The Biology of Cancer

• The cells of multicellular organisms are committed to the survival of the germ cells

• Cells collaborate for germ cell survival

• Mutations giving rise to “selfish behavior” jeopardizes the survival of the germ cells

• *Cancer is that selfish cell*
Cancer

• Reproduce in defiance of the normal restraints (proliferation)
  • proliferation gives rise to a tumor (neoplasm), a growing mass of abnormal cells
  • If the cells remain clustered in a single mass, it is benign and can be removed with surgery

• Invade and colonize territories normally reserved for other cells
  • A tumor is counted as cancer only if it is malignant, the ability to invade surrounding tissue
  • The formation of secondary tumors is called metastases
Classifications of Cancer

• Cancers are classified according to the tissue and cell type from which they arise

• Cancers arise from:
  – Epithelial cells: carcinoma
  – Connective tissue/muscle: sarcoma
  – Hemopoietic cells: leukemias
### Incidence of Cancers

<table>
<thead>
<tr>
<th></th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both Sexes</td>
<td>Male</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td>1,372,910</td>
<td>710,040</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>29,370</td>
<td>19,100</td>
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<tr>
<td>Tongue</td>
<td>7,660</td>
<td>5,050</td>
</tr>
<tr>
<td>Mouth</td>
<td>10,070</td>
<td>5,370</td>
</tr>
<tr>
<td>Pharynx</td>
<td>8,590</td>
<td>6,520</td>
</tr>
<tr>
<td>Other oral cavity</td>
<td>3,050</td>
<td>2,160</td>
</tr>
<tr>
<td><strong>Digestive system</strong></td>
<td>253,500</td>
<td>134,370</td>
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<tr>
<td>Esophagus</td>
<td>14,520</td>
<td>11,220</td>
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<tr>
<td>Stomach</td>
<td>21,860</td>
<td>13,510</td>
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<tr>
<td>Small intestine</td>
<td>5,420</td>
<td>2,840</td>
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<tr>
<td><strong>Colon</strong></td>
<td>104,950</td>
<td>48,290</td>
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<tr>
<td>Rectum</td>
<td>40,340</td>
<td>23,530</td>
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<tr>
<td>Anus, anal canal, &amp; anorectum</td>
<td>3,990</td>
<td>1,750</td>
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<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>17,550</td>
<td>12,130</td>
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<tr>
<td>Gallbladder &amp; other biliary</td>
<td>7,480</td>
<td>3,330</td>
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<tr>
<td>Pancreas</td>
<td>32,180</td>
<td>16,100</td>
</tr>
<tr>
<td>Other digestive organs</td>
<td>5,210</td>
<td>1,670</td>
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</tr>
<tr>
<td><strong>Respiratory system</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>9,880</td>
<td>7,920</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>172,570</td>
<td>93,010</td>
</tr>
<tr>
<td>Other respiratory organs</td>
<td>2,350</td>
<td>1,490</td>
</tr>
<tr>
<td>Bones &amp; joints</td>
<td>2,570</td>
<td>1,480</td>
</tr>
<tr>
<td>Soft tissue (including heart)</td>
<td>9,420</td>
<td>5,530</td>
</tr>
<tr>
<td>Skin (excluding basal &amp; squamous)</td>
<td>66,000</td>
<td>37,580</td>
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<tr>
<td><strong>Melanoma-skin</strong></td>
<td>59,580</td>
<td>33,580</td>
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<tr>
<td>Other nonepithelial skin</td>
<td>6,420</td>
<td>4,000</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
<td>212,930</td>
<td>1,690</td>
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<tr>
<td></td>
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<td>Male</td>
</tr>
<tr>
<td><strong>Genital system</strong></td>
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<tr>
<td>Uterine cervix</td>
<td>321,050</td>
<td>241,570</td>
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<tr>
<td>Uterine corpus</td>
<td>40,880</td>
<td>40,880</td>
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<tr>
<td>Ovary</td>
<td>22,220</td>
<td>22,220</td>
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<tr>
<td>Vulva</td>
<td>3,870</td>
<td>3,870</td>
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<tr>
<td>Vagina &amp; other genital, female</td>
<td>2,140</td>
<td>2,140</td>
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<tr>
<td><strong>Prostate</strong></td>
<td>232,090</td>
<td>232,090</td>
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<tr>
<td>Testis</td>
<td>8,010</td>
<td>8,010</td>
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<tr>
<td>Penis &amp; other genital, male</td>
<td>1,470</td>
<td>1,470</td>
</tr>
<tr>
<td><strong>Urinary system</strong></td>
<td>101,880</td>
<td>71,090</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>63,210</td>
<td>47,010</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>36,160</td>
<td>22,490</td>
</tr>
<tr>
<td>Ureter &amp; other urinary organs</td>
<td>2,510</td>
<td>1,590</td>
</tr>
</tbody>
</table>
## Incidence of Cancers

<table>
<thead>
<tr>
<th>Category</th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both Sexes</td>
<td>Male</td>
</tr>
<tr>
<td>Eye &amp; orbit</td>
<td>2,120</td>
<td>1,090</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>18,500</td>
<td>10,620</td>
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<tr>
<td>Endocrine system</td>
<td>27,650</td>
<td>7,550</td>
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<tr>
<td>Thyroid</td>
<td>25,690</td>
<td>6,500</td>
</tr>
<tr>
<td>Other endocrine</td>
<td>1,960</td>
<td>1,050</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>63,740</td>
<td>33,050</td>
</tr>
</tbody>
</table>
Cancer and Genetics

• If a single abnormal cell is to give rise to a tumor, it must be able to pass on its abnormality to its progeny

• These heritable traits are passed on by DNA
Causes of Cancer

• Carcinogenesis: generation of cancer causing…
• Mutagenesis: changes in DNA sequence

• Types of carcinogens:
  – Chemical: local changes in nucleotide sequence
  – Radiation: causes breaks in chromosomes
  – Viruses: introduce foreign DNA
Mutations of DNA

• In a lifetime, each gene (<25,000) undergoes about $10^{10}$ individual mutation events.

So why does cancer occur so infrequently?
Mutations of DNA

• In a lifetime, each gene (<25,000) undergoes about $10^{10}$ individual mutation events.

**So why does cancer occur so infrequently?**

• Almost all of the mutations are removed or corrected
• 1 mutation is not enough. Several must accumulate in the genome with age
Growth of Cancer

• Cancerous growth often depends on deranged control of cell differentiation or cell death

• Result is cells dividing out of control
Metastases

• Cancer cells loosen their adhesion and escape the tissue of their origin

• This involves:
  – Breaking away from tissue of origin
  – Burrowing through tissue to a blood supply or lymphatic vessel
  – Entering circulation
  – Leaving circulation
  – Possessing the ability to live in a new environment
Drugs

• Most chemotherapeutic agents impair mitosis and thus target rapidly dividing cells, e.g. cancer, hair, intestinal lining

• Disadvantage to chemotherapy:
  – Those cells that survive are resistant to that drug and have been artificially selected for further growth
Molecular Genetics of Cancer

• Cancers are not genetically identical, even within a single tumor!

• All cancers involve the disruption of normal restraints on cell proliferation often occurring in a small subset of genes
Molecular Genetics of Cancer

• Proliferation can be regulated by “start” site of cell division or by apoptosis
  – Make a stimulatory gene hyperactive
    • Oncogene: dominant effect (1 allele needed)
    • Proto oncogene: normal alleles
      – Oncogenes are identified by placing them into normal cells (transformation) and seeing if the normal cells become tumor cells
  – Make an inhibitory gene inactive
    • Recessive effect (requires both alleles)
    • Both gene copies must be inactivated or deleted
    • The lost gene is a tumor suppressor gene
      – Tumor suppressor genes are harder to identify
Retroviruses

• Example is the Rous sarcoma virus (chickens)
  – Viral RNA is copied to DNA by the host’s cell machinery and inserted into the host cell’s genome. Can then be inherited by subsequent generations
Retroviruses

• Proto oncogenes can be converted into an oncogene upon incorporation into a retrovirus
  – Gene sequence altered so it codes for a protein with abnormal activity
  – Gene brought under control of powerful promoters/enhancers in the viral genome that causes its product to be made in excess or during inappropriate circumstances
Identification of Oncogenes *in vitro*

- Transform normal fibroblast cells with DNA from human tumors
  - Identified the *same* oncogenes as identified from retroviral transformations
  - 25% of all cancers result from mutated members of the *ras* gene family
Proto Oncogenes can become Oncogenes

• Deletion or point mutation in coding sequence
  – Hyperactive protein made in normal amounts

• Gene amplification
  – Normal protein greatly overexpressed

• Chromosome rearrangement
  – Nearby strong enhancer/promoter causes normal protein to be overproduced
Oncogene Collaboration

• A single oncogene is not usually sufficient to turn a normal cell into a cancer cell

• Oncogene collaboration
  – The synergistic action of 2 or more specific oncogenes are required to make a cell cancerous
    • E.g. transform mouse egg with ras and/or myc oncogenes
Tumor Suppressor Genes

• Tumor suppressor genes protect cells
• Loss of tumor suppressor genes leads to cancerous condition
• Problem: It is harder to find something that is missing!
Tumor Suppressor Genes: Rb

- Retinoblastoma (Rb): cancer of the retina
  - Deletion of a region of chromosome 13
  - Cancerous cells have defect in both alleles
  - Other somatic cells are deficient in one allele

  - Rb gene is missing also in cancers of the lung, breast and bladder
  - Loss of the Rb gene is a major step in the progression toward malignancy
Tumor Suppressor Genes: Rb

- Rb is found in all cells and acts as a “brake” on the cell division cycle
- Regulation is by phosphorylation
- Rb protein (pRb) alternates between the phosphorylated and unphosphorylated states in every cell cycle, being unphosphorylated when not cycling
Tumor Suppressor Genes: Rb

• When unphosphorylated, pRb is bound strongly to gene regulatory proteins preventing them to promote DNA replication in the nucleus

• When pRb is removed, DNA replication and cell division goes unchecked
DNA Tumor Viruses

• DNA tumor viruses activate the cell’s DNA replication machinery as part of their survival

• Viral DNA fails to replicate and becomes incorporated into the host’s genome

• If the viral gene that activates the host’s machinery for DNA replication is transcribed, this gene can act as an oncogene
DNA Tumor Viruses

• Large T antigen from the SV-40 genome
  – binds protein products of two key tumor suppressor genes of the host cells
  – These genes are disabled permitting the cell to replicate its DNA and divide uncontrolled

• One of these proteins is pRb
• The other is p53
p53 Mutations

• p53 binds to DNA and induces the transcription of a 21 kD protein (p21)

• p21 binds to G₁ cyclin and CdK2 proteins, which serve to drive the cell past the G₁ checkpoint in the cell cycle, and blocks them
  – Cell is prevented from progressing into S phase and DNA replication
p53 Mutations

• When DNA damage occurs, there is an increase in p53 expression:
  – Delays the cell in G1 so repairs can be made
  – Cell may enter apoptosis and die

• If the cell lacks p53, damaged DNA continues to replicate and is transmitted to the daughter cells

• Mutations of the p53 gene are found in 50% of all cancers
Colorectal Cancer

• Arise from the epithelium lining the colon and rectum

• Begins with a benign tumor (adenoma) of the gut epithelium forming a protruding mass of tissue (polyp)

• Progression of the disease is very slow (10-35 years)
  – 75% of colorectal cancers have inactivating mutations in the p53 tumor suppressor gene
  – 50% have a point mutation in a ras proto oncogene
Order of Events Leading to Colorectal Cancer

1) Inactivation of APC (adenomatosis polyposis coli), a tumor suppressor gene
   - Detected in small polyps
   - Increase rate of cell proliferation

2) ras activation
   - Rare in small polyps, common in larger ones

3) DCC (Deleted in Colorectal Carcinoma) & p53 mutations
   - Common in malignant tumors
   - Loss of p53 allows the cells to divide uncontrollably and accumulate still further mutations
Methods: Immunohistochemistry & ELISA

Direct

Indirect
The assay is based on measurement of activity of lactate dehydrogenase (LDH) which is a stable enzyme normally found in the cytosol of all cells but rapidly releases into the supernatant upon damage of plasma membrane. The assay is fast and sensitive. Results can be analyzed by spectrophotometry at 500 nm.
ADCC Results

PBMC 50:1 (Peripheral Blood MonoCytes)

mAb CHO 31.1
mAb UPC-10 (control)
Complement-Dependent Cell-Mediated Cytotoxicity (CDC)
In vivo ADCC: Efficacy Study

Tumors established with 1x10^6 cells
Dose = 400 ug/injection
PBMC = 2x10^7 cells

Tumor Cell Line
LS174T or ASPC-1

Target cell

CHO31.1
or

Human IgG1 (Control)

Human effector cells
(PBMC)

or

Saline (Control)

Athymic: NCR-nu

2 Dose
Day 0

3 Dose
Day 0

Day 5 & 9

Day 5, 8 & 11

Day 6 & 10

Day 6, 9 & 12

Measure Tumor Size
Day 0-24

Controls

Day 27
Animals Euthanized
Results of Animal Studies

Mean Tumor Volume (mm$^3$) vs. Day

- CHO31.1 + PBMC
- hlg + PBMC
- CHO31.1
- hlg
MAb CHO31.1 Inhibits Growth of ASPC-1 Tumors \textit{in vivo}

Left: CHO + PBMC (3 Doses) \hspace{1cm} Right: Control (Human IgG)
**mAb CHO31.1 Binding Affinity: Cell Based ELISA**

0.75x10^5 cells/well

Goat Anti-Human IgG (H+L) AP Conjugate secondary mAb

ELISA was evaluated using GraphPad Prism

Experiments were carried out in duplicate with values indicated as means; bars, SE.

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**Tumor Cell Lines:** *SWS 900 & LS174T*

**mAb:** *CHO31.1 & Human IgG1*

![Graph showing binding affinity with B_max = 0.98 and K_d = 0.44 nM.](image)

- SW900 + Human IgG1
- SWS900 + CHO31.1
- LS174T + Human IgG1
- LS174T + CHO31.1

Red line indicates nonlinear regression curve
Flow cytometry is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid.