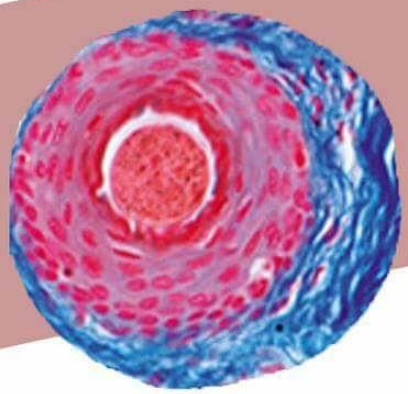
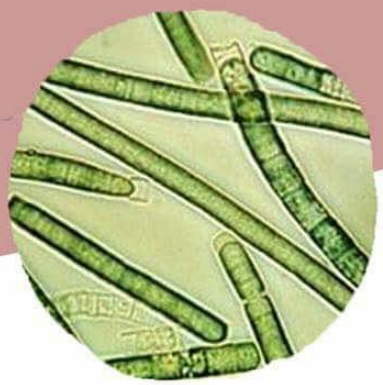
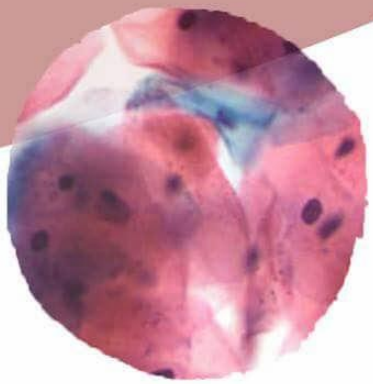
INTRODUCTION TO PATHOLOGY

Done by Obada Zalat

Corrected by Sufian Alhafez

Sheet# 21

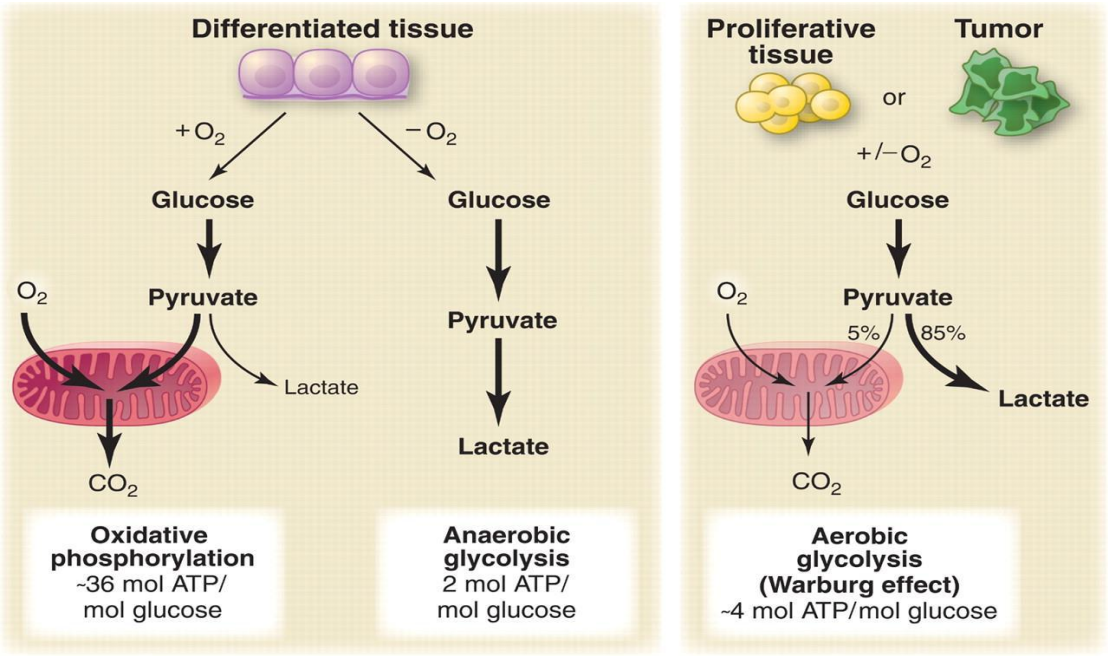
The hallmarks of cancer

❖ Reprogramming energy metabolism

-Even if there is plenty of O2 a shift occurs to glycolysis, this is called Warburg effect (aerobic glycolysis), embryonic and cancer cells use aerobic glycolysis in order to divide too fast since aerobic glycolysis provides pyruvate which is used as a carbon backbone to build lipids and other metabolites needed for nucleic acid synthesis so there will be increase in glucose uptake , also we will see increase in amino acids uptake to build proteins(remember that actively dividing cells must double their DNA, proteins, membranes and organelles before division).

-In normal differentiated adult cells, when O2 is present glucose is converted to pyruvate, which will be further oxidized, and we will have 36 ATP per Glucose molecule by oxidative phosphorylation, and if there is no O2 anaerobic glycolysis will be active and we will have 2 ATP per Glucose molecule , but in embryonic and cancer cells which use aerobic glycolysis, less ATP than that in oxidative phosphorylation will be produced (the exact number of ATP is not important, some books mention 2 ATP others mention 4 ATP per Glucose),so aerobic glycolysis is less efficient than oxidative phosphoylation, and to compensate for the reduced number of ATP molecules produced per Glucose molecule through aerobic glycolysis, cancer cells increase their uptake of glucose so they manifest

"glucose hunger".



-What is the role of oncogenes and tumor suppressor genes in aerobic glycolysis ?

Growth factors such as PDGF and EGF stimulate Akt (an intermediary in RAS signaling) and affect Pi3 kinase and that result in enhancement of glycolysis and increase in glucose uptake as a result of increasing glucose transporter proteins in the membrane of the cell, they also inhibit pyruvate from going down through oxidative phosphorylation pathway. Tumor suppressors like PTEN and TP53 reduce glucouse uptake, and cause reduction in aerobic glycolysis.

*The book mentions(page 196) that tumor suppressors stimulate aerobic glycolysis , but the right sentence is: **mutated** tumor suppressors stimulate aerobic glycolysis.

-Cancer cells are in “glucose hunger” state, so we can benefit from that by injecting the patient with non-metabolized

derivative of glucose (fluorodeoxyglucose), and observe what tissues will take it extensively and rapidly to detect tumor cells, this method is known as Positron Emission Tomography (PET).When we apply PET to the patient, there will be aggregation of glucose in the brain and bladder NOT for cancer reasons, but why?

-the fluorodeoxyglucose is a non-metabolized derivative of glucose, so it will be excreted by the bladder. Brain is a glucose hungry tissue without having a tumor. So we cannot use this method to diagnose tumors in these areas.

❖ Evasion of the Immune system

-Immunosuppressed patients have a higher risk for developing cancer, this is because when any transformed cell is detected by the immune system it will be destroyed, and this doesn't happen in immunosuppressant patients. That means, for cancer to show up in immunocompetent patients, it needs to avoid detection by the immune system of these patients.

-There is a higher percentage of cancer patients who are immunocompetent than those who are immunosuppressed, and this is because immunosuppressed patients represent a small portion of the whole population, even though they have high risk and (relatively) high incidence of cancer.

-There are several mechanisms as to how cancer cells can avoid the immune system:

-first, your cell normally has a surface protein called MHC (Major histocompatibility complex) or HLA (human leukocyte antigen), this protein presents bits and pieces of proteins from inside the cell on the surface, if an immune cell (in this case "T

cell") comes for this cell and reads the antigen of that bit protein and finds that this bit of protein is a normal protein , it will ignore the cell and go away, if it finds a protein that is mutated or viral protein (a protein that it has never seen before), it will recognize the cell as an abnormal cell and it will kill it.

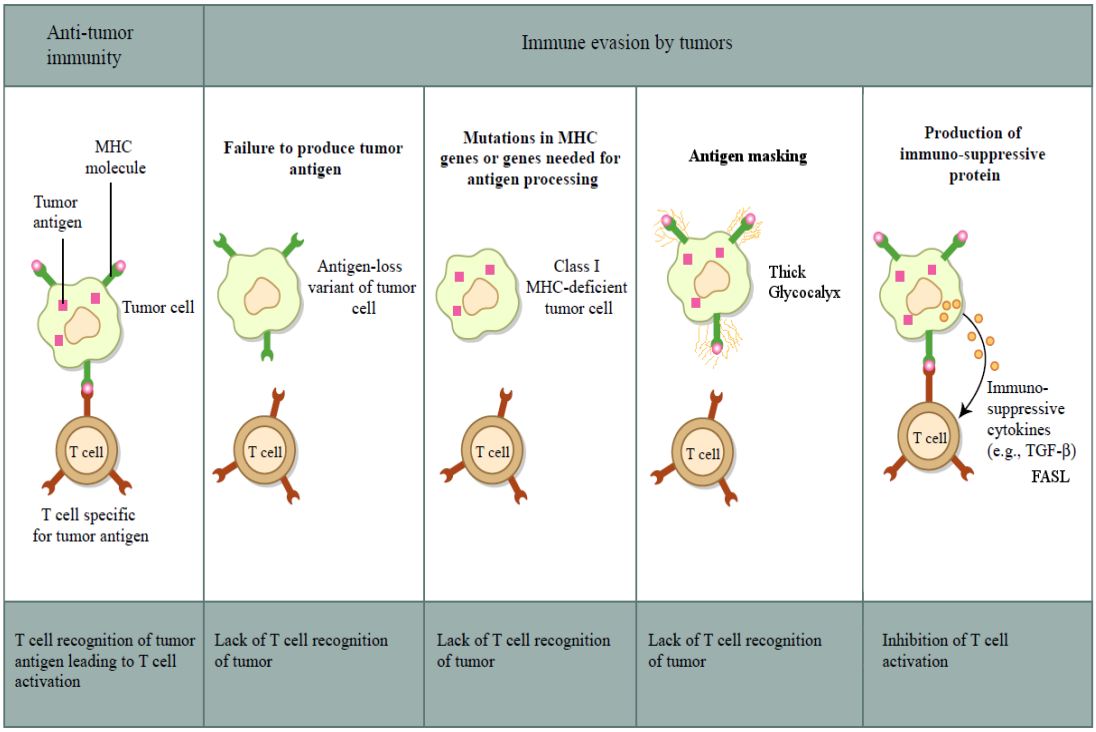
So based on that, what can cancer cells do to evade detection by T cells?

1-they could lose the antigen and no longer present it on MHC, T cells will kill tumor cells that produce an antigen and the cells that lose it will not be killed and this is an example of natural selection.

2-Mutation in MHC molecule, so cells no longer can be detected by T cells (there is another type of T cells that can detect this type of cancer cell "will be discussed later")

3-Antigen masking, some cells can produce glycoproteins (branched sugars connected to proteins, they are present on the surface of the cell), but some cancer cells produce excessive amount of these glycoprotein that they mask surface protein (surface receptors including MHC-proteins), therefore even if MHC is presenting an abnormal antigen or a foreign antigen, it cannot be detected by T cells.

4-Some cancer cells can produce immunosuppressive proteins such as FASL which induces apoptosis in T cells and therefore they kill T cells before the T cells can kill them, also although TGB-b in early stages of cancer inhibits the growth, later on cancer cells will produce TGF-b in order to inhibit T cells.



❖ Genomic instability

-Bridge-fusion break cycle is an example of genomic instability that we discussed in the previous lecture.

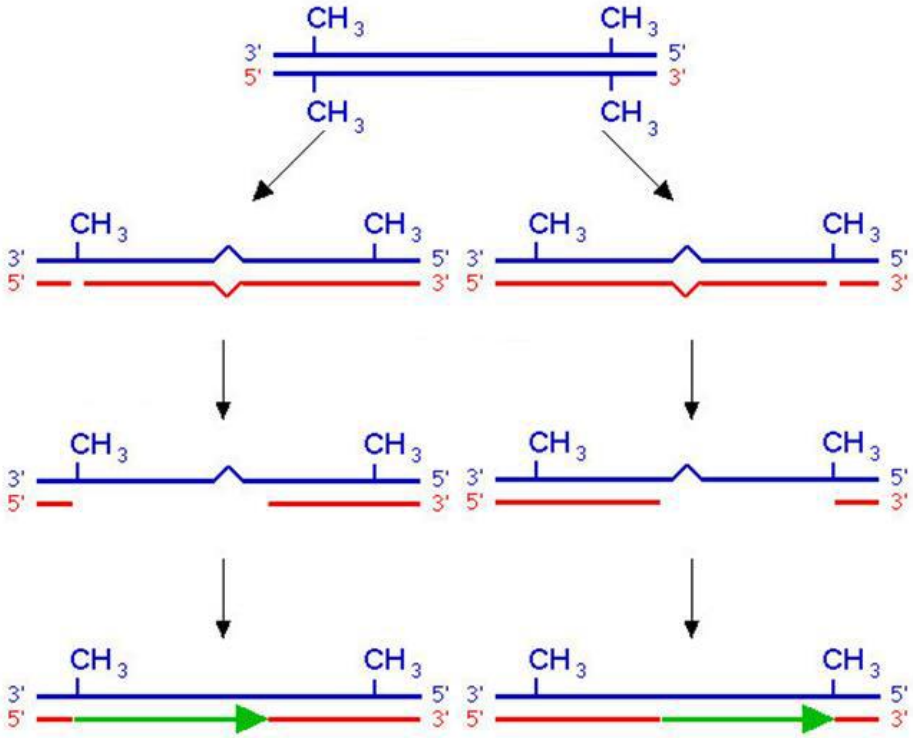
-If the cell has a mutation in P53 then it will have genomic instability, because P53 induces DNA repair in the cell, so mutations in any DNA repair pathway will induce genomic instability.

-There are multiple repair responses to DNA damage, we have mismatch repair pathway, recombination repair pathway and nucleotide-excision pathway. So let's talk about these pathways:

1) Mismatch repair

-It is active during replication of DNA, so as we know, in DNA replication you have the original strands, you separate the strands and make a copy of the strands, at some point there could be an error in that copy where G isn't matched with C or A isn't matched with T, and to repair this you end up removing the

mismatchednucleotide, and inserting the proper nucleotide. But how do these enzymes know which nucleotide to remove (how do they know which is the original strand and which is the new strand)?
Methylation of the DNA is hereditable, so when the cell separates the two parent strands, they retain the methyl groups, and when you copy the other strand, the new strand doesn't have the methyl groups yet, so the methyl groups identify the parental strand, and nucleotides that don't match up with the parental strand will be considered abnormal and will be removed.



-IF you have a mutation in one of the genes of the mismatch repair pathway, you end up with (among other diseases) a disease called HNPCC (hereditary Nonpolyposis Colon Cancer).

- HNPCC is an inherited cancer disease without polyps formation, patients with this disease also known as patients with lynch syndrome, have DNA mismatch repair pathway defects and they end up with a mutator

phenotype. In particular in the colon when they lose the second copy of the DNA repair gene (so mismatches will not be repaired), they end up with characteristic mutations such as TGF-b receptor II mutation and BAX mutation(Just because the environmental toxins in the colon predispose these particular types of mutations). Even though the syndrome is an autosomal dominant syndrome, the patient will get the tumor after losing the second copy, but this doesn't mean that you must lose both copies in all DNA repair genes to have a cancer, some DNA repair genes function in a haploinsufficienct manner where the loss of one copy is enough to promote cancer.

-Mismatch repair genes are responsible for maintaining your microsatellites. Microsatellites are one to six DNA nucleotide repeats, and certain number of repeats is unique for each individual, and the location of these repeats is unique for each individual. These repeats give us DNA finger print, no two people have identical DNA finger print, this print is very useful in crimes (who killed whom for example) by microsatellites investigation (check how many microsatellites, where are they and check more than one location to make sure that you identify the person correctly) , but this method is not
useful when you have a person who has DNA mismatch repair pathway abnormality and a person who has HNPCC syndrome (lynch syndrome), their microsatellites are unstable, because they are not being maintained by the proper pathway, if he has a finger print today…few months later he will have a different print.

-These patients with HNPCC (unlike patients who get polyps like FAP (Familial Adenomatous Polyposis) patients or sporadic tumors who have a predilection to get a left

colon cancer) are more likely to get tumor in the right side of the colon.

2) Nucleotide Excision repair

-this pathway is important in removing pyrimidine bridges that occur because of UV radiation, so you can expect somebody who has mutated nucleotide excision repair pathway to be sensitive to UV, which means these patients even with normal exposure levels to UV will end up with skin cancers.

-These patients have a disease called Xeroderma pigmentosum (Xero=dry, derma=skin and pigmentosum=pigmented; due to the damage and the inflammatory reactions that can occur), these patients frequently develop skin cancer due to sun exposure, because UV light will cause pyridimine dimers and cross links either on the same strand or between the two strands.

3) Homologous recombination repair

-This is different from bridge-fusion pathway that we talked about.

-These patients (who have a mutation in this pathway of repair) could have various autosomal recessive syndromes such as Bloom syndrome, ataxia-telangiectasia (mutation in ATM) and fanconi anemia.

- The whole point of homologous recombination is that if you have a double stranded break on one chromosome and you don't know how the damage has occurred, your cell doesn't know how to fix it, so to fix this break you go to the second chromosome, and read from the second homologous copy of that chromosome, to recreate the

damage on the first chromosome, so you end up with re-combination event, where you mix between two chromosomes, and you copy one to the other to get a properly functional chromosome without mutations.

-as mentioned previously, If a patient has an abnormality in homologous re-combination repair pathway, he can get one of these three syndromes (Bloom syndrome, ataxia-telangiectasia (mutation in ATM) and fanconi anemia), based on which gene is mutated.

-The phenotype in patients with these disorders is complex and includes, in addition to predisposition to cancer, certain feature, for example: Bloom syndrome patients have developmental defects, in Fanconi anemia they have anemia, in ataxia-telangiectasia they have cerebral degeneration. So mutations in each one of these genes (in addition to inducing a mutator phenotype and cancer) will induce a particular characteristic that identifies these patients.

**There are two proteins BRCA1 and BRCA2 …..the doctor said we have to study the paragraph (page 197) that talks about these proteins….especially to know the answer to these questions:
1-how do they relate to breast cancer?
2-how are these particular genes different from other genes in relation to how they produce cancer?

**Also the doctor said that you have to know what is the role of PARP inhibitors in cancer in relation with BRAC1 and BRAC2.

***There may be a question in the final exam about* these proteins**

- patients with Fanconi anemia, in addition to having anemia and mutations that predispose them to tumors, are also sensitive to DNA cross-linking agents such as nitrogen mustard and alkylating agents (alkylating agents can be used as warfare agents and can be used as chemotherapeutic agents) so we can't treat Fanconi anemia patients who have a tumor by these alkylating agents because you could end up making things worse.

#The last vestige of genomic instability: Lymphoid neoplasms

-There are 84 genes responsible for producing antibodies, yet we produce 10^16 different types of antibodies, that happens due to recombination events (genomic rearrangement), and this done by two proteins RAG1 and RAG2, they responsible for bringing those DNA strands together and cutting and removing section that you don't need to create antibodies (VDJ recombination), so you can imagine if you have mutations in RAG1 and RAG2, you could get various forms of genomic instability.
Additionally, there is another enzyme called activation-induced cytosine deaminase (AID), when a B cell is activated (after encountering an antigen) there will be activation of cytosine deaminase (AID), this enzyme's whole purpose is to induce hypermutations, it changes cytosine with Uracil , so you exchange G-C base with U-A base, lymphoid cells do that in order to mature the immunoglobulins and to switch between Igm and Igg.
Also, this mechanism is used to improve the function of the antibody(Remember that the first binding between antibody and antigen is somehow weak), as the B cell matures the binding becomes stronger, so this enzyme(AID) is involved in changing the antibody from

weak binder to a very good binder, but this is not a directed process, it is a random mutagenesis (natural selection); if the mutations in some cells cause the antibody to become less attached to the antigen, these cells will no longer be activated and no longer be stimulated, at the end they will die off. Cells that receive mutations that make antibody bind better to the antigen will receive a growth stimulatory signal and will be naturally selected.

-If you have inappropriate activation of AID in cells (lymphoid cells or other cells) you will have mutations.

❖ Tumor-promoting inflammation

-who comes first inflammation or tumor?
-actually, the two are correct.

-If you have inflammation, you induce angiogenesis, fibroblast, fibrosis, cytokines, FGF, PDGF, and ECM degradation products and everything we took in the past which can be used by the cancer cells for their own purposes such as growth, changing differentiation and metastasis. So we can have inflammation that results in stimulation of growth and we end up with a cancer.

-If you have a mutation first that results in growth and then these cells induce stroma to think that there is an inflammatory reaction going on and so it will recruit white blood cells and then the cancer cells use those cytokines,.,..ect (that are produced from inflammatory cells) for its own purpose.

As it turns out you have various inflammatory conditions that can induce cancer such as: Barrett's esophagus (when a patient has a heart burn, the acid reflux causes inflammation and metaplasia and this is a fertile soil for cancer), ulcerative colitis (it is one of two types of inflammatory bowel disease, the recurrent inflammation in the bowel is an independent risk factor for getting colorectal cancer, so these patients have high incidence of colorectal cancer), *H.pylori* gastritis (an ulcer in the stomach which can induce stomach cancer) , hepatitis B and C (these viruses damage the liver and the attempts of the liver to fix the damage, through inflammation, proliferation, could induce liver cancer) and chronic pancreatitis; due to alcohol consumption (could lead to pancreatic cancer).

- However, In tumors that we don't know previous inflammatory reactions that induce them, we will find inflammatory cells infiltrating the tumor, even though the patient has no symptoms and we don't know that the patient has inflammation, but where did they come from and how did the tumor bring them?, one of the inflammatory mediators is called COX-2 (produces prostaglandins), as it turns out more than 80% of colorectal cancers over express COX-2, inhibition of COX-2 is an active area of research to prevent colorectal and lung cancers.

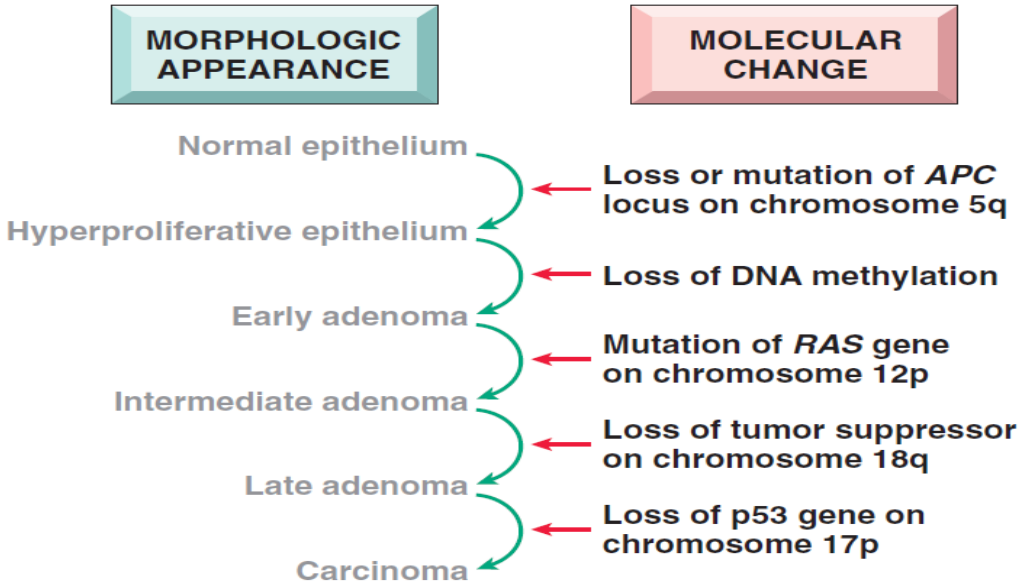
-Growth factors that are produced from inflammatory cells induce cells to survive, proliferate, migrate, change their differentiation, and induce proliferation through promotion of cell cycle entry (through over expression of cyclins), relieve cell cycle blocks(through affecting Rb,TGF-b…), inhibit apoptosis, and induce protein synthesis.

-Other than growth factors, inflammatory cells can produce free radicals, which can cause DNA damage and mutations.

❖ Multistep carcinogenesis

-you can notice that in these hallmarks of cancer, one hallmark leads to the other and they may not all be present at the same time, but you will frequently find a combination of these hallmarks in various cancers. This tells you that carcinogenesis is a multistep process.

-For example, in colorectal cancer, you start with a mutation in APC this is also followed by loss in the DNA methylation (epigenetic effect), and then you have an activating mutation in RAS pathway, and you could lose a Tumor suppresser on chromosome 18 (affecting TGB-b pathway) and finally you get loss in p53 that enhances mutation, these are well documented events in the progression from a polyp to a tumor in colorectal cancer.



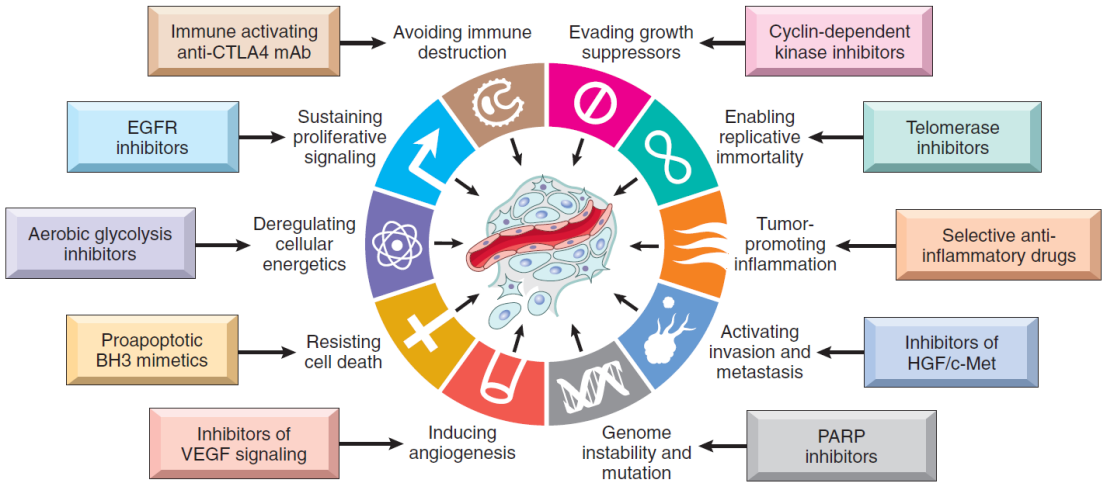
#Why do we care about the hallmarks?

Because every single hallmark has produced an anti-tumor drug that specifically targets that molecular change.

*this is list of anti cancer drug…..> the target molecular change

1-PARP inhibitors, they target Genome instability.

2-inhibitors of HGF/c-Met(HGFR), they target invasion and metastasis hallmark.
3-selective anti-inflammatory drugs(COX-2 inhibitors), they target tumor-promoting inflammation.
4-tolemarase inhibitors(if we inhabit telomerase you push the cancer into mitotic catastrophe), they target Enabling replicative immortality hallmark.
5-CDKI (CDK4 is one of the 4 major mutated genes in cancer so inhibiting it could prevent cancer), they target evading growth suppressors hallmark.
6-immune activating CTLA4 mAb, they target avoiding immune destruction hallmark.
7- EGFR inhibitors, they target sustaining proliferative signaling hallmark.
8-aerobic glycolsis inhibitors (turn off Warburg effect), they target deregulating cellular energetics hallmark.
9-pro-apoptic BH3 mimetics (induce apoptosis and kill the cancer), they target resisting cell death hallmark.
10-inhabitors of VEGF signaling, they target inducing angiogenisis hallmark.



The end of the hallmarks of cancer

"Life is short. Smile while you still have teeth"