

NAME _____ TA _____

7.013 Spring 2005 Problem Set 6

FRIDAY April 29th, 2005

Problem sets will NOT be accepted late.

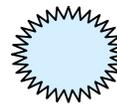
Question 1

You are given the following cancer cell line derived from a patient's cervical tumor. Your job to characterize what caused the tumor in this cell line. You compare this cell line to a cell line derived from the same patient's normal cervical cells.

Normal Cervical Cell



Cervical Cancer cell

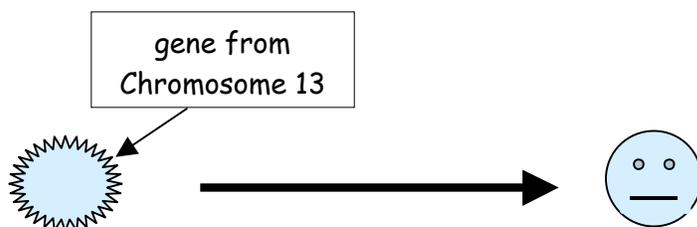


a) First you expose the two cell lines to DNA damaging UV radiation and look at the effects. You find that normal cervical cells die after UV exposure, but the cancer cells do not. Which of the following are suggested by this experiment? Circle all that apply.

- i) a tumor suppressor gene is mutated in this tumor.
- ii) an oncogene is mutated in this tumor.
- iii) a gene involved in apoptosis is mutated in this tumor.
- iv) a gene involved in DNA repair protein is mutated in this tumor.
- v) a gene involved in metastasis is mutated in this tumor.
- vi) a gene involved in angiogenesis is mutated in this tumor.

b) You look at the location of commonly known cancer genes in the two cell lines by analyzing genetic markers and you determine that there is a loss of heterozygosity on chromosome 13 only in the cancer cells. Using a virus you introduce a wild-type copy of a gene from chromosome 13 known to be involved in carcinogenesis into the cancer cell line.

You find that introduction of this gene copy slows down the rate of cell division in the cancer line.



Which of these hypotheses is suggested by this observation? (Circle all that apply.)

Chromosome 13 contains...

- i) an apoptotic factor that is mutated in the cancer cell line.
- ii) an oncogene that is mutated in the cancer cell line.
- iii) a tumor suppressor gene that is mutated in the cancer cell line.
- iv) a gene coding for a DNA repair protein that is mutated in the cancer cell line.
- v) a gene required for metastasis.
- vi) a gene required for angiogenesis.

c) A colleague discovered a gene that is mutated in late-stage (aggressive) cervical tumors. He gives you a vector containing a wildtype copy of this gene that you transfect into your original cervical cancer cell line. You observe no differences in growth, cell death or any other properties in standard tissue culture tests.

You inject these transfected cells, as well as the original cancer cell line (not transfected) into different groups of mice. You observe...

- Primary Tumors of similar size develop in both groups of mice.
- The original cancer cell line kills all the mice within 2 month of transfection and dissection of these mice show tumors throughout body.
- The transfected cancer cell line does not kill the mice within the 6 months of transfection.

Which hypotheses are suggested by these observations?

The gene codes for a protein that...

- i) inhibits cell division.
- ii) promotes cell division.
- iii) prevents apoptosis.
- iv) prevents metastasis.
- v) repairs DNA.
- vi) promotes angiogenesis.

e) Since Human Papilloma Virus (HPV) infection is associated with cervical cancer, you obtain a HPV-infected cancer cell line and a corresponding uninfected normal cell line from a patient. You determine that there is no p53 protein in the infected cancer cells, although the RNA is expressed normally.

When you infect the normal cell line with HPV, you find that the p53 protein levels drop dramatically, yet the p53 RNA levels remain the same. Which of the following could explain how HPV might be involved in cervical cancer.

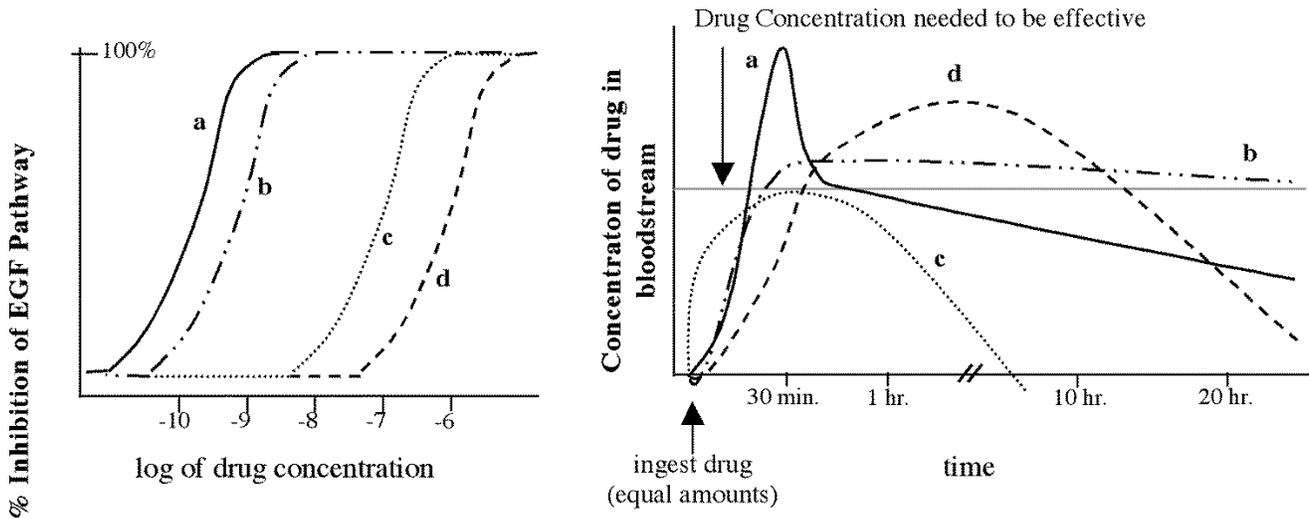
- i) HPV causes the degradation of the p53 protein.
- ii) HPV codes for an oncogene that is expressed at high levels.
- iii) HPV integrates into *p53* locus, thereby knocking out the *p53* gene.
- iv) HPV promotes tumorigenesis by suppressing the immune system of the infected patient.
- v) HPV codes for a protein that degrades a DNA repair protein, leading to mutations in the p53 gene.
- vi) HPV encodes a *p53* gene to be expressed at high levels.

e) Your colleague has come up with an idea to devise a gene therapy for cervical cancers with HPV. His idea is to introduce a wild-type copy of the gene encoding the p53 gene into the HPV infected cancer cells. Will this work?? Explain why or why not.

f) Iressa is a drug that was recently designed to inhibit the EGF signaling pathway. If you were to discover that adding Iressa to cancer cells from some tumors **killed them immediately**, what might you suspect about EGF signaling in these tumors? Choose the best answer.

- i) EGF receptor signaling in these cancer cells is needed for the cells to divide.
- ii) EGF receptor signaling in these cancer cells prevents apoptosis.
- iii) EGF receptor signaling in these cancer cells promotes cell growth and apoptosis.
- iv) EGF receptors from these cancer cells bind to Iressa, leading to increased EGF receptor signaling.

g) In the final stages of generating Iressa, investigators performed a battery of tests to compare various versions (a, b, c, and d) of the drug to find one with all the properties necessary for effective therapy. Shown below are two of the tests performed. Given that low concentrations and long duration of effective dose in the blood are most critical in choosing a drug, from these tests, which compound would you choose as your drug?



- i) drug a
- ii) drug b
- iii) drug c
- iv) drug d
- v) all drugs are equally effective.

Question 2

You are biking along Mass Ave, enjoying the weather and watching the teeming masses of prospective students. Amidst your nostalgia, you fail to notice a car-sized pothole, take a rough fall, and end up with a nasty cut on your left knee.

a) Over the course of several hours, the cut gradually swells up and becomes red and tender. You conclude that cells from your immune system are being recruited to the site of injury by cytokines such as interleukins and other messenger molecules. Circle the predominant white blood cell type(s) entering the injury region at this time.

B-cells CD4+ Helper T-cells CD8+ Killer T-cells Macrophages Mast Cells Neutrophils None

Two days after the initial injury, the cut is still red and swollen, as a result of white blood cells leaving the bloodstream and entering the tissues at the injury site. This is normally caused by pro-inflammatory cytokines that bind to receptors on the endothelial cells lining nearby blood vessels, causing these cells to express integrin. When integrin binds its receptor the white blood cells stick on the side that faces the bloodstream.

c) White blood cells have a receptor for the integrin, and binding to it causes them to stick to the walls of the blood vessel, exit the bloodstream, and enter the surrounding inflamed tissue. A small number of individuals are homozygous for an integrin deletion mutation. Which of the following effects would this have? (Circle all correct answers.)

- i) Increased bleeding time
- ii) Reduced heat & swelling after an injury
- iii) Increased vulnerability to bacterial infections
- iv) Increased vulnerability to viral infections
- v) Severe immunodeficiency: needs to live in a germ-free bubble to survive
- vi) None of the above

d) Chronic granulomatous disease is a disorder caused by a mutation in the NADPH oxidase gene. With these mutation neutrophils and macrophages can't produce the highly reactive oxygen radicals necessary for killing phagocytosed bacteria. What are the consequences of this? (Circle all correct answers.)

- i) Increased bleeding time
- ii) Reduced heat & swelling after an injury
- iii) Increased vulnerability to bacterial infections
- iv) Increased vulnerability to viral infections
- v) Severe immunodeficiency: needs to live in a germ-free bubble to survive

Question 3

a) Cells present antigens on MHC I and MHC II molecules. The following table lists several different antigen types, and two different cell types that will present the antigen. For each box, write I, II, BOTH, or NEITHER to indicate which MHC molecule(s) the cell will use to present the antigen in the question.

| Antigen | Macrophage | Muscle cell |
|---|------------|-------------|
| A viral protein produced by a virus that has infected the cell. | | |
| A novel cancer protein produced within the cell. | | |
| A cytoplasmic protein normally found in the cell. | | |
| A protein from a phagocytosed bacterium. | | |
| A lipid from a phagocytosed bacterium. | | |

b) It is known that during viral infections, viral proteins activate CD8+ T-cells via MHC I complexes. However, CD4+ T-cells are also activated. Since CD4+ T-cells are being activated via interactions with MHC II, what might be occurring to allow viral proteins to be expressed on MHC II cells? MØs are phagocytosing viruses, or B cells

c) Patients with DiGeorge's syndrome have a developmental abnormality that results in partial or total lack of development of the thymus. These patients are capable of handling bacterial infections, but are very vulnerable to viral infections. What types of immune cells are missing or defective in these patients? (Circle all correct answers.)

Neutrophils Macrophages CD4+ T-cells CD8+ T-cells B-cells None

d) HIV selectively infects and destroys CD4+ T-cells. While it is the CD8+ "Killer" T-cells that destroy intracellular invaders, CD4+ "Helper" T-cells are required for Killer T-cell activation. Which of the following effects would this have? (Circle all correct answers.)

- i) Increased bleeding time
- ii) Reduced heat & swelling after an injury
- iii) Increased vulnerability to bacterial infections
- iv) Increased vulnerability to viral infections
- v) Severe immunodeficiency: needs to live in a germ-free bubble to survive

Question 4

a) Our ability to recognize a wide range of antigens depends upon the generation of a wide variety of immune receptors. This occurs through a process called VDJ recombination, where different combinations of DNA (the V, D, and J sequences) are shuffled to make random receptors. Adenosine deaminase is an enzyme required in this process, without which functional immune receptors cannot be created. What cells would be absent or defective in a patient lacking a functional adenosine deaminase?

B-cells CD4+ Helper T-cells CD8+ Killer T-cells Macrophages Mast Cells Neutrophils None

Put a check in the appropriate boxes to indicate which immune receptor binds to which antigen or antigen complex.

| Antigen / Antigen Complex | B-cell receptor | CD4 T-cell receptor | CD8 T-cell receptor |
|------------------------------|-----------------|---------------------|---------------------|
| Free-floating antigens | | | |
| Antigens presented by MHC I | | | |
| Antigens presented by MHC II | | | |

b) The following figure shows a complete antibody protein, with two heavy chain peptides forming a dimer in the inner part and two light chain peptides hanging on the outer part.

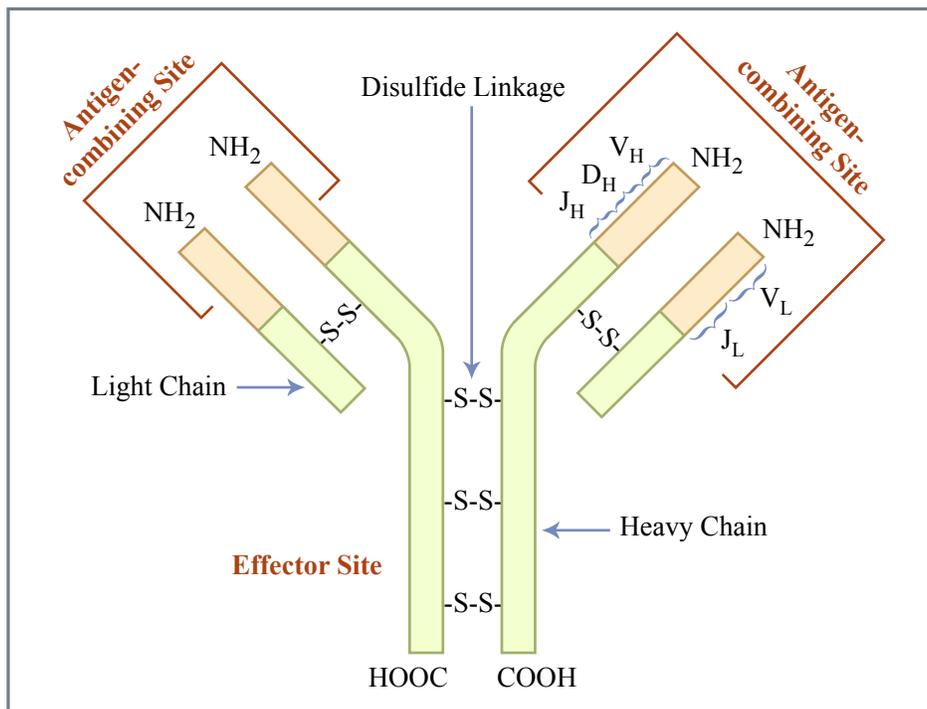


Figure by MIT OCW.

Light chains do not have a D segment.

c) If there are 300 V_L segments and 5 J_L segments, how many different light chains can be produced by VJ recombination?

d) If there are 200 V_H segments, 15 D_H segments, and 4 J_H segments, how many different heavy chains can be produced by VDJ recombination?

e) If the two heavy chains have to be the same, and the two light chains have to be the same, but their VDJ recombination is independent of each other, how many different antibodies can be produced?

f) B-cell and T-cell immune receptors are produced by undergoing an analogous VDJ recombination process to antibody production. Each B-cell or T-cell the body makes has a unique immune receptor. Each immune cell expresses its unique receptor, and only its unique receptor because of.... (Circle one.)

- A unique combination of transcription factors
- A unique change in the DNA coding for the receptor
- A unique way of translating the mRNA coding for the receptor
- All receptors are produced, but only the unique receptor gets expressed at the cell surface

g) What happens to *developing* T-cells that express receptors that recognize self-antigens?

h) What happens to *mature* T-cells if they express receptors that recognize self-antigens?

i) What happens to *mature* T-cells that express receptors that recognize foreign antigens?