“Cancer is, in essence, a genetic disease. Although cancer is complex, and environmental and other nongenetic factors clearly play a role in many stages of the neoplastic process, the tremendous progress made in understanding tumorigenesis in large part is owing to the discovery of the genes, that when mutated, lead to cancer.”

Bert Vogelstein (1988)
Cancer Genetics: I

Lecture Goals

• Types of Genetic Alterations in Cancer
• Evidence that Mutations Cause Cancer
• Multistage Model of Carcinogenesis
• Oncogenes, Tumor Suppressor Genes, DNA Repair Genes

Cancer Arises From Gene Mutations

Germline mutations

Somatic mutations

Somatic mutation (eg, breast)
Types of Genetic Alterations in Cancer

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

Subtle Alterations

- Small deletions
- Insertions
- Single base pair substitutions
  - (Point mutations)
Point Mutations

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>THE BIG RED DOG RAN OUT.</td>
</tr>
<tr>
<td>Missense</td>
<td>THE BIG RAD DOG RAN OUT.</td>
</tr>
<tr>
<td>Nonsense</td>
<td>THE BIG RED.</td>
</tr>
<tr>
<td>Frameshift (deletion)</td>
<td>THE BRE DDO GRA.</td>
</tr>
<tr>
<td>Frameshift (insertion)</td>
<td>THE BIG RED ZDO GRA.</td>
</tr>
</tbody>
</table>

Point mutation: a change in a single base pair

Chromosome Number Changes

- Aneuploidy
  - somatic losses or gains
- Whole chromosome losses often are associated with a duplication of the remaining chromosome.
- LOH
  - loss of heterozygosity
Chromosome Translocations

- Random translocations
  - breast, colon, prostate (common epithelial tumors)
- Non-random translocations
  - leukemia, lymphoma

FISH

- Certain chromosomal translocations are easily detected by FISH
- Fluorescent in Situ Hybridization
  - probes on different chromosomes fluoresce
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 proliferation
• Generally in advanced tumors

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
Review: Types of Genetic Alterations in Cancer

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

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

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 prob of cancer
- Malignant tumors are clonal
Multi-Step Carcinogenesis (eg, Colon Cancer)

- Loss of APC
- Activation of K-ras
- Loss of 18q
- Loss of TP53
- Other alterations

Adapted from Fearon ER. Cell 61:759, 1990

Tumors Are Clonal Expansions

Adapted from ASCO Tumors Are Clonal Expansions.
“No inkling has been found…of what happens in a cell when it becomes neoplastic, and how this state of affairs is passed on when it multiplies…. A favorite explanation has been that [carcinogens] cause alterations in the genes of cells of the body, somatic mutation as these are termed. But numerous facts, when taken together, decisively exclude this supposition.”

Peyton Rous (1966)
in Les Prix Nobel

“The search for genetic damage in neoplastic cells now occupies a central place in cancer research…. Cancer may be a malady of genes, arising from genetic damage of diverse sorts -- recessive and dominant mutations, large rearrangements of DNA and point mutations, all leading to distortion of either the expression or biochemical function of genes.”

J. Michael Bishop (1987)
Science 1997; 235:305-311
Oncogenes, Tumor Suppressor Genes, and DNA Repair Genes

- Oncogenes
- Tumor Suppressor Genes
- Retinoblastoma and the “2-hit Hypothesis”
- DNA Repair Genes

**Oncogenes**

- Normal genes (regulate cell growth)
- 1st mutation (leads to accelerated cell division)
- 1 mutation sufficient for role in cancer development
Oncogenes Activated in Non-viral Human Cancers

• Gene fusions / translocations
• Point mutations

Effects of Oncogenes are Dominant

• Positive effect on growth
  – even in the presence of a normal (inactivated) version of the gene

• Example
  – Oncogenes derived from growth factor receptors confer the ability to bypass the growth factor requirement…independent growth.
Examples of Oncogenes

- RAS - activated in many cancers (colon)
- c-MYC - overexpressed in colon ca
  - amplified in lung, rearranged in lymphoma
- RET - MEN 2a
- MET - hereditary papillary renal cancer
- CDK4 - familial melanoma
- BCR/ABL - chronic myelogen leuk t(9;22)
- BCL2 - follicular lymphoma t(14;18)

Tumor Suppressor Genes

Normal genes (prevent cancer)

1st mutation (susceptible carrier)

2nd mutation or loss (leads to cancer)
Tumor Suppressor Genes

Key Attributes

- Familial Cancer Syndromes
- Inactivation in Common Human Cancers
  - Loss of Heterozygosity
- “Recessive” at a cellular level
- Two-hit hypothesis

Tumor Suppressor Genes

Familial Cancer Syndromes

- Most familial cancer syndromes are related to Tumor Suppressor Genes
  - Retinoblastoma, FAP, Li-Fraumeni, Familial Breast-Ovarian, VHL, Melanoma, Tuberous Sclerosis...
- Only 3 known syndromes related to Oncogenes
  - RET, MET, CDK4
- Few DNA repair syndromes
  - XP, AT, Bloom, Fanconi, Werner, HNPCC
Tumor Suppressor Genes

- Loss of Heterozygosity (LOH)
- 2 copies of each gene
- 1 is lost or inactivated
- Only 1 remains…
  - no longer heterozygous
  - one copy of a defective gene, same as no gene

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
The Two-Hit Hypothesis

First hit

First hit in germline of child

Second hit (tumor)

• Retinoblastoma - tumor of retinal stem cell
• Average age – unilateral 26 months, bilateral 8 months
• Affects 1 in 20,000 live-born infants
• Males and Females equally affected
• Familial more likely to be bilateral, younger
Features of Retinoblastoma

- 1 in 20,000 children
- Most common eye tumor in children
- Occurs in heritable and nonheritable forms
- Identifying at-risk infants substantially reduces morbidity and mortality

Genetic Features of Heritable Retinoblastoma

- Autosomal dominant transmission
- \textit{RB1} gene on chr 13 (first tumor suppressor gene discovered)
- Penetrance >90%
- Prototype for Knudson’s “two-hit” hypothesis
### 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>

#### Presentations of Retinoblastoma

- **Nonheritable**
  - ~60%
  - All Retinoblastoma
- **Heritable**
  - ~40%
  - Bilateral ~80%
- **Unilateral**
  - ~20%
- **Trilateral (rare)**
  - Heritable Retinoblastoma
“The data presented here and in the literature are consistent with the hypothesis that at least one cancer, retinoblastoma, can be caused by two mutations.... One of these mutations may be inherited as a result of a previous germinal mutation.... Those patients that inherit one mutation develop tumors earlier than do those who develop the nonhereditary form of the disease; in a majority of cases those who inherit a mutation develop more than one tumor.”

A. Knudson
PNAS 1971, p.823

Knudson’s “Two-Hit” Model for Retinoblastoma

<table>
<thead>
<tr>
<th>Normal</th>
<th>Predisposed</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 intact copies</td>
<td>1 intact copy 1 mutation</td>
<td>Loss of both copies</td>
</tr>
</tbody>
</table>

Modified from *Time*, Oct. 27, 1986

ASCO
The $RB1$ Gene

- Large gene spanning 27 exons, with more than 100 known mutations
- Gene encodes Rb protein which is involved in cell cycle regulation

Adapted from Sellers W et al. J Clin Onc 15:3301, 1997

Long-Term Survival of Children With Heritable Retinoblastoma

DNA Repair Genes

• DNA repair genes
  – targeted by loss of function mutations

• Differ from tumor suppressor genes:
  – TSG directly involved in growth inhibition or differentiation
  – DNA repair genes are indirectly involved in growth inhibition or differentiation

DNA Repair Genes

• Inactivation of DNA repair genes
  – increased rate of mutation in other cellular genes
  – proto-oncogenes
  – tumor suppressor genes

• Accumulation of mutations in the other cellular genes is rate limiting…
  – tumor progression is accelerated
DNA Repair Genes

- Nucleotide Excision Repair
- Mismatch Repair
- Somatic Mutational Disorders

Nucleotide Excision Repair

- Xeroderma Pigmentosa
  - individuals are extremely vulnerable to UV light
- NER
  - removes wide array of unrelated DNA damage
- Repairs helix-distorting chemical adducts
  - adducts induced by carcinogens like
    - benz[a]pyrene
    - UV light
Nucleotide Excision Repair

Mismatch Repair

- Hereditary NonPolyposis Colorectal Cancer
  - increased incidence of cancers of the colon, endometrium, ovary, stomach, and upper urinary tract
  - autosomal dominant
- HNPCC due to germline mutations in mismatch repair genes
  - hMSH2, hMLH1, MSH6, (PMS1, PMS2)
DNA Mismatch Repair

Base pair mismatch

Normal DNA repair

Mutation introduced by unrepaired DNA

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