Revised reading assignments (Assignment Set 7)

For your “personal enrichment” only:
Chapter 12 pgs 228-233

Required:
Chapter 11 pg. 203 (The three stages....) -209
Look at big themes; don’t get bogged down in memorizing the details of any specific pathway
Six Diabolical Superpowers of Cancer

1. Growth Even in the Absence of Normal "Go" Signals
   Most normal cells wait for an external message before dividing. Cancer cells (image) often counterfeit their own pro-growth messages.

2. Growth Despite "Stop" Commands Issued by Neighboring Cells
   As the tumor (yellow) expands, it squeezes adjacent tissue, which sends out chemical messages that would normally bring cell division to a halt. Malignant cells ignore the commands.

3. Evasion of Built-In Autodestruct Mechanisms
   In healthy cells, genetic damage above a critical level usually activates a suicide program. Cancerous cells (magenta) bypass this mechanism, although agents of the immune system (orange) can sometimes successfully order the cancer cells to self-destruct.

4. Ability to Stimulate Blood Vessel Construction
   Tumors need oxygen and nutrients to survive. They obtain them by co-opting nearby blood vessels to form new branches [brown streaks] that run throughout the growing mass.

5. Effective Immortality
   Healthy cells can divide no more than 70 times. Malignant cells need more than that to make tumors. So they work around systems—such as the telomeres (yellow) at the end of chromosomes (blue)—that enforce the reproductive limit.

6. Power to Invade Other Tissues and Spread to Other Organs
   Cancers usually become life-threatening only after they somehow disable the cellular circuitry that confines them to a specific part of the particular organ in which they arose. New growths (orange and yellow) appear and eventually interfere with vital systems.

Accessible info on cancer biology and cancer treatment:
http://www.cancer.gov/cancerinfo/
How many somatic cells is an adult human made of?
• An adult human has somewhere around $10^{14}$ cells
• In the mature organism some cell types divide continually (such as epithelial cells and cell lining the GI tract)
• Other cell types divide rarely
• Since too few or too many cell divisions could produce chaos in a particular organ, the growth and division of each cell type is very carefully controlled
• Cancers result when single cells in the body and change their behavior relative to neighboring cells

  ❖ Early frog embryo cells: 30 minute cycle
  ❖ Human intestinal epithelia cells: 12 hour cycle
  ❖ Human liver cells: about 1 year
  ❖ Other vertebrate cells (such as neurons) exist for months or day or years without growing or dividing
  ❖ A yeast cell can complete a full cell cycle in 90 min. (Single-celled eukaryotes must also carefully regulate their cell cycle)
Somatic cells exist in a “social” setting where they need to be responsive to cues from neighboring cells

Cancer cells can be thought of a rogue cells that no longer obey the rules of the social contract

1. Most cells “decide” whether or not to divide only after receiving signals from neighboring cells, either positive signals that stimulate division or negative signals that prevent proliferation. Many tumor cell, by contrast, make their own stimulatory signals

2. Loss of contact inhibition. Normal cells stop dividing when they come in contact with one another. Tumor cells, which have lost this property, climb all over each other to produce piles of cells -- a significant departure from the highly ordered pattern seen in normal tissues

3. Normal cells dies or commit suicide when starved of growth factors or when heavily damaged by toxins or X-rays or UV light. Many cancer cells do not exhibit this property.
Cancer cells differ from normal cells in the following ways:

1. *The cells mutate so that they can dodge the cellular signals that suppress growth [or that encourage suicide of genetically abnormal cells]*
2. *The cells acquire their own growth-signalling pathways, independent of the external signals that normal metazoan cells are dependent on*
3. They develop limitless potential to proliferate: normal cells can divide only about 70 times before their telomeres (remember?) become so shortened that the chromosomes are damaged and the cell dies
4. Solid tumor cells create their own network of blood vessels *(to supply the growing monster with food and oxygen)*
5. Finally the most dangerous tumor cells are those that can travel to distant sites in the body (metastasis). Nine of ten cancer deaths result from metastases.
Cancers are diseases in which unremitting clonal expansion of somatic cells kills by invading, subverting and eroding normal tissues

A cancer is an aggregate of cells that are clonal descendants of an initial aberrant founder cell

Figure 23–11. Molecular Biology of the Cell, 4th Edition.

- A tumor develops through repeated rounds of somatic mutation and proliferation, giving rise eventually to a clone of fully malignant cancer cells.
- Mutations that enhance proliferation increase the chance of that the next step in tumor progression will occur by increasing the size of the cell population at risk of undergoing another mutation.
**Tissue and organ function in multicellular organisms depends absolutely on the ability of cells to properly interact and communicate with each other**

**Signals from the environment**

An animal cell’s dependence on multiple extracellular signals. Each cell type displays a set of receptor proteins that enables it to respond to a corresponding set of signal molecules produced by other cells. These signal molecules work in combination to regulate the behavior of the cell. As shown here, an individual cell requires multiple signals to survive (blue arrows) and additional signals to divide (red arrows) or differentiate (form a specialized cell type -- green arrows). If deprived of appropriate signals, a cell will undergo a form of cell suicide or programmed cell death (apoptosis).
Cancer cells reproduce *in defiance of* normal restraints on cell division

Chart of the major signalling pathways relevant to cancer in human cells, indicating the cellular locations of some of the proteins modified by mutation in cancers.

[Red dots: individual signalling proteins]

Rate of cell proliferation in multicellular organisms is controlled by *growth promoting and growth suppressing* signal transduction pathways (Text figure 19.12 in text)
**Reception:** signalling molecule binds to receptor protein (which may be membrane bound or intracellular)

**Transduction:** receptor protein’s activity is altered by binding the signalling protein: the signal is “converted” into a form that can bring about a specific cellular response

**Response:** many possible levels of cellular response
Text figure 19.12b

What would the effect of loss-of-function mutations in a growth inhibiting pathway be?
What would the effect of loss-of-function mutations in a growth promoting pathway be?

How then can mutations in a growth promoting pathway result in increased cellular proliferation?
Gain-of-function mutation!

• increased level of wild-type gene product
• altered function of protein: *unregulated activity of protein*
• altered temporal or spatial expression of gene product

Gain-of-function mutations are always dominant to the wild-type allele

Why is this important in this context?
How would a gain-of-function mutation “operate” in a signal transduction pathway that stimulates cell division?

**BASIC PRINCIPLE OF SIGNAL TRANSDUCTION:**

*Normal (wild-type proteins)*

NO SIGNAL : NO TRANSDUCTION: NO RESPONSE

SIGNAL : TRANSDUCTION: RESPONSE

*Cancer Cell (at least one GF mutant protein)*

NO SIGNAL : TRANSDUCTION: RESPONSE
How is a signal transduced?

Many possible mechanisms

Look at one example that involves:

• Allostery
• Protein phosphorylation: the covalent addition of a phosphate group to a side chain of a protein (such as a tyrosine)

Phosphorylation: is catalyzed by enzymes called protein kinases
From first principles?

Why would the addition (or subtraction) of a phosphate affect the activity of a protein?
Addition of a phosphate group to a polypeptide will cause a \textit{change} in the tertiary structure: \textit{for example, by attracting a cluster of positively charged amino acid side chains (see next page)}

Such a change occurring at one site in the protein can in turn alter the protein’s tertiary shape elsewhere.

\textbf{In other words, we are controlling the activity of a protein by changing its shape.}
phosphorylation/dephosphorylation of a protein as a control mechanism has many advantages:

- It is rapid, taking as little as a few seconds.
- It does not require new proteins to be made or degraded.
- It is easily reversible.

The extensive use of this control mechanism is apparent by the large number of known kinases and phosphatases. Even in a simple organism like yeast, approximately 3 percent of its proteins are kinases or phosphatases. Some of these enzymes are extremely specific, potentially phosphorylating or dephosphorylating only a few target proteins, while others are able to act broadly on many proteins.
Activation of a receptor tyrosine kinase:

- Binding of signalling molecule causes two receptor molecules to associate into a dimer
- Dimer formation brings the kinase domains of each receptor into close contact and they phosphorylate each other on several tyrosine side chains

NOTE: membrane fluidity is key here
Activated tyrosine kinases transduce the signal to Ras

Virtually all receptor tyrosine kinases activate \textit{Ras}: a small protein that is bound by a lipid tail to the cytoplasmic face of the plasma membrane

Allosteric control of Ras:
- Inactive when GDP bound
- Active when GTP bound
- After a delay, Ras switches itself off by hydrolyzing GTP to GDP
Ras triggers a phosphorylation cascade
Gain of function mutations in Ras are found in many cancers ~ 40%?

Ras is a type of oncogene (cancer causing gene)
Most cancers result from the accumulation of multiple mutations in a clone of somatic cells

*Mutation is a random process:*

The *number of mutations* and the *specific genes* involved depends on the specific cell or tissue type under examination
In addition to genes that function in growth promoting and growth inhibiting pathways, a third category of genes (not directly controlling rate of cell division) is mutated in cancer cells: 

*guardians of the genome*

What specific roles would these genes have?
→ Mutations in the DNA replication and repair machinery

→ Mutations in the feedback control machinery that prevents the cell from progressing through the cell cycle with damaged DNA

Nature 432: 316  Nov. 18, 2004
“All life on earth must cope with constant exposure to DNA-damaging agents such as the Sun's radiation. Highly conserved DNA-repair and cell-cycle checkpoint pathways allow cells to deal with both endogenous and exogenous sources of DNA damage. How much an individual is exposed to these agents and how their cells respond to DNA damage are critical determinants of whether that individual will develop cancer.** These cellular responses are also important for determining toxicities and responses to current cancer therapies, most of which target the DNA. “

** see table on next page
**Inherited cancer syndromes:** families with several cases of common cancers that fall into a recognized pattern of cancer types. Spectrum ranges from families with multiple cases at a young age to two to three cases at older ages.

**NOTE: MOST CANCERS ARE NOT DUE TO INHERITED MUTATIONS**

Germline mutations (inherited from parent) predisposing individuals to cancer

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>DNA repair defect</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroderma pigmentosum</td>
<td>inability to repair bulky lesions that distort the DNA helix (such as UV-induced damage)</td>
<td>genome instability: point mutations, skin cell death and skin cell cancers</td>
</tr>
<tr>
<td>Li Fraumeni syndrome (very rare)</td>
<td>loss-of-function in p53 gene function is to stop/slow progression through the cell cycle in response to DNA damage and to stimulate apoptosis</td>
<td>multicancer syndrome: sarcomas, breast cancer, brain tumors</td>
</tr>
<tr>
<td>HNPCC</td>
<td>defect in post-replication mismatch repair</td>
<td>genome instability: point mutations</td>
</tr>
<tr>
<td>BRCA 1 &amp; 2</td>
<td>chromosome instability due to the inability to repair double-strand breaks in the DNA</td>
<td>genome instability: chromosome aberrations, very high risk of breast cancer, increased risk of other cancers</td>
</tr>
</tbody>
</table>
Spectral karyotyping. This metaphase image of a breast cancer cell line (below) was obtained by 24-colour fluorescence in situ hybridization. Each chromosome is labelled with a different combination of fluorescent dyes and the final image is interpreted by software that colours each pixel to show which chromosome is most likely to be present at that point.

Remarkable genomic instability.

See Text figure 8-13  NORMAL karyotype
Chromosome painting by in situ hybridization with different-labelled probes
The probes used for the two different karyotypes are obviously different
Genetic instability and tumor production. Cells that maintain an “optimal” level of genetic instability may be the most successful in the race to form a tumor.

a) In normal cells, the intrinsic amount of genetic instability is low. When such normal cells hit a selection barrier—low levels of oxygen or a scarcity of proliferation signals, for example—they are very unlikely to be mutable enough to produce a cell that continues to proliferate.
b) In tumor cell precursors, an increased level of genetic instability makes it likely that at least one cell will contain the requisite genetic alteration to pass the selection barrier and continue the process of tumor progression. This genetic instability is retained in the lineage and can be measured in the resulting tumor.
c) If the level of genetic instability is too high, many of the cells suffer deleterious mutations and either proliferate more slowly than their neighbors or are eliminated by cell death. This excessive mutability can lead to extinction of the cell lineage.
### Classes of genes mutated in cancer cells

<table>
<thead>
<tr>
<th>Proto-oncogenes (becomes oncogene when mutated)</th>
<th>Tumor suppressor genes</th>
<th>Guardians of the genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of normal gene on cell growth and division</td>
<td>Stimulates/ Activates</td>
<td>Inhibits/ Represses</td>
</tr>
<tr>
<td>Oncogenic mutation in gene</td>
<td>Gain-of-function</td>
<td>Loss-of-function</td>
</tr>
<tr>
<td>Genetic behavior of oncogenic mutation</td>
<td>increased or unregulated activity or expression of the gene or gene product (hyperactive gene or product)</td>
<td>gene product is absent or its expression or activity is decreased</td>
</tr>
<tr>
<td>Phenotypic effect of oncogenic mutation</td>
<td>Dominant</td>
<td>Recessive</td>
</tr>
<tr>
<td></td>
<td>One mutant allele is sufficient for the cancer phenotype</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased stimulation of cell proliferation</td>
<td>Decreased inhibition of cell proliferation</td>
</tr>
<tr>
<td></td>
<td>CANCER</td>
<td>CANCER</td>
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