The Molecular Biology of Cancer

- Cells become cancerous when they:
  - Lose their ability to control cell division; and
  - Gain the ability to invade and colonize other tissues.
- These changes are heritable.
- This is termed “transformation” since it is a change in the cells’ phenotype.

Tumors

- A tumor is a mass of dividing cells that is inappropriate in time or place.
- Benign tumors grow by cell division, but are usually not as harmful because they don’t spread throughout the body and look relatively normal...

Benign vs. Malignant Tumor

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Malignant Tumors

- Cells from malignant tumors spread by metastasis (i.e. - they metastasize).
- Metastatic tumors are less differentiated, so they less resemble normal tissues.
- Metastatic tumors constitute cancer.

Stages in Tumor Growth

- Human liver containing metastases from lung cancer
By the way…
Where do you think those tumors come from?

Transformation

• The transformation process could be **genetic**, reflecting a change in DNA sequence, or **epigenetic**, reflecting a heritable change in gene expression pattern.

Mutagens are carcinogenic

• It appears that most cancers derive by genetic means. One of the many arguments in favor of this theory is that **chemical carcinogens** have a strength that correlates well with their **mutagenicity**.

Some mutagens must be activated in the body

• Also, many compounds that are known to be carcinogenic in the diet are not necessarily mutagenic in tissue culture. Those compounds must be activated by oxidases in liver, so again, carcinogenicity corellates with **ultimate** mutagenicity.

Rous sarcoma virus (RSV) showed relationship between transformation and genes.

• Peyton Rous, Rockefeller, early 1900’s
• RNA-containing virus
• Infection causes tumors
• Some mutants of RSV lack tumor-forming capability
• Since mutations define genes, a gene must be required for tumor formation.

The Oncogene Theory

• Each normal genome contains **proto-oncogenes**, which may be altered by mutagenesis into oncogenes.
Oncogenes

- RSV contains src, an oncogene
- src is homologous to c-src, a proto-oncogene found in all cells
- If Oncogene theory is correct, chemically-mutagenized tumors must also contain oncogenes.

Cell growth in culture

- Primary cells divide for a time in culture, but then reach crisis stage and stop cell division.
- Cells that continue to divide beyond crisis are termed “immortal.”
- Rodent cells are more capable of immortalization than human cells.

Properties in transformation

- Immortalization - continuous cell division without reaching "crisis" or "senescence"
- Transformation - failure to observe the normal constraints of growth, such as by cell-cell contact inhibition
- Metastasis - gain of the ability to invade other tissue

Properties of normal cell lines:

- Anchorage dependence
- Growth factor dependence
- Contact inhibition
- Cytoskeletal organization
Assays for transformed cells

- **Anchorage-independence.** Cells are plated onto dishes containing a layer of agarose, which is soft and doesn't support normal cell adhesion. If cells survive and grow they can be detected by staining and observing colonies of survivors.

- **Focus-forming assay.** Cells are plated at relatively high density and allowed to reach confluence (all cells touch each other). If some cells continue to divide and grow, "foci," or groups of cells, form piled up on one another. These foci are easily stained since they are denser than the surrounding layer of cells.

- **Nutritional independence.** Cells are cultured in the absence of serum or growth factors.

- **Tumor formation.** Immunocompromised mice ("nude mice") are injected with cultured cells. If a tumor forms (a lump in the mouse's belly), cells are considered tumorigenic.

Weinberg Experiment

- Mouse 3T3 cells are immortal, but relatively non-transformed
- Ej cells are from human bladder tumor.
- If Ej cells contain an oncogene, transfer of oncogene to 3T3 cells should transform the 3T3 cells.
Result

- A single gene was isolated, containing a human Alu sequence, that could transform mouse 3T3 cells.
- This gene was ras.
- When compared with proto-oncogene ras, a single nucleotide difference could be found, producing a single amino acid difference in ras protein.

Proto-oncogenes

- All cells have proto-oncogenes.
- Proto-oncogenes are often important in signal transduction or regulation of cell division.

Proto-oncogenes

All cells have proto-oncogenes, which are often important in signal transduction or regulation of cell division. Proto-oncogenes are genes that can become cancer-causing genes when mutated. They are considered normal genes that are often essential for normal cell growth and development.

DNA-containing transforming viruses

- Adenovirus is a good example.
- Very common - 80% population has antibodies to virus.
- Infection causes flu-like symptoms.
- Rodents nonpermissive to infection.
- Immune clearance in animal.
- Cultured cells from rodents cannot replicate viral DNA.

Viral oncogenesis

- DNA-containing viruses
  - Adenovirus
  - SV40
  - polyoma
- RNA-containing viruses
  - MMLV
  - FeLV
  - RSV

Adenoviral tumors

- Infection of newborn rats (lacking mature immune system) causes tumors.
- Only viral proteins expressed are E1A and E1B (“immediate early” genes).
- Encode family of nuclear regulatory proteins by alternative splicing.
- Mutation of E1A and E1B eliminates tumors.
Hallmarks of DNA viral oncogenes

- DNA viruses generally carry oncogene, which functions in its normal viral life cycle to regulate viral gene expression.
- DNA viral oncogenes generally expressed early, before DNA replication.
- DNA viruses generally cause tumors only when they cannot replicate.

RNA tumor viruses

- At least two patterns:
  - Acute transforming viruses carry own oncogene
  - Integration of virus may activate cellular proto-oncogene

Acute Transforming Virus

- Contain mutated form of cellular proto-oncogenes.
- Thought to arise from recombination with cellular genome.

A few examples

<table>
<thead>
<tr>
<th>Virus</th>
<th>Name</th>
<th>Species</th>
<th>Tumor</th>
<th>Oncogene</th>
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</thead>
<tbody>
<tr>
<td>Rous sarcoma</td>
<td>RSV</td>
<td>Chicken</td>
<td>Sarcoma</td>
<td>src</td>
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<td>Harvey murine sarcoma</td>
<td>Ha-MuSV</td>
<td>Rat</td>
<td>Sarcoma &amp; erythro-leukemia</td>
<td>H-ras</td>
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<td>Feline sarcoma</td>
<td>SM-FeSV</td>
<td>Cat</td>
<td>Fibrosarcoma</td>
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<td>Abelson leukemia</td>
<td>MuLV</td>
<td>Mouse</td>
<td>B cell lymphoma</td>
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</tbody>
</table>

RNA viruses without transforming genes

- Classic examples are Feline Leukemia Virus (FeLV, for which we now test most domestic cats) and Mouse Mammary tumor virus (MMLV).
- These viruses have the ability to activate a cellular proto-oncogene.
- Often integrate into first intron of myc.

Activation of c-myc

- Myc has three exons, first encodes a long untranslated region.
- Integration causes enhanced transcription from LTR promoter.
- Protein produced is identical to proto-myc.
- Level of protein is high and unregulated.
Activation of c-myc

Anti-oncogenes

• Genes that normally function to *repress* tumor growth.
• When mutated, allow tumor growth.
• First example was retinoblastoma protein (Rb).

Retinoblastoma

Anucleation “cures” sporadic RB

• Precursors to retinal cells are retinoblasts. A malignant tumor of a retinoblast is called a retinoblastoma.
• With anucleation, Rb patients could grow to be adults and reproduce. This led to the first cases of "familial" Rb, and the older version was called "sporadic" Rb.

Hypothesis

• Knudsen proposed that Rb requires two events or mutations.
• Sporadic Rb is rare because it requires two mutations.
• Inheritance of one mutation increases frequency of observing tumors.

Mutation rates

• Furthermore, sporadic Rb seldom affects both eyes and is monoclonal.
• Familial Rb commonly bilateral and polyclonal.
• Propose that *two copies of a gene* must be *inactivated* by mutation to cause tumor.
• Gene involved functions if only one copy present.
**Discovery of Rb gene**

- Search of chromosome 13 library led to discovery of marker (segment of DNA).
- Marker present in normal humans, lacking in some tumor DNAs.
- Marker hybridizes with one 4.7 kb mRNA on Northern blot, this mRNA is missing in many tumors.

**Rb Protein**

- mRNA cloned as cDNA, sequence, predicts a single 110-114 kd protein.
- Western blots found that every Rb tumor lacks wild-type Rb protein.
- Rb protein functions by suppressing tumor formation.
- It inhibits cell cycle unless it is specifically inactivated by growth factor signaling.

**Tumor suppressors**

- Rb protein - functions by binding E2F and cyclins to prevent the cell cycle from proceeding from G1 to S (DNA synthesis).
- Normal stimulation of cell cycle requires that cyclin-CDK complexes phosphorylate Rb to remove it from its inhibitory role.

*Therefore, inactivation of Rb protein, either by binding viral oncoproteins or by mutation of the Rb gene, blocks a pathway that controls cell division, allowing uncontrolled cell division.*

**Another tumor suppressor**

- p53 originally identified in complex with E1A or SV40 T antigen.
- Found as nuclear phosphoprotein.
- Known to function as a transcription factor.
- Found in high levels in tumors (hint-this is probably mutated p53).
p53 in human disease

- Over 51 types of human tumors have been found to carry p53 mutations
- Found mutated in 70% of colorectal cancer, 50% of lung cancers and 40% of breast cancer.
- Mutated p53 is a good predictor of serious disease.


p53 knockouts

- Homozygous knockouts are viable and develop normally.
- But...

p53 knockouts

- Homozygous knockouts are viable and develop normally.
- They develop tumors by 3 months.

What does p53 do?

- p53 levels low in normal cells.
- Stimulation of p53 leads to inhibition of cell cycle and/or apoptosis
  - p53 is transcription factor to induce expression of p21, an inhibitor of G1 cyclin and Cdk2 protein.
  - If p53 cannot stop cell cycle, it induces apoptosis.

p53 in DNA Damage/Repair

- Cells irradiated with ultraviolet light or gamma rays show increased levels of p53 protein
- Irradiated cells stop cell division or die by apoptosis (programmed cell death).
- Cells from p53 knockouts fail to stop cell division or die and accumulate mutations.

When is p53 stimulated?

- After DNA damage by radiation.
- Upon stimulation of cell cycle by an “inappropriate” signal (e.g. oncogene).
- If p53 stops cell division, it must normally be inhibited to allow appropriate cell division.
- What is the mechanism of p53 regulation?
p53 is main target of several pathways to control cell cycle

Therefore, mutations in p53 are strongly selected
- Mutant p53 is induced by DNA damage & oncogenes.
- Mutant p53 is unable to control cell cycle
- Mutant p53 is unable to stimulate apoptosis.
- With p53 as a check-point in cell transformation, any mutation in p53 would be easily transformed.

Cellular Transformation
- Oncogenes usually mutations of regulatory gene products.
- DNA viruses transform using viral regulatory gene products.
- RNA viruses may carry cell-derived oncogene or may affect cellular proto-oncogene.

Cellular Transformation
- Since p53 is tightly regulated, and since p53 normally functions to control cell division, mutants in p53 are good predictors of serious disease.