporadic
- Family clusters
- Hereditary

15% - 20%
5% - 10%

Ovarian Cancer
- Sporadic
- Family clusters
- Hereditary

5% - 10%
SUMMARY: General Etiology and Pathogenesis

Acquired (environmental) DNA damaging agents:
- chemicals
- radiation
- viruses

NORMAL CELL

DNA Damage

Failure of DNA repair

Mutations in the genome of somatic cells

Inherited mutations in:
- Genes affecting DNA repair
- Genes affecting cell growth or apoptosis

- Activation of growth-promoting oncogenes
- Alterations of genes that regulate apoptosis
- Inactivation of cancer suppressor genes

Expression of altered gene products and loss of regulatory gene products

Clonal expansion
- Additional mutations (progression)
- Heterogeneity

Malignant neoplasm
Hallmarks of Pathways to Malignant Cancer

1. Cancer cells acquire **self-sufficiency** in the signaling processes that stimulate division and growth.

2. Cancer cells are **abnormally insensitive** to signals that inhibit growth.

3. Cancer cells can evade **programmed cell death** (apoptosis).

5. Cancer cells develop ways to grow themselves.

6. Cancer cells acquire the ability to invade other tissues and colonize them.