# 7.016 Recitation 17 – Fall 2018

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


## Summary of Lectures 25 (11/9) & 26 (11/14):

Cancer is a complex set of diseases that results due to uncontrolled increased in cell number either due to uncontrolled cell proliferation and/ or decreased cell death. Tumor arises from uncontrolled cell division and can either be benign, malignant or metastatic.

Cancer results from the accumulation of more than one mutation in the same cell; accumulation of these mutations take time therefore it is often regarded as a disease of old age. Most of these mutations arise spontaneously within somatic cells, therefore most cancers are sporadic, but some cancers may also arise due to familial predisposition. 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 into a cancer causing form, which are often detected by modified or standard Ames tests.

**Oncogenes, proto- oncogenes and Tumor suppressor genes:** Tumor suppressors genes and proto- oncogenes are 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 alleles of a tumor suppressor gene must loose their function to transform a normal cell to a cancerous type. The wild-type function of a proto-oncogene is to promote the cell cycle in any cell that is supposed to be actively growing and dividing. If only one allele of a proto-oncogene has a gain a function mutation, it results in uncontrolled cell division and it is now referred to oncogene.

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) that carries the v-src gene. Other examples include the avian leukemia virus that causes leukemia and human papilloma virus (HPV) responsible for cervical cancer.

**Retinoblastoma:** This is a tumor of the retina that arises in the precursor photoreceptor cells. It is a rare childhood tumor that occurs at a frequency of 1/20,000 children and is seen from birth – 8 years of age. It can either be:

- **Sporadic:** Occurs spontaneously in children who have no family history of retinoblastoma. The tumor usually appears in one eye and if the eye is removed the child is at no risk of developing the disease.

- **Familial:** These children have a genetic predisposition of developing retinoblastoma and the predisposition appears in an autosomal dominant fashion. These children show the development of multiple tumors in both eyes and are also at a high risk of developing osteosarcoma and other tumors.

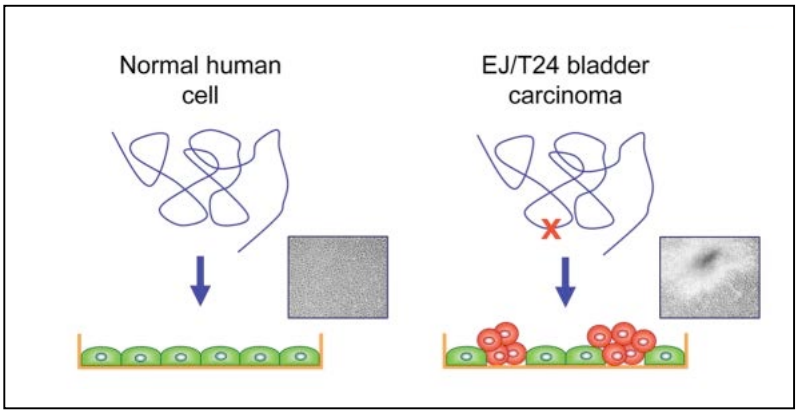
Here the Rb, a tumor suppressor gene shows a homozygous loss-of-function mutation. As a result the inhibitory effect of pRb protein on E2F transcription factor is lost, this allows the cell to move from G1-> S phases without any regulation resulting in deregulated, uncontrolled cell division causing retinoblastoma.

In Familial retinoblastoma, 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 germ-line 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 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.

**Questions**

**1.** Cancer is caused by mutations in proto-oncogenes and tumor suppressor genes. *Ras* was the first oncogene to be discovered in Weinberg's laboratory at MIT based on the following experiment when they asked the question whether introducing DNA from chemically transformed cancer cells into a normal healthy cell could alter the behavior of the latter, converting them from normal cells into cancer cells. They did this using a cell line focus formation assay as diagrammed and described below.

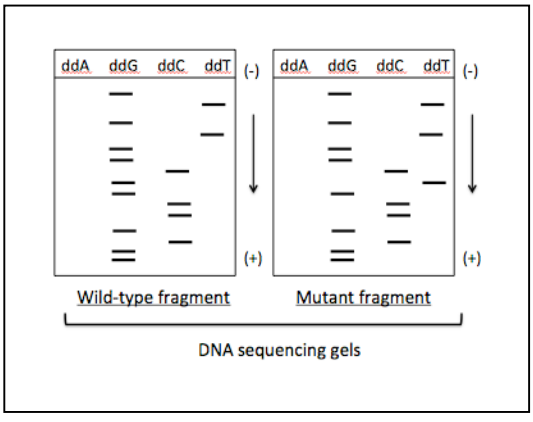



**Experiment:** Genomic DNA from human bladder carcinoma cells was isolated and cut into small pieces and each piece was put into a mouse 3T3 fibroblast cell line. The 3T3 cells were grown in petri-plates. Only the mouse cell that took up the mutant allele of the Ras oncogene could grow and divide enough to form a focus (a colony of cells).

**a)** Which of these describe mutated form of RAS gene?

- **i.** It is proto-oncogene that has undergone a recessive, loss-of function mutation.
- **ii.** It is a proto-oncogene that underwent a dominant, gain-of-function mutation
- **iii.** It is a tumor suppressor gene that underwent a gain-of-function mutation
- **iv.** It is a tumor suppressor gene that underwent a homozygous loss-of-function mutation
- **v.** It is a tumor suppressor gene that underwent a heterozygous loss-of-function mutation

**b)** You come across the coding sequence corresponding to amino acids **10-14 in exon 1** of the mutant and wild-type form of Ras gene and get the following profile.




Give the sequence of corresponding template strand used to sequence the wild type and mutant allele of Ras gene and label its 5' and 3' ends.

**c)** Identify the mutation in the mutant allele as….

- **i.** Frame shift mutation
- **ii.** Silent mutation
- **iii.** Nonsense mutation
- **iv.** Missense mution

**d)** In this experiment, only one mutation was necessary to make the 3T3 cells over-proliferate. We know, however, that cancer results from an accumulation of mutations. Why then did this experiment work?

**2.** Ras is a GTPase that is active in the GTP-bound form but inactive in the GDP-bound form
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.

You identify a cell that has a wild-type copy of RAS gene and mutant copy that shows a constitutively active GTPase activity. Would this cell have a cancerous phenotype? Why or why not?

**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.*

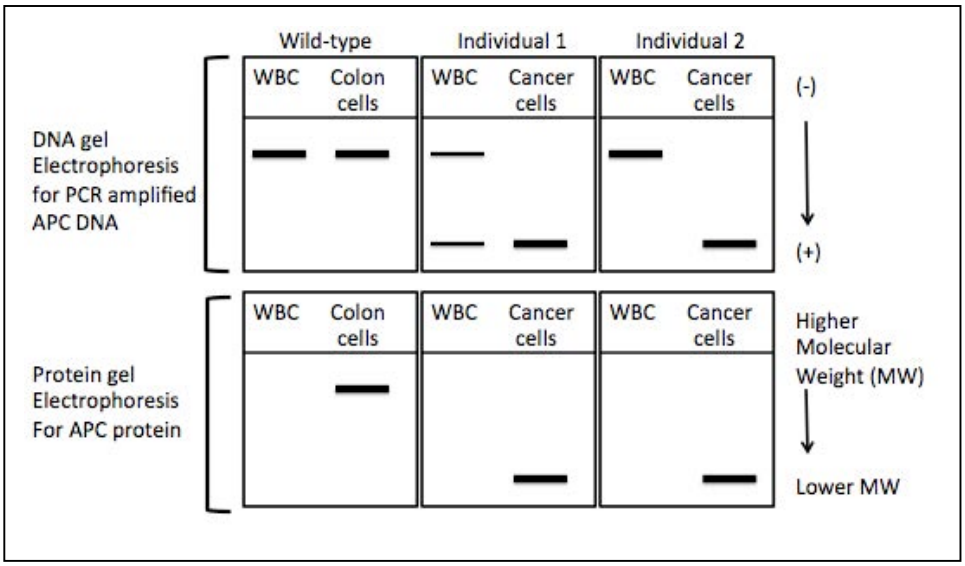
| Gene | Normal function of **encoded** protein | Wild-type version of this gene functions as a ***proto- oncogene or tumor suppressor gene?*** | The mutant allele, introduced into the cell line, encodes a… | Phenotype (***cancerous or non-cancerous***) of the resulting cell that has received **one copy** 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.** Complete the table for each of the following chemotherapeutic drugs.

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

**5.** 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.

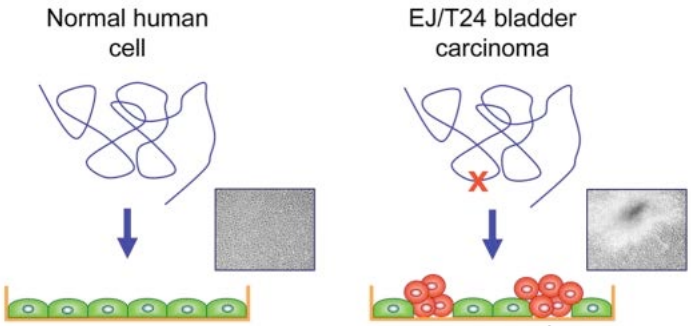
**b)** One of these two individuals **does not** have FAP but still develops colon cancer. Given the data above, which individual would this be? Explain how this individual got colon cancer.

**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 | | | |

**The key**

**1.** Cancer is caused by mutations in proto-oncogenes and tumor suppressor genes. *Ras* was the first oncogene to be discovered in Weinberg's laboratory at MIT based on the following experiment when they asked the question whether introducing DNA from chemically transformed cancer cells into a normal healthy cell could alter the behavior of the latter, converting them from normal cells into cancer cells. They did this using a cell line focus formation assay as diagrammed and described below.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109

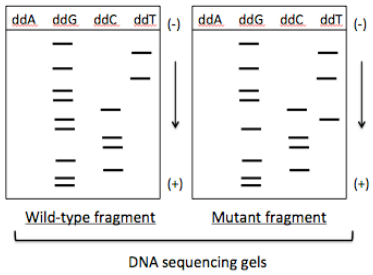
**Experiment:** Genomic DNA from human bladder carcinoma cells was isolated and cut into small pieces and each piece was put into a mouse 3T3 fibroblast cell line. The 3T3 cells were grown in petri-plates. Only the mouse cell that took up the mutant allele of the Ras oncogene could grow and divide enough to form a focus (a colony of cells).

**a)** Which of these describe mutated form of RAS gene?

- **i.** It is proto-oncogene that has undergone a recessive, loss-of function mutation.
- **ii.** It is a proto-oncogene that underwent a dominant, gain-of-function mutation
- **iii.** It is a tumor suppressor gene that underwent a gain-of-function mutation
- **iv.** It is a tumor suppressor gene that underwent a homozygous loss-of-function mutation
- **v.** It is a tumor suppressor gene that underwent a heterozygous loss-of-function mutation

*In the experiment, introduction of one mutant allele promotes cell proliferation and cancer phenotype. So the mutant allele should confer a trait that is dominant to the wild type.*

**b)** You come across the coding sequence corresponding to amino acids **10-14 in exon 1** of the mutant and wild-type form of Ras gene and get the following profile.




Give the sequence of corresponding template strand used to sequence the wild type and mutant allele of Ras gene and label its 5' and 3' ends.

*Since sequence shown is for the coding/ non-template strand it should…*

*Wild type allele: 5'CACACCGCCGGCGCC3'*
*Mutant allele: 5'CACACCGACGGCGCC3'*

**c)** Identify the mutation in the mutant allele as….

- **i.** Frame shift mutation
- **ii.** Silent mutation
- **iii.** Nonsense mutation
- **iv.** *Missense mutation*

*The 3rd codon is being changes from 5'GGC3' → 5'GTC3'. This point mutation corresponds to an amino acid change from Glycine → Valine. Since the identity of a single amino acid residue is changed, this is an example of a nonsense mutation.*

**d)** In this experiment, only one mutation was necessary to make the 3T3 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.*

*The 3T3 cells although immortal, retained some normal growth properties such as contact inhibition, dependence on survival factors and did not propagate when deprived of substratum (e.g., did not form colonies in soft agar) or to form tumors when inoculated into immunocompromised mice. However, these cells did display high sensitivity to mutant Ras induced "focus formation", such that morphologically altered cells that were no longer contact-inhibited grew as easily visualized foci of transformed cells over the background monolayer of untransfected "normal" cells. The ability of NIH/3T3 cells to become morphologically and growth-transformed by a single viral oncogene provided a sensitive one-hit biological assay for the activated oncogenes that were speculated to be present in DNA obtained from tumor but not from normal cells. NIH/3T3 cells have therefore been the longtime workhorse cell culture model for these studies, and were instrumental in characterizing* RAS *and many other oncogenes.* ***(Reference: Cox and Channing, PMC, Aug 2010)***

**2.** Ras is a GTPase that is active in the GTP-bound form but inactive in the GDP-bound form
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.

You identify a cell that has a wild-type copy of RAS gene and mutant copy that shows a constitutively active GTPase activity. Would this cell have a cancerous phenotype? Why or why not?
*No, this cell would not exhibit a cancerous phenotype. The constitutively active GTPase will keep the RAS in its GDP bound inactive state and the wild-type allele will encode a normal functional RAS protein. So there is unrestricted cell proliferation.*

**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.*

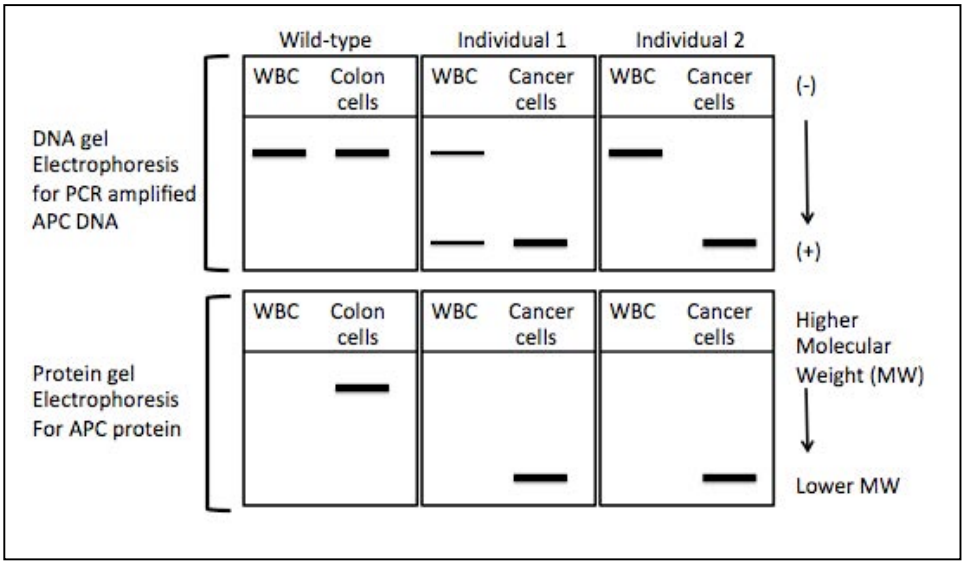
| Gene | Normal function of **encoded** protein | Wild-type version of this gene functions as a ***proto- oncogene or tumor suppressor gene?*** | The mutant allele, introduced into the cell line, encodes a… | Phenotype (***cancerous or non-cancerous***) of the resulting cell that has received **one copy** 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.** Complete the table for each of the following chemotherapeutic drugs.

| Drug | Normal function | Which process is 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.* |

**5.** 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 (LOH)*** |
| #2 | +/+ | -/- | *Same as above* |

# 7.016 Introductory Biology
Fall 2018