7.013 Problem Set 7

This completed problem set must be turned into the wooden box outside 68-120 by 4:40 pm on Thursday, April 24. Problem sets must not be late. Solutions will appear on the web after the due time.

Question 1
In the following experiment, cells removed from 3 different mouse lines were placed into petri plates. These cells were then subjected to UV light, which damages DNA.

a) Indicate the resulting effect on the UV irradiated cells in the adjacent boxes. (Assume there are no further p53 mutations.)

<table>
<thead>
<tr>
<th>p53</th>
<th>After UV, isolated cells will (LIVE or DIE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-/-</td>
<td>LIVE</td>
</tr>
<tr>
<td>+/-</td>
<td>DIE</td>
</tr>
<tr>
<td>+/+</td>
<td>DIE</td>
</tr>
</tbody>
</table>

b) Will a woman get breast cancer if she loses both normal copies of p53 in a single breast cell? Why or why not?

Not necessarily. A loss of p53 function alone does not automatically generate cancer. The accumulation of multiple genetic changes (or successive alterations) must occur to transform a cell into one capable of giving rise to a tumor.

c) The p53 protein is often referred to as "the guardian of the genome," and is the most commonly mutated protein found in human tumors. Briefly explain why the function of p53 makes it so critical in cancer prevention.

p53 monitors the overall health of a cell, including how abnormal the genetic material is and the degree of cell proliferation. It can trigger a halt and repair (if possible) or else cell death. This prevents mutant cells from giving rise to increasing numbers of mutant daughter cells, which could cause disease, including cancer. Without functional p53 watching over a cell, it is more likely to acquire successive alterations, and become transformed.
Consider the following hypothetical signal transduction pathway by which an extracellular growth factor induces a cell to grow and divide:

Upon binding of the growth factor (ligand L) to the receptor kinase A, receptor A dimerizes and autophosphorylates.

The kinase B is then able to bind the phosphorylated cytoplasmic domain of receptor A, leading to a change in the conformation of kinase B that makes it active.

Kinase B then phosphorylates the kinase C, making it active.

Kinase C then phosphorylates the transcription factor D.

The phosphorylated D then enters the nucleus where it binds the promoters of genes involved in promoting the transition from G1 to S phase of the cell cycle.

These genes become actively transcribed, leading to cell proliferation.
Given the previous information, which of the following gene mutations, if homozygous, would you expect to contribute to the development of cancer? Briefly explain why.

a) A missense mutation in the gene coding for kinase B that generates a protein whose three-dimensional structure is identical to kinase B in its activated form.

Cancer. This would give a constitutively-active pathway, because protein B would constantly be able to phosphorylate kinase C, regardless of whether ligand was present or not.

b) In the cells that normally produce the ligand L, a gene rearrangement occurs such that a constitutively active promoter is placed upstream of the gene encoding L.

Cancer. The ligand would always be produced and would be continuously inducing cells to proliferate.

c) A deletion in part of the extracellular domain of A that allows it to dimerize in the absence of ligand.

Cancer. The signal transduction pathway would be activated independently of the presence of ligand. This would lead to unregulated cell proliferation.

d) A missense mutation in C that changes the serine that is normally phosphorylated by B to alanine, which cannot be phosphorylated.

No cancer. These cells would not be able to proliferate normally in response to the ligand.

e) A nonsense mutation in the gene encoding transcription factor D, causing the loss of the factor’s nuclear signal sequence.

No cancer. The signaling cascade would still be able to successfully phosphorylate factor D, but factor D would be unable to enter the nucleus and bind to its target gene promoter without a nuclear signal sequence.
Question 3

The family pedigree of M shows a number of individuals (shaded) acquiring Retinoblastoma.

The figure below depicts chromosome 13, where the \textit{Rb} gene maps. The pair of chromosomes is shown from DNA isolated from \textit{normal} tissue from all the family members with cancer.

Normal Tissue from Cancer Patients
Chromosome \#13 pair

Chromosomes isolated from Tumor Tissues

1. The \textit{Rb} locus exhibits a loss of heterozygosity.
2. The tumor may be the result of a large deletion.
3. The tumor may be the result of nondisjunction.
4. The tumor may be the result of a \textit{de novo} point mutation.
5. The tumor may be the result of mitotic recombination.
6. The tumor may be the result of amplification.
b) Compared with this family, the new mutation resulting in M’s tumor is (more, as, less) likely to occur in the general population. Circle one.

c) Compared with this family, if this particular mutation did occur in someone from the general population, it would it be (more, as, less) likely to cause tumor formation. Circle one.

d) Is the cancer phenotype of a single mutant Rb allele (Rb\(^{-}\)) dominant or recessive (circle one) at the cellular level?

**Question 4**

A cancer patient, Mr. X, has a history of cancer in his family. Both he and the other cancer survivors in his family developed cancer later in life. Using genetic linkage analysis, you map this family’s disease to a region of chromosome 8. You isolate DNA from a tumor sample taken from Mr. X and find that both copies of chromosome 8 have a deletion in a gene whose function has not yet been characterized.

a) Is the normal gene likely to be a tumor suppressor gene or a proto-oncogene? Explain.

*The normal gene is likely a tumor suppressor gene. Cancer results when both copies are mutated.*

In order to determine the exact region of the gene that is deleted, you sequence both the normal gene in an unaffected individual and the mutant gene from the copy of chromosome 8 that Mr. X inherited from his mother (who also had cancer). You find that the mutant gene has a deletion that completely eliminates exon 11, as shown below.

![Genetic diagram showing deletions in exons](image-url)
b) Using PCR diagnosis technique (with primers able to amplify the 1000 base wildtype region), you analyze the blood of Mr. X and his father (who married into the pedigree so was not a carrier). Indicate on the gel what the expected product sizes will be. In lane 1, you load the reaction using the blood Mr X’s father. In lane 2, you load the reaction using the blood of Mr. X. (Assume that the blood of Mr. X does not contain tumor tissue.)

![Genetic Analysis Diagram]

<table>
<thead>
<tr>
<th>LANE 1</th>
<th>LANE 2</th>
<th>LANE 3</th>
</tr>
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<tbody>
<tr>
<td>1000bp</td>
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<tr>
<td>900bp</td>
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<tr>
<td>500bp</td>
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<tr>
<td>400bp</td>
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c) You discover that in the tumor sample taken from Mr. X, the copy of chromosome 8 inherited from his father contains a deletion that eliminates the entire gene described above. How did this deletion arise?

*This is a somatic mutation that arose in a single cell of Mr. X, which eventually developed into the tumor. The deletion is therefore not present in his father.*

d) Indicate in the gel above in Lane 3, what the products would be if DNA from this tumor sample were used as the template in the PCR-based test.

e) Is the cancer phenotype dominant or recessive? Explain briefly.

*Recessive. Both copies (alleles) must be mutated to show the cancer phenotype. (Increased susceptibility to cancer, however, is a dominant phenotype because it is due to mutation of only one allele.)*
Question 5

You have discovered a novel gene that you believe is involved in the control of normal cell proliferation based on sequence comparison with other known regulators of proliferation. You predict that a mutation in this gene could lead to the development of human tumors.

Therefore, you isolate DNA from a tumor sample taken from a patient, Mr. Z, in order to determine whether there is a mutation present in the novel gene. You perform PCR analysis on this DNA using primers that amplify a region of the novel gene and discover that the resulting product has the same size as amplified DNA isolated from a person without this cancer. You digest the PCR product from the tumor sample to completion with a variety of restriction enzymes and find that most of these show no difference in the number or size of the bands on the gel. However, a complete digest with HindIII shows the following band pattern:

<table>
<thead>
<tr>
<th>Unaffected Person</th>
<th>Mr. Z</th>
</tr>
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<tbody>
<tr>
<td>800bp</td>
<td></td>
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<tr>
<td>700bp</td>
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<td>600bp</td>
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<td>200bp</td>
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<td>100bp</td>
<td></td>
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</tbody>
</table>

a.) In Mr. Z, what type of mutation does the novel gene contain?

*Point mutation that eliminates a single HindIII site on one copy of the novel gene.*

b.) Name 2 possible causes of this mutation.

*Error in DNA replication, UV induced lesion, Chemical mutagens, superoxides Others that will be mentioned by Tyler in lecture*

c.) Is the normal gene likely to be a tumor suppressor gene or a proto-oncogene? Explain.

*It is likely a proto-oncogene. A point mutation causes the protein to be continuously active.***

d.) You later discover that the wildtype allele of this novel gene encodes a kinase that phosphorylates a transcription factor. The phosphorylated transcription factor activates the expression of genes required for cells to initiate DNA synthesis. Explain how the mutation in the novel gene can lead to the development of cancer.

*The point mutation causes the kinase to be continuously active. As a result, the transcription factor remains in its phosphorylated and active state. Genes involved in DNA synthesis would therefore be expressed at high levels. Cells would tend to divide more rapidly, leading to cancer.*
Question 6

Depicted below is a G-protein called Rap, which is a key player within a certain signaling cascade relevant to cancer. This signaling cascade triggers cell division in response to the binding of a specific growth factor to its receptor.

Growth factor binds its Receptor at cell surface leads to...

a) Which of these genes, when wildtype, might act as a Tumor Suppressor Gene?

Rap  |  GAP  |  GEF  |  GTP
-----|-------|-------|-------
none of the above  |  all of the above

b) Which of the following mutations in Rap might cause a cell to become cancerous?

i) A missense mutation causing Rap protein to continuously hydrolyze GTP

ii) A missense mutation causing Rap to spontaneously and continuously bind to GTP

iii) A missense mutation causing Rap protein to signal to target proteins independent of GTP/GDP binding