Molecular Biology
lecture -12-

In chapter 18 we talked about transformation of normal cells into cancer cell and it acquires having a mutated proto-oncogene to become an oncogene (gain of function) and mutated TSG like TP53 (loss of fxn) so that uncontrolled cell proliferation happens. And we said that in order to have full transformation into malignancy; cancer cell should be able to avoid apoptosis, because cell can commit suicide.

Once cell knows that its mutations can't be repaired, DNA damage triggers apoptosis so it activates activators of apoptosis like Bad and Bax or inhibit BCL-2, or when telomere length is reduced to a certain limit so cell can't live anymore and commit apoptosis. So, cancer cells activate telomerase to avoid apoptosis, in the case telomere length is shorter than its length in other cells but with a constant length .. Why it's shorter ?! Because until cell managed to activate apoptosis , telomere get shorter by fast division of the cell.

#regulation of apoptosis:

In growth factor dependent cells: they count on continuous supply of GFs to live. so let's take the PDGF/Akt /Bad pathway :

Here when PDGF binds it receptor, it activates PI-3 kinase which activates Akt (protein kinase B) , which then phosphorylate Bad (BH3 only proapoptotic) to become inactive so apoptosis won't occur.

Bad is inactive when phosphorylated and active when dephosphorylated if GF depletion >> apoptosis.

So, for cancer cells to be apoptosis resistant they become GF independent >>to have sustained phosphorylated Bad even if no GFs are there. How ?!

By a mutation in the receptor so that it remains bound to the GF (normal receptors are reversibly binding their ligands) , or a mutation that constitutively

avtivatePI-3 kinase or Akt without need of GFs , so that Bad is always phosphorylated >> not active → NO apoptosis

#we already said that in order to have full transformation cell has to accumulate 4-7 mutations and some say 5-10 mutations. And each cancer is a different disorder which means they have different combination of mutated oncogenes and TSGs, consequently cancers differ in proliferation rate, growth of tumor, drug resistance , metastasis capability .

But it's very difficult to have all these 4-7 mutations in the same cell in specific locations ,, it needs hundreds of years to have , 60 years isn't enough . so how would a 60-yeared man have them ?!

Here are the theories explain the likelihood of incidence of these mutations :

1) Clonal expansion theory:

expansion of a mutated population arising from a single cell , so that all cells contain the same original mutation and any of them can have further secondary mutations, what does that mean ?! each cell after every mutation has an increased division rate to give 10 cells for example each have the original mutation of the mother cell >> any of these 10 get a 2^{nd} hit >> it has now more proliferation rate to give 100 cells >>one of them get the 3^{rd} mutation >> it gains more proliferation potential to give 1000 cells >> then one gets 4^{th} mutations ,, so now it accumulates 4 mutations to make it malignant and so on . but some say that even this theory doesn't explain likelihood of mutations.

2) Cancer stem cells theory :

- Bulk of tumor is made of heterogeneous cells (different types) , some are more malignant than the others and the most malignant ones are in the core of the tumor >> cancer stem cells . They reproduce cancer after chemotherapy when tumor bulk shrinks to become very small. They are the most malignant, the most drug resistant, and have the most dangerous mutations.
  Now scientists try to target them and with the chemo therapy (to get rid of tumor bulk) they think they could solve the problem of cancer.

But how to recognize these cells?! by special markers (CD44, CD133 EpCAM ,found exclusively on their surface or heavily on their surface but in small amounts on other normal cells. We produced molecules that can bind these receptors or markers (targeted of smart drugs) like Monoclonal Abs and Herceptin is an example .
But sometimes these markers are only signaling markers and not for proliferation >> in this case we use them to recognize stem cell and pour the drug inside the cell , like Aptamers molecules ; they carry the toxic drug in a liposome to target a cell with a specific marker of cancer stem cells . It's like chemotherapy but with specific targets instead of giving toxic materials in the blood and harms many cells.

- Also, this theory argues that cancer stem cells was actually normal stem cells because they already continuously dividing and proliferating and have switched on oncogenes.
  So, transforming a stem cell into a cancer cell is easier than transforming a normal cell.

 # Would benign tumors transform into malignant ones?! Mostly no, but in some cases they transform into malignant ones.
For ex: (Figure 18.17 slide 281) mutations in epithelial lining of GIT esp. when consuming junk food, a mutation in APC → hyper proliferative epithelium ,, another mutation → early adenoma ,, a mutation in RAS for ex → intermediate adenoma ,, another mutation in one of the TSGs → late adenoma ,, and finally wen a mutation deactivates p53 → then carcinoma (malignant) would happen.

So, if a person with polyp in his GIT and he has 1st degree relatives with colon cancer, then it's dangerous and should be removed because it can transform into carcinoma.
Medical advice: The first degree relatives of a patient of colon cancer who is younger than 50-55 years must do regular Laparoscopy annually after age 30.

 # In the last statistics of incidence of cancer in Jordan:

- Brest cancer is cancer number one in females.
- Colon cancer is cancer number one in males .

— Lung cancer is catching up fast or almost equal to colon cancer in both males and females.
Smoking increase susceptibility to have lung cancer 10 times.

Now we'll move to the next chapter which is**:**

Recombinant DNA technology

(genetic engineering or applied molecular biology)

It's a group of techniques which enables manipulating DNA like reading DNA, identify mutations, producing proteins as insulin, etc. . This technology is accelerating very fast, for ex : for DNA sequencing , the used to use sanger method which read 400 nucleotides in each run . it needs 10 years to finish human genome sequencing and millions of dollars. A new technique appeared in 2010-2011 which enables us to know DNA sequence in 10 hours with a cost of 600 – 700 JD , it's called next(2^{nd}) generation sequencing . recently another technique is discovered which is the 3^{rd} generation sequencing . now scientists expect that sequencing would be by portable nano chips which have million holes ; a DNA string is read in each hole by an electrical current >> bases make resistance in this electrical field and according to its magnitude , it's T, A, C, or G, and the computer save the sequence . it only needs 3-4 hours , 100-200 dollar.

We'll start by techniques of obtaining genes or DNA fragments:

For ex: producing insulin using a bacterial genome: 1^{st} we have to obtain insulin gene>> cloning it into the plasmid of a bacteria and ligate it by DNA ligase >>replication of plasmid and production of insulin.

But, how to obtain the gene of insulin?! By three ways :

1) **By using restriction enzymes** which are bacterial enzymes cut DNA at specific sequences called restriction sites or sequences, each restriction enzyme has a specific restriction site . bacteria need them in order to

protect them from foreign DNA. Bacteria protect their restriction sites by hiding them using methylation .

These enzymes named according to bacteria from which this enzyme is taken , for ex: **EcoRI** is a restriction enzyme comes from Escherichia **coli RY13** bacteria. Restriction sites are 4 or 6 or 8 **palindromic** bases, which means we can read them from 5'- 3' in the two complementary strands in the same way with the same sequence of bases . for ex: AGCT, GGCC

5'- AGCT - 3'

3'- TCGA – 5'

Q: Is GGAA a restriction site for EcoRI ?

A: No, because it is not palindromic !

Note: You don't have to remember the sites from the slides but knowing that it must be palindromic would help you to answer if you had a question in the exam.

*restriction enzymes can cut bluntly (**straight**) or in zigzags(**sticky ends**) which have single stranded overhang in each end and it's easier to ligate into bacterial plasmid than blunt ends and more cohesive because overlapping sticky ends form hydrogen bonds between complementary strands and phosphodiester bonds are formed by DNA ligase. It only needs half an hour but blunts ends to be ligated needs 16 hours on average.

Q: Why do the bacteria have restriction enzymes?

A: To protect itself from the foreign DNA.

Q: How do the bacteria protect its own DNA from restriction?

A: By methylation of the restriction sites on its DNA.

2**) By reverse transcriptase**: get DNA by RNA so that we get the short copy of DNA without introns, bacteria can't deal with splicing DNA sequence to get rid of introns.

Spliced RNA >>> Viral Reverse transcriptase* >>> replicate DNA >>> cloning of DNA into bacterial plasmid.

*from retro viruses.

3) **Chemical synthesis** by DNA synthesizer which have all 4 bases . 1st we go to Gene Bank to get insulin gene sequence. This device reads gene sequence, and put bases according to it and ligate them chemically to build that gene.

 # Techniques to identify DNA sequence: it's used in diagnostic medicine and to compare genes found in humans, plants, and animals. We have two ways to identify DNA sequence:

1) **Direct** by sequencing of DNA, we use **Sanger method** . The only problem is that it reads only 400 nucleotides in each run except if you know the exact location of the mutation in a certain exon which consists of 50 bases for example, so we only need to do sequencing for 1 run of only 50 bases.
2) **Indirect** by using probes which are ssDNA or ssRNA complementary sequences to the gene we want to identify its sequence; they are labeled by a radioactive or a fluorescent material. We use this way if we know exact location of the mutation.

1st we denature DNA by heat or alkali, we put it with the probe in a dry environment; we shouldn't put them in a solution and do immobilization of target DNA on a solid support. Then we do washing. If they bind then we see the signal >> and it's not mutated, it's a normal gene.

Sometimes we make two probes, a mutated and a normal one. If only the normal sequence binds then there's no mutation. It only the mutated sequence binds then there's a mutation . But, If both bind then it's heterozygous.

***how to do Immobilization ?!** By **Electrophoresis** , we isolate DNA bands according to their sizes>>> then plotting DNA from gel into transparency or nylon membrane to obtain DNA sequence .

How to do electrophoresis?! we put DNA fragments broken down by restriction enzymes in a jelly material with pores under electrical current , we have 2 electrodes ; anode and cathode , DNA fragments travel from anode to cathode

because of its negative charge of the phosphate group , and the smaller the DNA fragment , the faster its movement .

We control gel density and contents in order to control pores size to be suitable to sizes of DNA fragments we have by agarose (1% or 3%) and polyacrylamide (4% or 10 %).

Also, we do staining of the gel so that we can see DNA by a fluorescent material which is ethidium bromide . DNA catch the stain and by UV light box we can see DNA fragments in a bright orange color.

"الوطن ليس جغرافيا ؛ إنه قيمة ؛ الحب والكرامة والفداء والإباء والعدل.. الوطن إيمان المخلص وتضحية العاشق الوطن ثبات على المبدأ في ضجّة البائعين ، وتشبث بالحرية.. في سوق النخاسين .. الوطن أنت وأنا وأولئك الذين يجمعهم الضمير النقي والغاية الشريفة" ..

أيمن العتوم.