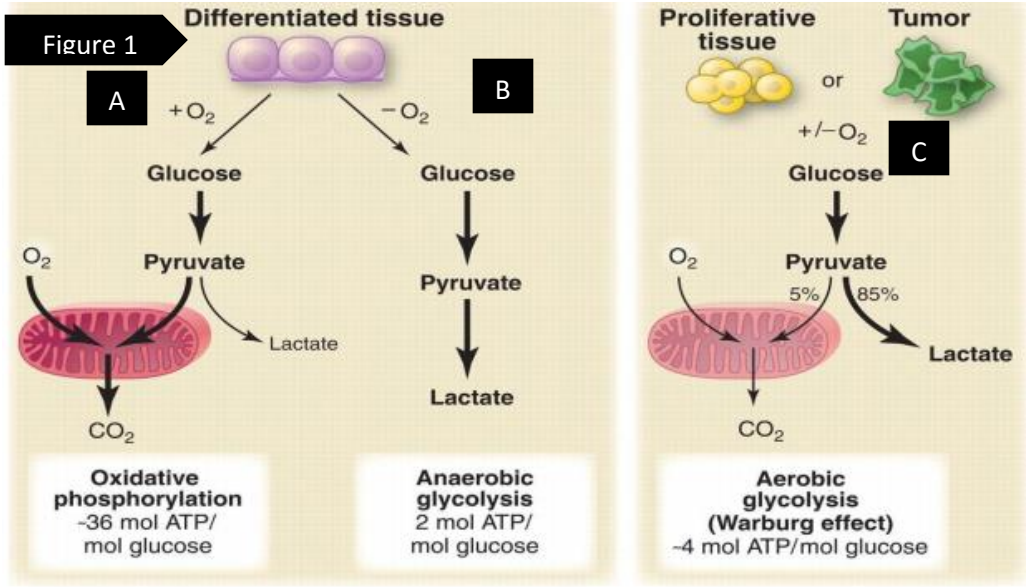
Sheet #20
Reprogramming energy metabolism

this is so common in cancer cells>>it become a hall Mark
Reprogramming energy metabolism figure1 this give an impression that sth is changed,so what is it?
Even if there is plenty of O2 a shift occur to glycolysis. This is called Warburg effect (aerobic glycolysis)
What is the problem with that?
aerobic glycolysis is less efficient than mito. Oxidative phosphorylation. The former produce 2 ATP while the later 36 ATP .. so I end up with less energy even when the requirement to make a higher energy is there. That's the problem.
but when talking about CANCER we are talking about A LOT of growth so from where these tumors obtain energy?
a state of "Glucose hunger" occur, the cells will take up glucose in HIGH amount and just break it by glycolysis rapidly .. it won't proceed to oxidative phosphorylation which appear to be a waste of time to tumor cells. This pattern of glu. Usage is obtained by shunting pyruvate toward biosynthetic pathways at the expense of the oxidative phosphorylation and ATP production (sth in Biochem. Just memorize it)
example on a tumor with Reprogramming energy metabolism : Burkitt lymphoma (this tumor grow very rapidly)

Figure 1



A: Normally in the presence of oxygen cells make oxidative phosphorylation and yield 36 molecule of ATP.
B: Normally in the absence of oxygen cells make Anerobic glycolysis and yield 2 ATP.

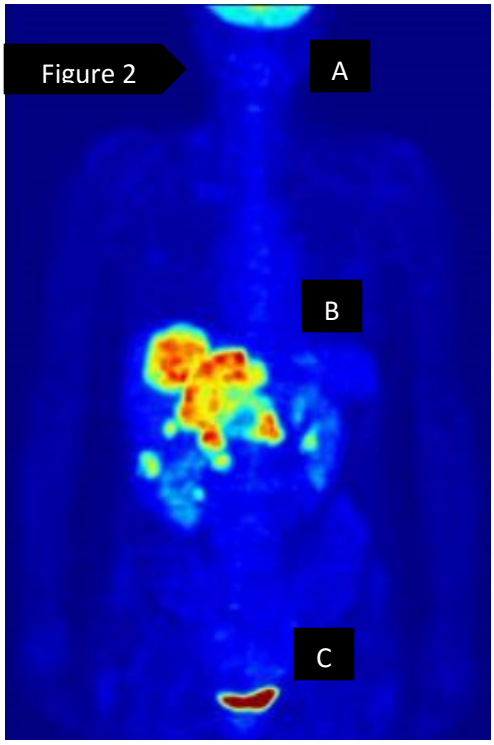
C: in cancers cells abnormally in the presence of oxygen make glycolysis and yield 2 ATP. (aerobic glycolysis /Warburg effect) notice the pyruvate shunt

let's overthink a little bit about this Reprogramming of energy :
do any other cells use this method? Yes , the embryonic cells use the same method (aerobic glycolysis)
this give an indication that this method is used when cells want to grow rapidly

The clinical importance of Knowing this :
we can use these data for tumor diagnosis , we benefit from the "glucose hunger" state. So what exactly is done that they inject the patient with non-metabolized derivative of glucose , and observe what tissue take it extensively and rapidly , this method is known as Positron Emission Tomography (PET).
when u apply PET to the patient and observe the photo see figure2 it's normal to see aggregation of GLu

in the brain and the bladder because they take up glu. Normally at high levels (actually this represent the limitation of this method , as u can not use it to diagnose tumors in that areas)

the Hardware (genes) responsible for this reprogramming:
some oncogenes and tumor suppressors (TP53, PTEN, Akt) these stimulate glu. Uptake.



PET technique
A; Normal (!) uptake of glu by the brain
B: abnormal uptake of glu by the colon >>Cancer
C; Normal (!) uptake of glu by the bladder.

Evasion of the Immune System

this also so common in cancer that it is considered as a hall mark.
How does cancer evade Immune system ?
simply by tricking it.. they trick the immune system by representing themselves as Normal cells so the immune system won't attack them >> the tumor won't be recognized or eliminated .
I think we will talk further about this latter but by now u should know these things..

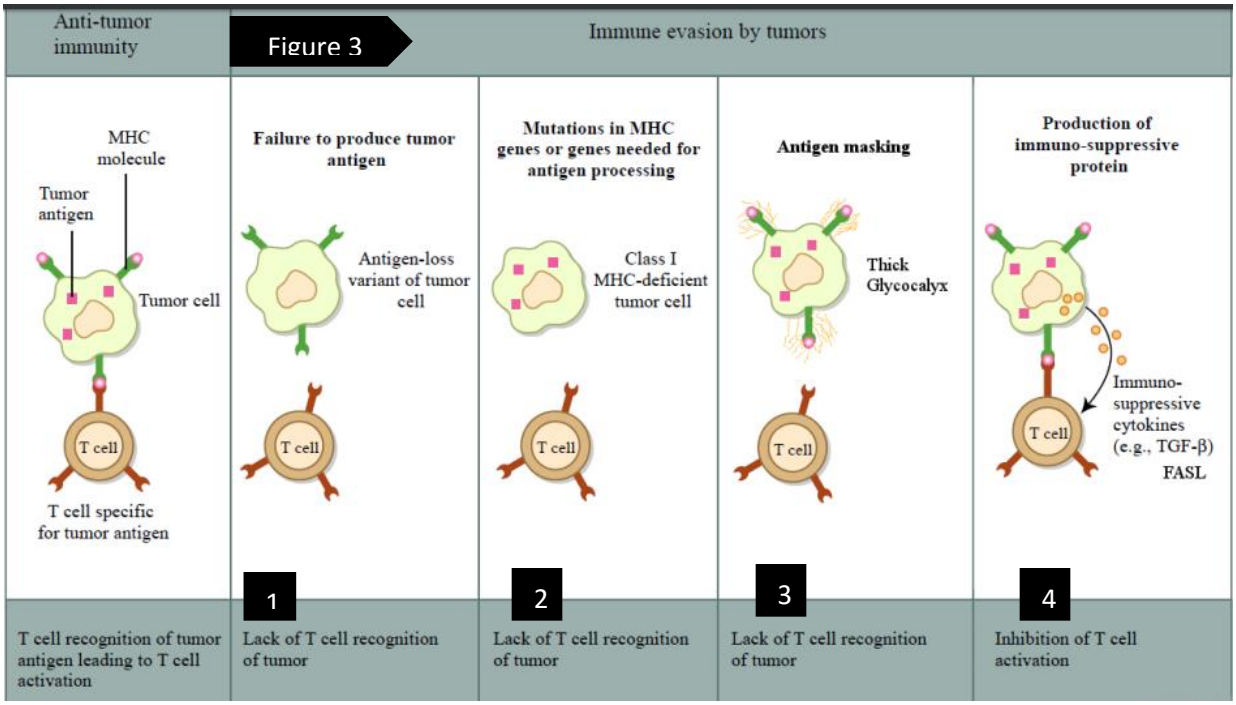
so normally cells would present on their surfaces specific proteins that tell the immune system what is the type of these cells , typically these represented proteins are sample of bits and pieces of proteins from inside the cell that is put randomly on the outside..
these antegens are been been put outside by agents called MHC (Major histocompatibility complex)..

the whole idea is : the antigens that are presented outside the cell reflex what is happening inside..
so if this cell get infected by a virus the immune system will know from the presented antigens
and in the same manner if any "cancerous" protein is over expressed on the surface of a cell the immune system must know and must kill it , but that's not the case in cancer.

Let's think as a cancer cell..
if u are a normal cell you don't express a lot of MYC protein, so the chance of having MYC on a high enough concentration on the surface of the cell to stimulate T-cells is very low.
Abnormally: If you have MYC over-expression, the chance of having bits of MYC being presented on the outside of the cell increases. T-cells will recognize these tumor cells and realize that something is abnormal, therefore killing those cells.

but this does not happen. Why?
because I am a bad evil genus cancer cell, I can trick the immune system *evil laugh hahaahaaaa !*
but how ? figure3
1. Loss of the antigen: if the antigen leads to the cell being recognized by T-cells and therefore killing them off, this will lead to killing of all cells that have the antigen, leaving the cancer cells without the antigen invisible to T-cells. ◊ These cells which don't produce the antigen will be naturally selected due to its ability to invade the immune system.
2. Mutation of MHC genes or any other gene involved in antigen processing: losing the expression of MHC proteins will make T-cells unable to identify those specific cancer cells(invisible).(another type of cells called natural killer cells can identify those cells as abnormal)
3. Producing a very thick glycocalyx: a glycocalyx is a coat of carbohydrates. If a cancer cell has a very thick glycocalyx, this means it could potentially mask the antigen, making it invisible to T-cells.

4. Production of immune-suppressive proteins: Ex: TGF-beta. - Death receptor pathway of apoptosis: FasL is expressed in activated T-lymphocytes and by ligation with Fas receptors that are present on neoplastic cells, apoptosis is induced. If there's a mutation in FasL that makes it unable to bind Fas, the cell will evade the immune system and won't die by apoptosis.



Genomic Instability as an enabler of malignancy

put in your mind that we are surrounding with massive amount of carcinogens (things cause cancer) but the body can deal with them, and very often correct what go wrong, thus cancer is a rare outcome. See figure4

our bodies have a very efficient repair system. However, sometimes this system is deficient>>genes that encode proteins involve in DNA repair are defective. And people born with such inherited defects are in a high risk of developing cancer. This whole situation is called Genome instability and it needs too defective copies of the gene involved. But to a lesser extinct, sometimes one defective copy of the gene would be enough to develop the situation (his is called haploinsufficiency) .in sporadic cancers (those not inherited) mutation could happen on genes involved in DNA repair –actually targeting these genes early in cancer is smart , as u can accumulate more and more mutations easily.

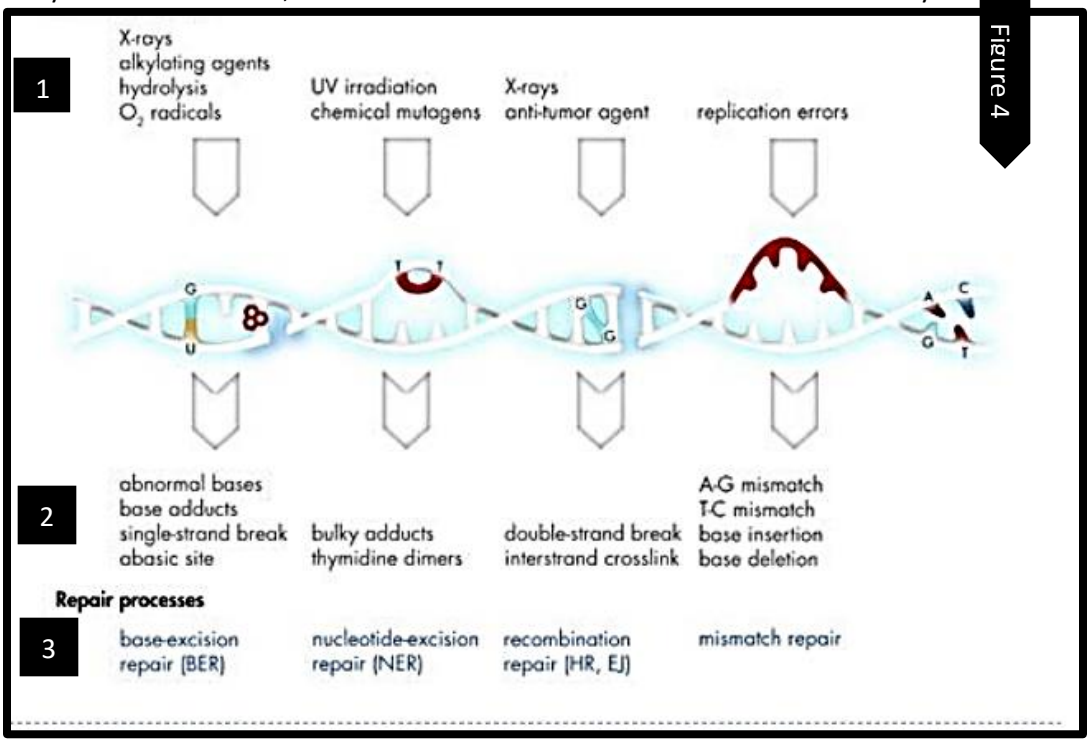


Figure 4

هذه صورةٌ جامعة
1: carcinogens and errors
2: what happen at the DNA level
3:rapair Mechanisim

Which pathways are we talking about when we mention genomic instability?
DNA repair pathways (p53) , which are
1. Mismatch repair pathway
2. Nucleotide excision repair pathway
3. Homologous recombination repair pathway

Now, details ☺

Mismatch repair pathway: figure5
When DNA is replicated, a mismatch in nitrogenous base paring will sometimes occur (example: C-T instead of C-G). This is where the mismatch repair pathway will work.

let's be more curious..
when this pathway work , how does it know which strand is the mother strand and which strand is the daughter strand? I mean if I am a mismatch repairing agent and I face a (C-T) pair, should I correct it to (C-G) pair or to (A-T) ? how can I know which base is the original and which one was mistakably added?

By methyl groups (remember: methyl groups are markers responsible for turning the gene on and off) When you replicate your DNA, the first thing you're going to replicate is your nucleotide sequence then you're going to copy the methylation from the mother strand to the daughter strand. So, that means you have a window before those methylation marks are copied over identifying any mismatched nucleotides therefore the DNA repair pathways will kick in taking out the mismatched nucleotide pair and inserting a new matched nucleotide pair.

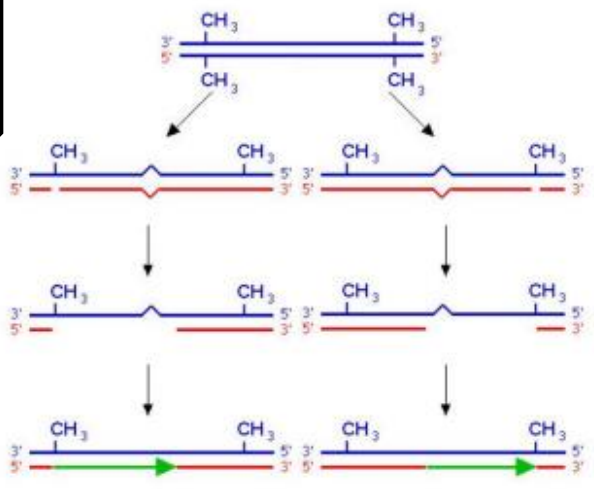
disorder related to a defect mismatch repair pathway:
a disease called HNPCC (Hereditary Nonpolyposis Colorectal Cancer) which is autosomal dominant.

Since we have problems in mismatch repair, we are inducing a mutator phenotype (random mutations). Mutations in TGF-beta type 2 receptors and Bax (pro-apoptotic protein that forms a channel with Bak allowing cytochrome C to leak out) induce colon cancer.

we must differentiate between HNPCC and FAP (Familial Adenomatous Polyposis), this table summarize the differences..

HNPCC (Hereditary Nonpolyposis Colorectal Cancer)	**FAP (Familial Adenomatous Polyposis)**
Mismatch repair pathway	APC—B-lecatinin
Nonplyposis	Polyposis
Predisposition of cancer: right	Predisposition of cancer: left

Figure 5



Mismatch repair pathway

the exact mechanism is not required (genetics course) but what I want u to notice is Methylation of the original strands.

Mismatch repair and Microsatellites instability figure6
- it is one of the interesting features about mismatch repair pathway >>it maintains your microsatellites.

Microsatellites :
are short repeats of DNA (1-6 nucleotides) embedded in your genome, that are the basis of DNA fingerprinting. The mismatch repair pathway is responsible for maintaining the length and location of theses microsatellites. - Mismatch repair pathway is responsible for microsatellite stability.
So if you come across an HNPCC patient, you won't be able to do DNA fingerprinting since their DNA repair pathway isn't working, leading to the continuous change of these microsatellites. The microsatellites in an HNPCC patient will either decrease or increase in size and change in location. This is called microsatellite instability. Microsatellite instability is a hallmark of HNPCC.

Related genes:
The growth-regulating genes that are mutated in HNPCC are those encoding: TGF-beta receptor type 2 BAX ,Others.

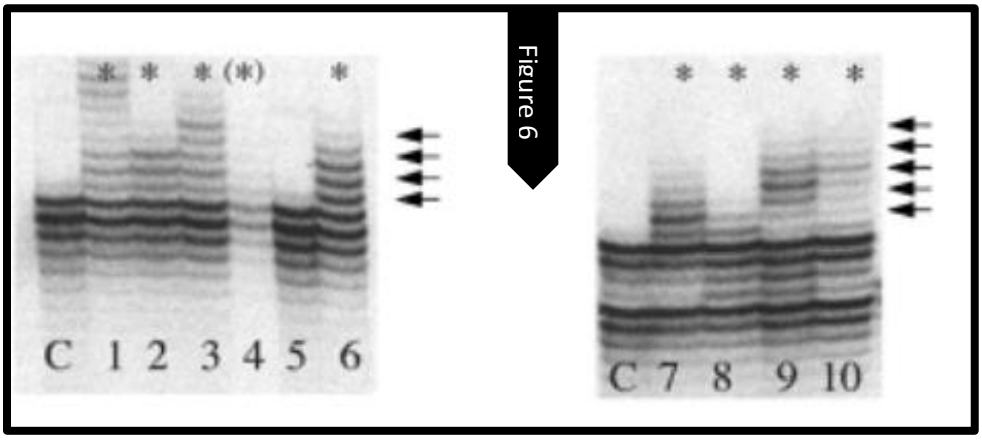
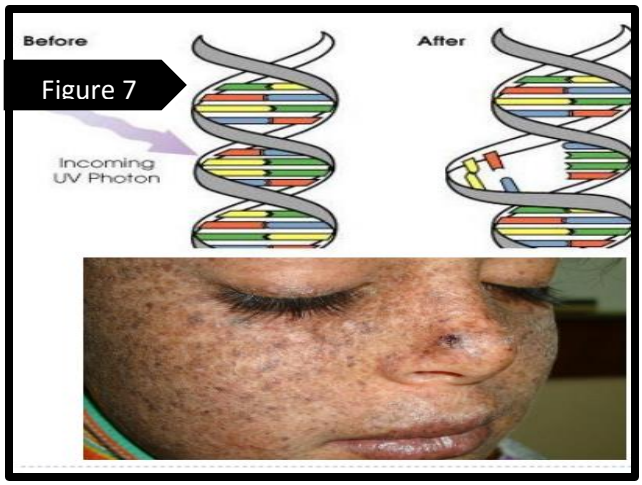


Figure 6

This figure represent microsatellites instability.
this is the same person with different microsatellites (locations and repeats) over time
-this does not happen in a normal person-

Nucleotide excision repair pathway figure7
UV radiation will cause pyrimidine cross links within a strand, so we will need to cut out those nucleotides and make a copy of the nucleotides from the other strand. These mutated cells don't need to be in the cycle.

related disorder –if this pathway is affected-
Unfortunately, in an autosomal recessive disease, if you lose one of the 4 or 5 the genes responsible for the nucleotide excision repair pathway, you will get xeroderma pigmentosum (pigmented dry skin).
Xeroderma pigmentosum patients are very sensitive to UV radiation because their nucleotide excision repair pathway, that repairs UV-induced DNA damage, is not active.
These patients frequently get various types of skin cancers because they're UV sensitive.



Notice how UV light cause crosslinking of pyrimidines , the Nucleotide excision repair pathway would fix this easily.
the picture below represent a Xeroderma pigmentosum patient , notice the skin pigmentation.

Homologous recombination repair pathway figure8

It's associated with DNA damage that's not limited to one strand but affects both strands of a gene (you have double-stranded breaks). How do you fix a double stranded break? You can use the other allele and start copying from it, this will result in:

A. Fully repaired allele that's is similar to the other allele.

B. Exchange of genetic material .

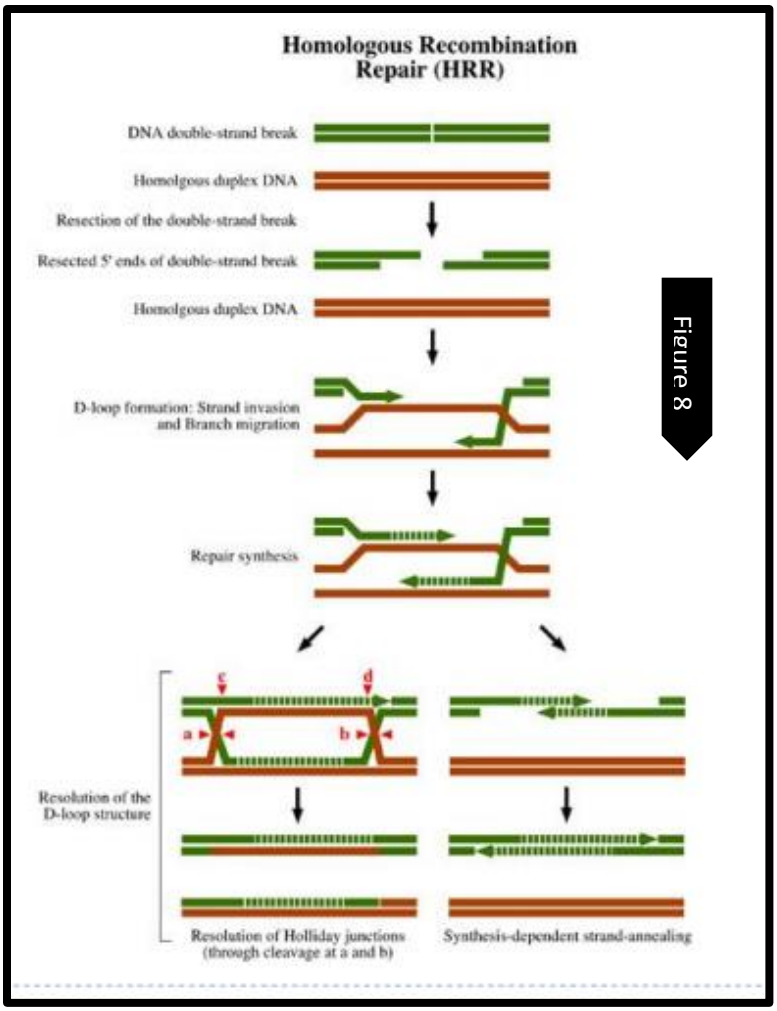


Figure 8

I don't Know if the exact mechanism is included.
you can watch this video

Related disorders –if this pathway is affected-:
Mutation of the homologous recombination repair pathway is considered as an autosomal recessive syndrome.(syndrome=more than one manifestation)
actually one of three diseases will result depending on which gene of the homologous recombination repair pathway is destructed.
The diseases are:
1. Bloom's syndrome: causes various cancers and development of abnormalities.
2. Ataxia-telangiectasia: causes various cancers and cerebral degeneration. The mutated protein responsible for this syndrome is directly responsible for p53 activity by affecting MDM2 and sending it off for degradation which will lead to the accumulation of p53. The accumulation of p53 will initiate DNA repair and if that doesn't happen, you'll end up inducing senescence or apoptosis.
3. Fanconi anemia: causes various tumors and anemia.

**There are 2 more DNA homologous repair proteins and they are BRCA1 and BRCA2. They are homologous repair proteins that are associated with familial breast cancer, unlike other DNA repair proteins they only occur in familial breast cancer but they do not occur sporadically

**There's a paragraph in the book that the doctor asked us to read it || **

Regulating genomic instability (recall what we have studied in genetics)

Which cells are we talking about when we mention regulating genomic instability?

Lymphoid cells..(antibodies producing cells)

again , let us over think ☺

There are 84 genes responsible for producing antibodies (fraction of these are genes of the antibodies their self and fraction for the T cells receptors proteins) yet we produce almost 10^16 various different types of antibodies. See figure9

so from only 84 genes we produce 10^16 type of antibodies, HOW? –by MIXING >>

1. For each gene you've got (V, J and D) regions and you end up recombining them to produce various forms.
2. Abnormalities: by translocations

related proteins to regulating genomic instability :

There are 3 proteins mentioned in the book that are responsible for regulating genomic instability RAG1 and RAG2 bring the V, D and J regions of DNA together, then they'll cut the interceding regions, then they join which ever variable diversity region you want to end up with in your immunoglobulin.

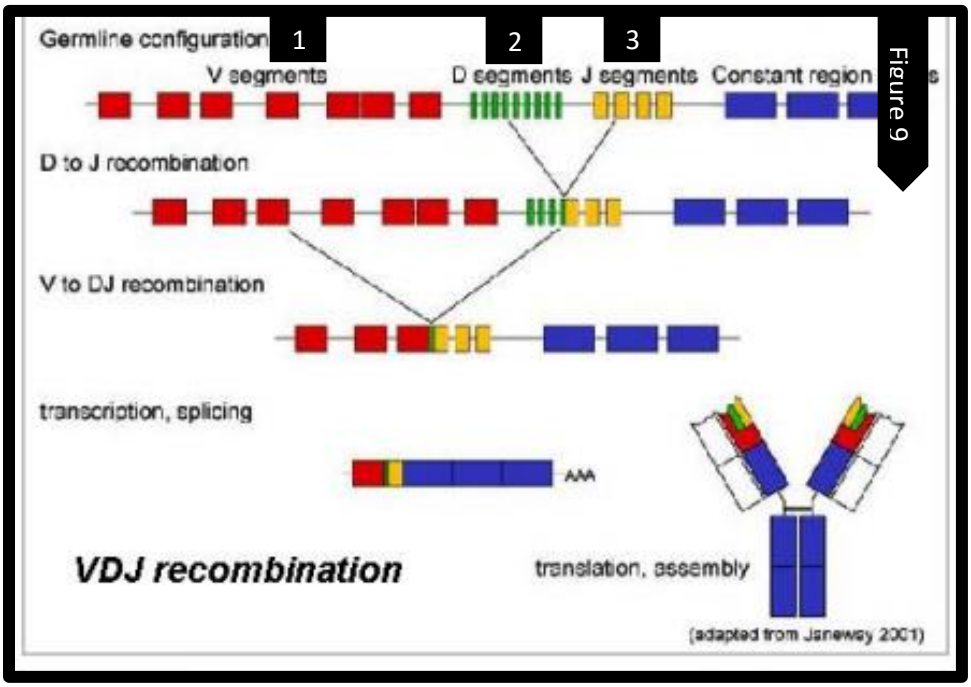


Figure 9

Notice the regions that are subjected to recombination
1: V rejoin
2: D rejoin
3: J rejoin

Activation induced cytosine deaminase

If you deaminate cytosine you'll end up with uracil; [remember: cytosine normally binds to guanine and uracil normally binds to thymine]. So you'll change a C-G pair to a U-T pair. This is used in your lymphoid cells to mature antibodies by increasing the affinity of the binding to the antigen which will lead to a better recognition leading to more activation. So you induce this enzyme to produce somatic hyper-

mutation in the gene responsible for producing that antibody.
And you end up with:
1. Antibody that is loosely bound: cells will lose the growth factor stimulation after binding to the antigen and those cells will die off.
2. Antibody that is tightly bound: better growth signals and you end up with a higher affinity antibody.

Note: If you inappropriately activate this enzyme, this will lead to lots of mutations causing lymphoid neoplasms because you're randomly changing C-G pairs to U-T pairs.

Tumors promoting inflammation

Inflammation is a physiological response that tend to kill pathogens, so Why would inflammation promote tumor?
Inflammation release "things" that may induce a bilateral damage, what are those "things"?

- ✓ ROS >>Free radicals= "BAD!"
- ✓ Growth factors induce proliferation and angiogenesis
- ✓ Survival factors inhibit apoptosis
- ✓ Inflammation will also produce mediators that induce chemotaxis, which will induce migration
  Those factors are bad for us and good for the cancer, which is why inflammation is frequently associated with cancer...

Again and again , let's overthink !

<u>which came first, inflammation or the tumor (chicken or egg issue)?</u>

- ✓ example when inflammation come first: (ulcerative colitis and colon cancer)
  Inflammatory bowel disease is a type of disease where you have recurrent inflammation in your intestines. One particular variation of this disease is called ulcerative colitis, meaning you have this type of inflammation in your colon for years and decades (recurrent). Unfortunately for those patients, because of the recurrent inflammation they have, they'll be at a much higher risk than the other normal part of the population to get colon cancer. In this case the inflammation came first and the tumor came second.
  other examples Barrett esophagus, H. pylori gastritis, HBV/HCV, & chronic pancreatitis
- ✓ when tumor come first:
  When you take a tumor that is not known to be associated with pre-existing inflammatory conditions and study it under a microscope, you will frequently find infiltrated immune cells and the same growth factors and cytokines that are associated with inflammation. So in this case it seems like the tumor itself has induced the WBCs to start the pathway for inflammation.

Why we consider inflammation and tumors associated?
Growth factors that are produced in inflammation and repair are the same growth factors that cancer will use, they are produced by lymphocytes, macrophages, storm all cells and parenchymal cells, this will induce cells to survive, proliferate and migrate.
Free radicals and reactive oxygen species will cause DNA damage and mutations, they will also cause

protein modification.
For example, tumor suppressors, PTEN can be modified because it has sulfhydryl groups and PTEN inhibits PI3 kinase/Akt thereby if you inhibit PTEN (on a protein level) you are releasing PI3 kinase/Akt signaling which will induce proliferation thus increasing glucose uptake and aerobic glycolysis.
Note : Inflammation induce cox-2 and this serve carcinogenic process A LOT..

Cancer is a multi-step process

So all the hallmarks come in steps not at once.
famous example colon cancer figure10
first: u will Lose APC gene/protein : APC is responsible for B-catenin which down-regulates E-cadherin, so the loss of APC will lead to more mobility.
secoundly: Loss of DNA methylation: turns on telomerase and turns off various tumor suppressors
then: Mutation of RAS: induces proliferation
after that: Loss of a tumor suppressors such as DPC4 or DCC this induces proliferation
finally : Loss of p53(the big boss) you end up with a carcinoma.

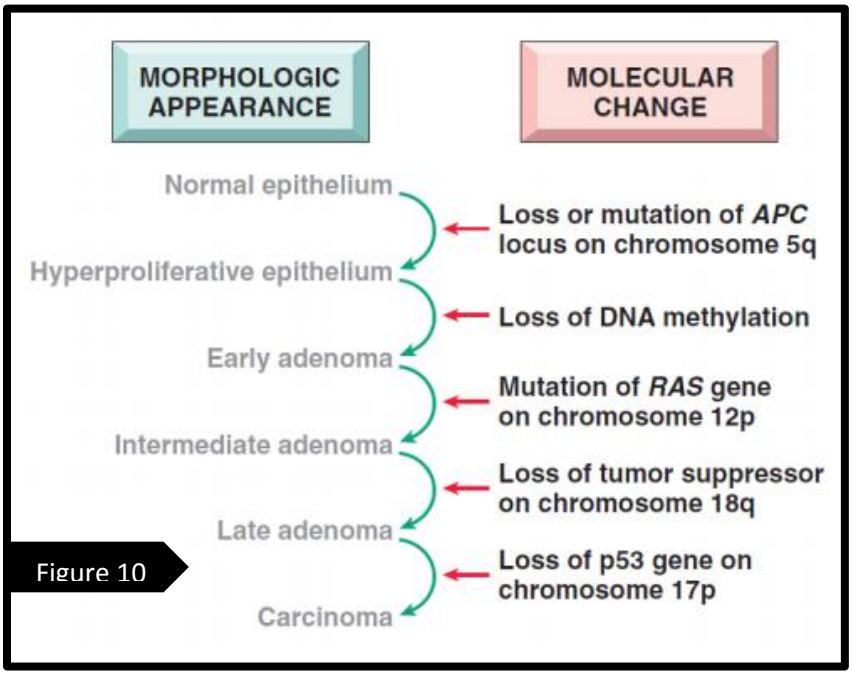
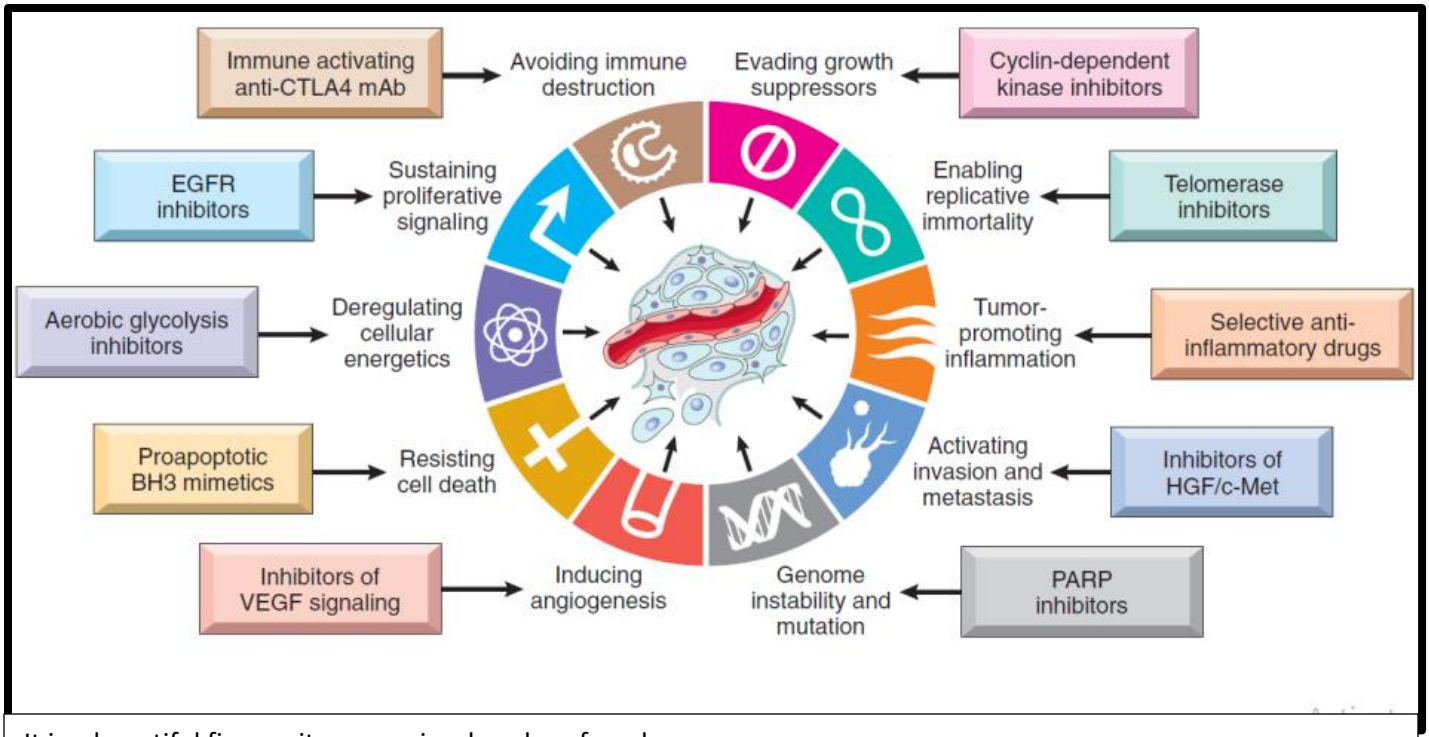


Figure 10

by this we concluded the hallmarks of cancer , but in medicine we always think applicably , how can we benefit from this Knowledge >>we cure cancer ! figure11



It is a beautiful figure.. it summarize decades of work.

Problem	Solution
Evading growth suppressors	Cyclic dependent kinase inhibitors
Activated telomerase	Telomerase inhibitors
Inflammation	Certain anti-inflammatory drugs such as cox-2 inhibitors (used in lung and colon cancers) Cox-1: responsible for mucosal well being Cox-2: responsible for inhibition of apoptosis in sites of inflammation
Glioblastoma	Inhibitors of HGF (produced by stromal cells in the brain) by blockage of c-Met or stopping the production of HGF
Genome instability (BRCA1 and BRCA2)	PARP inhibitors
Angiogenesis	Inhibition of VEGF signaling

END OF TEXT

Now revise yourself with this clip from the book.

SUMMARY

Genomic Instability as Enabler of Malignancy

- Persons with inherited mutations of genes involved in DNA repair systems are at greatly increased risk for the development of cancer.
- Patients with HNPCC syndrome have defects in the mismatch repair system, leading to development of carcinomas of the colon. These patients' genomes show MSI, characterized by changes in length of short tandem repeating sequences throughout the genome.
- Patients with xeroderma pigmentosum have a defect in the nucleotide excision repair pathway and are at increased risk for the development of cancers of the skin exposed to UV light, because of an inability to repair pyrimidine dimers.
- Syndromes involving defects in the homologous recombination DNA repair system constitute a group of disorders—Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia—that are characterized by hypersensitivity to DNA-damaging agents, such as ionizing radiation. *BRCA1* and *BRCA2,* which are mutated in familial breast cancers, are involved in DNA repair.
- Mutations incurred in lymphoid cells expressing gene products that induce genomic instability (RAG1, RAG2, AID) are important causes of lymphoid neoplasms.