7.013 Recitation 13 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lectures 21 (4/6), 22 (4/9) and 23 (4/11)

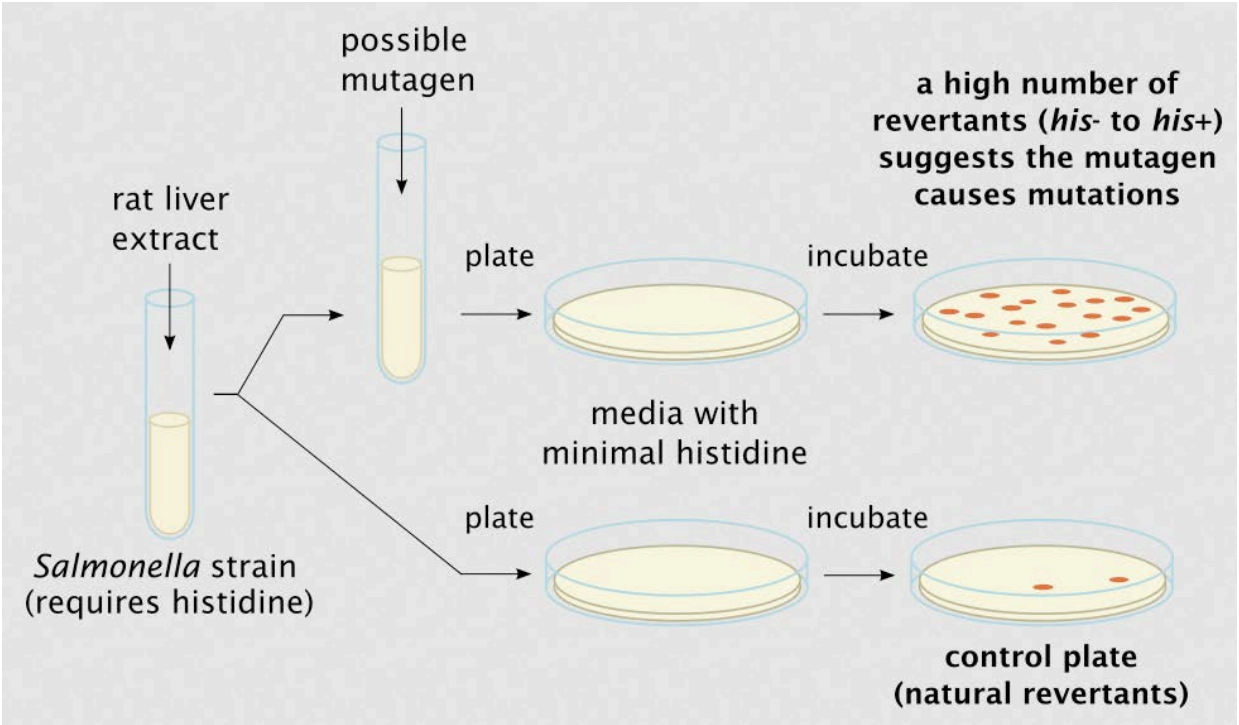
Cancer causing agents are called carcinogens and they are mostly mutagens or pro- mutagens (they do not cause cancer in their native form but can be metabolized by the enzymes, specially the enzymes in the liver into a cancer causing form).

Ames test: Ames test is a widely employed method that uses bacteria (His^-) or that cannot produce amino acid histidine and therefore need histidine in the growth medium to grow and divide) to test whether a given chemical can cause mutations in the DNA of the test organism (in this case the His- bacteria). More formally it is the biological assay to test the mutagenic (and hence the carcinogenic) potential of a test compound.

In Standard Ames test, you take your test compound and incubate it with the His- bacterial cells. Then you plate the treated bacterial cells on a plate in which the growth medium lacks the histidine. You look for the bacterial colonies that have reverted from the His- phenotype to His+ phenotype. The more the number of colonies the more is the mutagenic potential of the test compound.

There are some chemicals, which are not mutagenic in their original (native state). But once they are injested by organisms they may be metabolized into metabolites that can be mutagenic. Such chemicals are called PROMUTAGENS and they can be detected by the **modified Ames test**. Here the test compound is first treated with the liver extract, which supposedly should have the enzymes needed for metabolizing the test compound. The His- bacterial cells are then treated with the liver extract treated mixture of test chemical and its metabolites are then plated on the His- plate to look for the number of His+ bacterial colonies. Please check the following link:

testhttp://study.com/academy/lesson/the-ames-test-using-bacteria-to-test-for-carcinogens.html



Carcinogenicity assay: Here you inject the test chemical into mice regularly for a period of time and look for the development of tumor. You can also take the cells from the developed tumor, culture them in plates and see if they loose contact inhibition and grow on top of each other to foci. Ames test

(original and modified) using bacterial mutants is often used to detect the carcinogenic/ mutagenic potential of different chemicals.

Oncogenes, proto- oncogenes and Tumor suppressor genes: Tumor suppressors genes and proto- oncogenes are normal genes that work in a regulated fashion in a normal cell to properly control the cell cycle. The wild-type function of a tumor suppressor gene is to inhibit the cell cycle in any cell that is not supposed to be actively growing and dividing. Both homologous versions of a tumor suppressor gene must loose their function to transform a normal cell to a cancerous type. The wild-type function of an oncogene is to promote the cell cycle in any cell that is supposed to be actively growing and dividing. One of the two homologous versions of an oncogene must gain a function or increase its function for a cell to become cancerous. Normal cellular counterparts of the oncogenes are called the proto- oncogenes.

Some of these genes are carried by oncogenic viruses and are designated as v-oncogenes. The v–oncogenes can be linked to potent promoters that lead to their inappropriate and high level expression, leading to deregulated cell division. One example is the Rous sarcoma virus (RSV). This retrovirus infects the chickens, thereby causing them to acquire tumors. Here the viral genome contains a gene that it has stolen at some point from a host cell. This gene is an oncogene called src that is involved in cell signaling. The virus carries a mutant version of src that produces an overactive form of the normal cellular kinase src. When RSV infects a cell, the mutant src is transcribed and translated, creating an overactive cell signaling protein that promotes growth and division in chicken cells to form tumors. Other examples include the avian leukemia virus that causes leukemia and human papilloma virus (HPV) responsible for cervical cancer.

Retinoblastoma: This is a cancer of the retina. In Familial retinoblastoma, multiple tumors in the retinas of both eyes occur in the first weeks of infancy when the fetus inherits from one of its parents a chromosome that has its RB locus deleted or otherwise mutated. So in this form of the disease, a germline mutation plus a somatic mutation of the second allele leads to the disease. In sporadic retinoblastoma a single tumor appears in one eye sometime in early childhood before the retina is fully developed and mitosis in it ceases. In this form, both inherited RB genes are normal and a single cell must be so unlucky as to suffer a somatic mutation (often a deletion) in both in order to develop into a tumor. Such a double hit is an exceedingly improbable event, and so only rarely will such a tumor occur.

Cancer Therapy: Most cancer patients are treated with some combination of surgery, radiation, chemotherapy or immunotherapy. Radiation and chemotherapy have the disadvantage of destroying healthy as well as malignant cells and thus can cause severe side- effects. Drug design is a very expensive process. Drugs must be specific (i.e. they can't inhibit other proteins in addition to their targets or else they will cause side effects), must work at a low concentration (so that the amount that needs to be taken by the patient is feasible), and must not be metabolized by the patient either too quickly (so that taking the drug is ineffectual), too slowly, or into toxic byproducts. Gleevec is a drug that works against the type of cancer of the white blood cells called CML (chronic myelogenous leukemia). Almost all patients with CML contain cancerous cells that harbor a mutation that is a translocation between chromosomes #9 and #22. The genes at the two breakpoints of the translocation are Bcr and Abl (a kinase). The translocation causes a fusion of these genes to be created, such that a chimeric protein is made that is one continuous protein, half of which looks like part of BCR and half of which looks like part of ABL. The BCR-ABL fusion protein that is produced by this mutation functions aberrantly by phosphorylating proteins it normally shouldn't, thereby activating the growth and division of white blood cells. The drug Gleevec specifically inhibits the kinase activity of Abl, thereby ceasing the aberrant phosphorylation in CML patients and sending their cancer into remission.

The success of Gardasil is an example of preventive therapy for head and neck as well as cervical cancer. Heceptin is another example of immunotherapy for Her-2 positive breast cancer patients. Some less aggressive forms of breast cancer are not associated with an overexpression of the Her2 gene, so

they are not sensitive to Herceptin. Often the aggressive forms of breast cancer, are associated with an amplification of the Her 2 gene. The amplification of the Her2 gene correlates with the increased expression of receptor on cell surface, which increases the proliferation signal that is critical for tumor development. Herceptin is a monoclonal antibody that works on both the extracellular and the intracellular domains of the HER2 receptor. It does so by binding to the Her2 receptors that are expressed on the surface of cancerous cells thereby flagging these cells for destruction by the immune system. At the same time herceptin blocks the downstream signaling by the Her2 receptors thereby preventing tumor proliferation.

Questions

1. Weinberg's famous experiment: Ras was the first oncogene to be discovered. Ras is part of a cell signaling pathway. The input for this pathway is an extracellular protein growth factor, and the output is to induce transcription of genes necessary for the cell cycle to occur. Ras is a GTPase that is active in the GTP-bound form but inactive in the GDP-bound form. Ras was discovered in the Weinberg lab via the following experiment. Human tumor DNA was cut into pieces, and each different piece was put into a different mouse cell. The mouse cells were then grown in Petri plates. Only the mouse cell that took up the mutant allele of the oncogene could grow and divide enough to form a colony of cells.

a) Do you think that the mouse cells had their own versions of Ras before the experiment began? If yes, do you think that the mouse versions of Ras were wild-type or mutant?

b) In this experiment, it seems that there was only one mutation necessary to make the mouse cells over-proliferate. We know, however, that cancer results from an accumulation of mutations. Why then did this experiment work?

c) If a patient had a tumor that was caused in part by mutations in Ras, do you think it would be a good therapeutic decision to treat the cancer patient with a drug that targets and inhibits Ras?

d) Do you think it would be a good therapeutic decision to provide this cancer patient with a wild-type copy of the Ras gene?

e) Do you think that this experimental technique would work to identify tumor suppressor genes? Why or why not?

2. Complete the table for each of the following chemotherapeutic drugs.

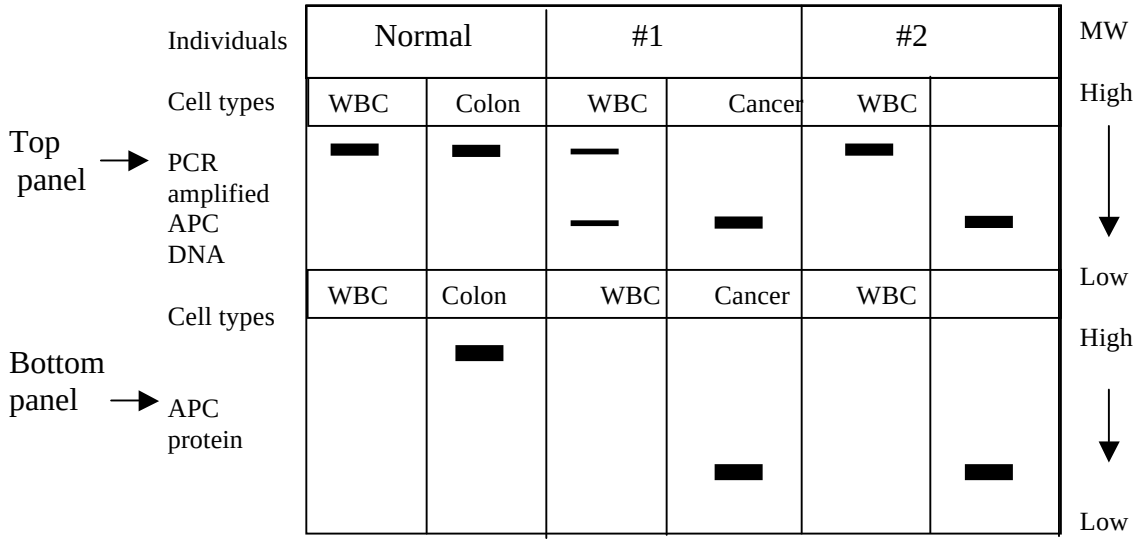
Drug	Normal function	Which process is **directly** inhibited: *replication, transcription, protein synthesis, division*? Choose **one** and **explain** your choice.
Methotrexate	Inhibits thymidine synthesis	
Cisplatin	Crosslinks double stranded DNA	

3. You introduce a single copy of the **mutant versions of the following genes** into an immortalized non-cancerous cell line. Complete the table for each introduced gene. ***Note:*** *Consider introduction of each gene separately.*

Gene	Normal function of **encoded** protein	Wild-type version of this gene functions as a ***proto- oncogene or tumor suppressor gene?***	The mutant allele, introduced into the cell line, encodes a…	Phenotype (***cancerous or non-cancerous***) of the resulting cell that has received **one copy** of the mutant gene.
fos	A transcription factor that promotes cell proliferation		fos gene product lacks the nuclear localization sequence	
Alk	A tyrosine kinase that promotes cell cycle progression		alk gene product has a constitutively (always) active kinase domain	

4. Familial adenomatous polyposis (FAP) affects nearly 1/8000 people in the USA. Patients having FAP are genetically **predisposed** to colon cancer. Mutations in the APC gene have been identified as the probable cause of FAP.

The following diagram represents the gel electrophoretic profiles of both the PCR amplified APC DNA (top panel) and APC protein (bottom panel) isolated from white blood cells (WBCs) and colon cancer cells of two individual patients. *(A profile of the APC DNA and APC protein in a normal individual is provided as a reference. Please note the intensity of the bands while answering this question).*



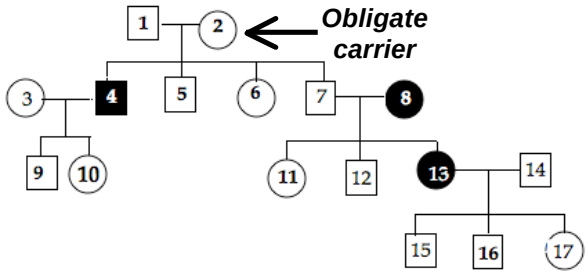
a) Explain why in the normal individual, the APC protein is detected only in colon cells even though the APC DNA is present in both colon cells and WBCs.

b) One of these two individuals **does not** have FAP but still develops colon cancer. Given the data above, which individual would this be? Explain how this individual got colon cancer.

c) Complete the following table based on the information provided in the gel profile above. *(Use the symbols '+' to represent the wild-type allele of the APC gene, '-' to represent the loss of function mutation and 'M' to represent the gain of function mutation. The genotype of the APC gene in a normal individual is provided as a reference).*

Individuals	Genotype of APC gene		Is the genotype of WBC different from colon cancer cells? If yes, explain why.
	WBC	Colon cells or Colon cancer cells	
Normal			
#1			
#2			

5. Mutations in the nuclear excision repair genes (NER) contribute to many malignancies, including neuroblastoma. The following is a human pedigree showing the predisposition to neuroblastoma due to a mutation in a NER gene. **Note**: All individuals who develop neuroblastoma are shaded. I*ndividuals marrying into the family (except for Individual 8) only carry the wild-type version of NER gene. Assume that no other mutation arises within the pedigree. Also assume* ***complete penetrance*** *except for* ***Individual 2*** *who is an obligate carrier (does not develop the disease but can pass the disease associated allele to the next generation).*



a) Give the **mode of inheritance** of predisposition to neuroblastoma.

b) Using the letters NER^{WT} or NER^{DIS}, give all possible genotypes of Individuals 1 and 2.

 # 1: _____

#2: _____

7.013 Introductory Biology

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