The key: 7.013 Recitation 13 – Spring 2018

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?
Yes, they had wild-type versions of Ras gene before the experiment was done.

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?
A cell line is already immortalized and is therefore predisposed to transformation unlike the wild-type cells. Hence a single mutation is enough to transform it to a cancerous cell line.

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?
Most likely, yes, since oncogenic mutation is a gain-of-function mutation.

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?
No, since adding a wild-type copy of Ras gene cannot suppress the phenotype caused by the gain-of-function mutation.

e) Do you think that this experimental technique would work to identify tumor suppressor genes? Why or why not?
No, it would not work. A mutation in a tumor suppressor gene is a homozygous loss-of-function mutation. Therefore introducing a mutated copy of the tumor suppressor gene into the cells that have two wild-type copies of the same gene will not work (the effect of wild-type allele is dominant to the mutant allele(s).

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

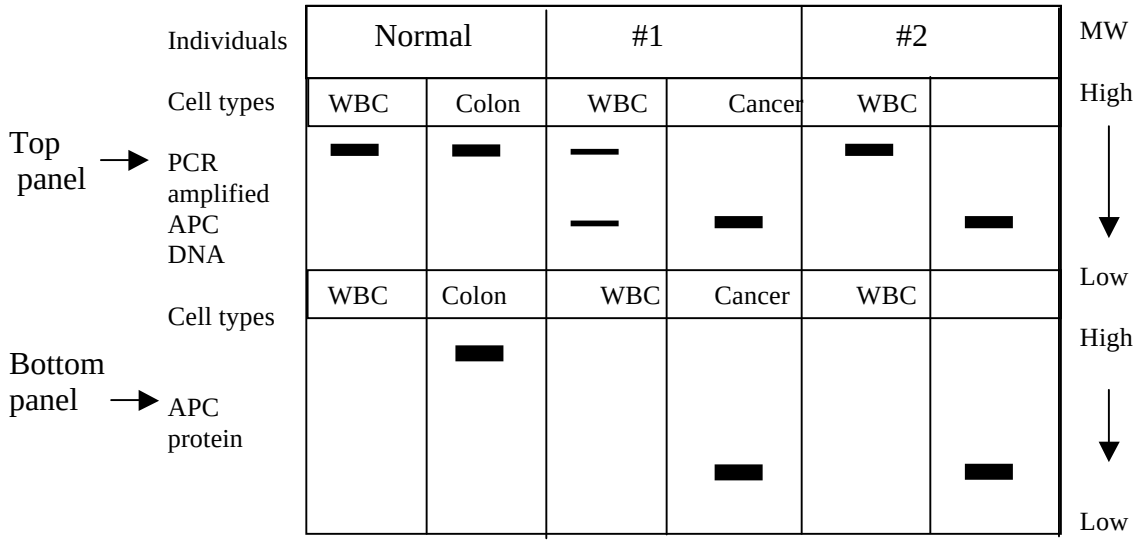
Drug	Normal function	Which process is **directly** inhibited: *replication, transcription, protein synthesis, division*? Choose **one** and **explain** your choice.
Methotrexate	Inhibits thymidine synthesis	*Replication and cell division, since T is one of the four bases of DNA and is needed for replication in S phase of the cell cycle.*
Cisplatin	Crosslinks double stranded DNA	*Replication and cell division, the DNA strands need to unwind in order to replicate during the S phase of the cell cycle.*

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	*Proto-oncogene*	fos gene product lacks the nuclear localization sequence	*Non-cancerous*
Alk	A tyrosine kinase that promotes cell cycle progression	*Proto-oncogene*	alk gene product has a constitutively (always) active kinase domain	*Cancerous*

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).*



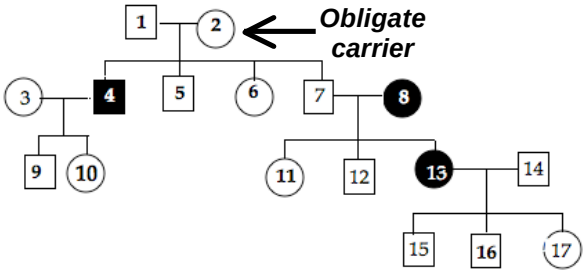
a) Explain why in the normal individual, the APC protein is detected only in colon cells even though the APC DNA is present in both colon cells and WBCs.
*All **somatic cells in an individual have the same DNA** and hence the same set of genes. However, each cell type in an individual expresses only **specific set of genes,** which regulate their shape, size and functions.*

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.
*Individual **#2** does not have FAP but sporadically develops colon cancer. This individual undergoes a spontaneous somatic mutation of both alleles of APC genes in colon cells producing a non-functional APC protein that leads to 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	+/-	-/-	***Yes**, APC gene shows **loss of heterozygosity***
#2	+/+	-/-	*same as above*

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.
Autosomal dominant

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

 # 1: *NER^{WT} NER^{WT}*

#2: *NER^{DIS} NER^{WT}*

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