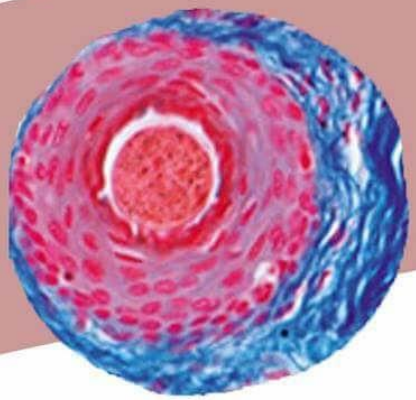
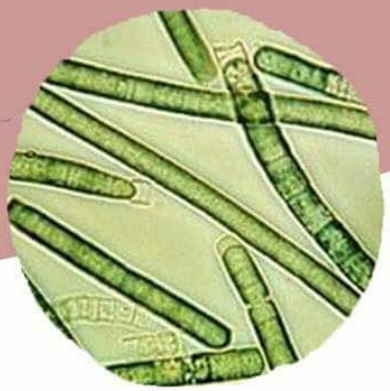
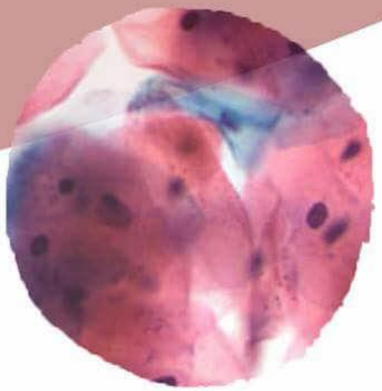
INTRODUCTION TO PATHOLOGY

Done by Renad Zakaria

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Sheet# 18

**This sheet was written from records, book and please refer to Dr. Main & Dr. Heyam's slides .(although its large sheet but with little effort it will be easy)

In the previous lecture we discussed that cancer starts as clonal some so cell gain the ability to invade, escape the immune system, metastasize and to stimulate angiogenesis… so with time it reach the final tumor that you actually excise and when it become malignant it will be very heterogeneous, based on that, the longer tumor grows it will be harder to treat; because of the mutations that occur with time. So the earlier you discover it the best treat you can provide.

The body puts a selection pressure on cancer and with the natural selection these cells can have a blood supply that changes their metabolism, same as angiogenesis. also response to natural selection…

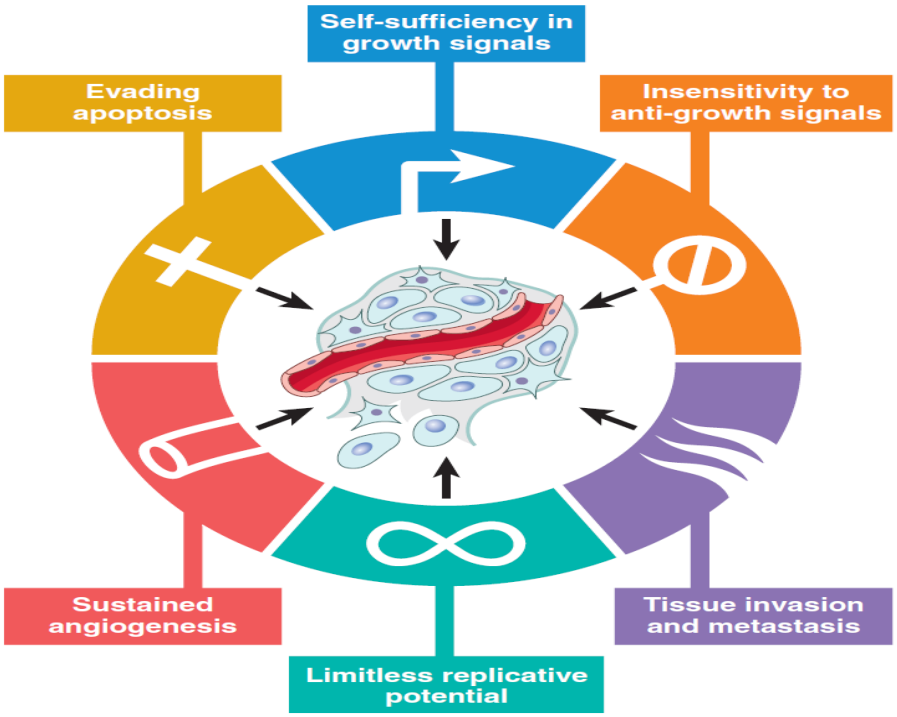
There is also immunologic selection pressure your T cells recognize abnormal tumor cells. some can't be recognized, so they will survive and evade the immune system and grow.

So after this explanation lets go back to the chemotherapy...When we give a drug to a cell that can resist it, it will survive,,,after the treatment, the tumor will regrow (recurrence), and then tumor become more aggressive and resistant to the drug and become fatal.

so what make these cancer cells become so aggressive and what characteristic they gain over time to give them the ability to grow, metastasize, resist drugs..etc ?!

this discovered before about 16 years by Douglas Hanahan and Robert Weinberg as the original hallmarks of cancer all that time they think that parenchyma the only that take place in caner, so you will notice five of the six hallmarks of the cancer are cancer parenchyma gain and completely independent of the stroma.

So what are they?

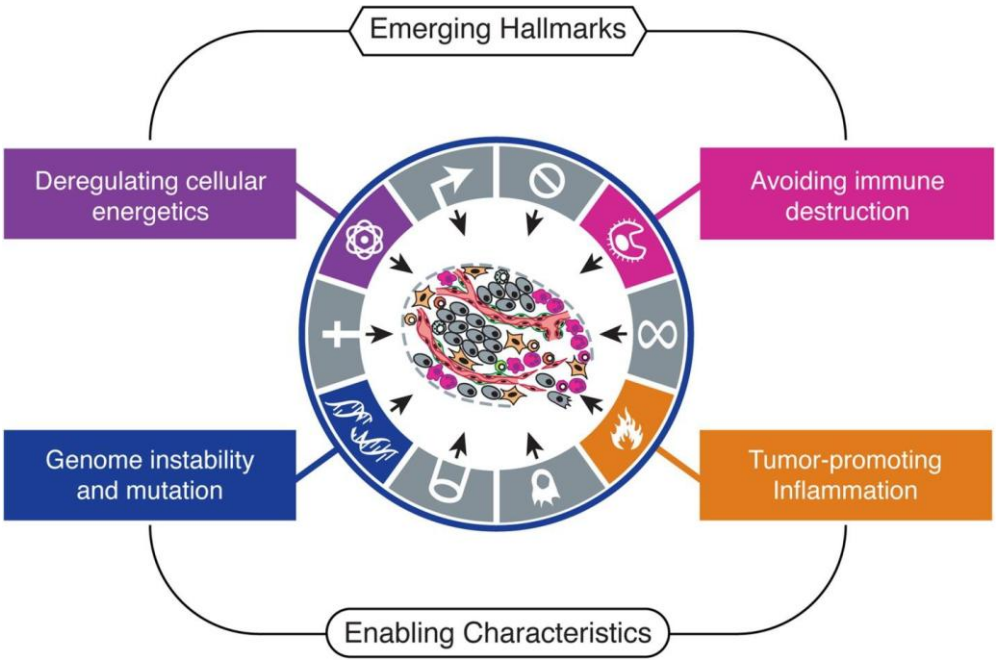


Follow the colors

- Tumor cell no longer require growth factor or produce its own to grow.
- Turning off pathway that induce senescence, turning off retinoblastoma, changing the ability of cell cycle to stop and turning off P53.
- They thought that's its function of tumor cell, nothing in stroma.
- Here we talk mainly about reactivation of telomerase.
- "Sustained angiogenesis" The only hallmark ''back then" concede for stroma to may have role in carcinogenesis by providing nutrients.

- Turning off P53 and intrinsic pathways.

In 2011 they revise and added 4 new hallmarks and they included: stroma's involvement, and included the immune system, and the relationship between inflammation and cancer, (look at the picture to know them we will discuss in future).



In the next few lectures we will discuss the Hallmarks of cancer :P

The first hallmark: Self-sufficiency in growth factors:

At first, how do cells grow?

Growth factor bind the **receptor** turning it on, then the receptor transmits the signal inside the cell through **transduction molecules** (they can be 2nd messenger, phosphorylated protein, attached to G protein). Finally these

signals transmit to the nucleus they affect **transcription factor** that turn on/off certain gene.

To make it easier; The elements that are involved in this hallmark are:

***1**-Growth factors (ligands) **2**-Receptor (transient activation) **3**-signal transducers **4**-Transcription factors **5**-The control of the cell cycle.*

Also, compare between the normal and the abnormal state.

1-Growth factor:

To achieve cell sufficiency through it:

a) Cells normally signal through paracrine effect, cell rarely produce its own growth factor and has a receptor for it (autocrine effect), otherwise it will end up with ***autocrine positive feedback loop*** (cell produces the GF and its receptor; more ligand production, more binding to the receptor so more activation).
-this feedback occurs for example in: Glioblastoma can produce PDGF and its receptor (remember it stimulates migration and proliferation in inflammation and repair). Sarcomas also produce TGFα and its receptor.

B) Abnormal interaction with stroma: In some cases, tumor cells send signals to activate normal cells in the supporting stroma, which in turn produce growth factors that promote tumor growth and here it called permissive stroma.

C) In some cases over express of growth factors takes place.

2- Receptors:

The receptor when receives its ligand, it turns on then eventually it will turn off, so we need mutations or over expression to keep it active:

A) *Mutation*: some point mutations can occur making the receptor independent of ligand (receptor is constitutively active) one of these receptor may be retinoblastoma receptor
example: EGF receptor mutations in colon/lung cancer. EGFR: Epidermal Growth Factor Receptor.

B) *Over expression:*
-- more common that mutations.
– Over expression of receptors will lead to massive signaling inside the cell (the cell will becomes hyper responsive to growth factors).
Examples: - ERBB1 (EGFR) in SCC of the lungs.
SCC: Squamous Cell Carcinoma
- ERBB2 (HER2/NEU) in breast cancer and adenocarcinomas of the lung, ovary and salivary glands.

*Note: Now, these receptors can be targeted by specific antibodies and their extracellular domains can be blocked. This is important in treating breast cancer.

3-signal transducers:

They transmit the signals from the activated receptors to the nucleus, initiating DNA transcription (they turn on/off certain genes). We will discuss two important members of this category:

1- RAS. 2- ABL.

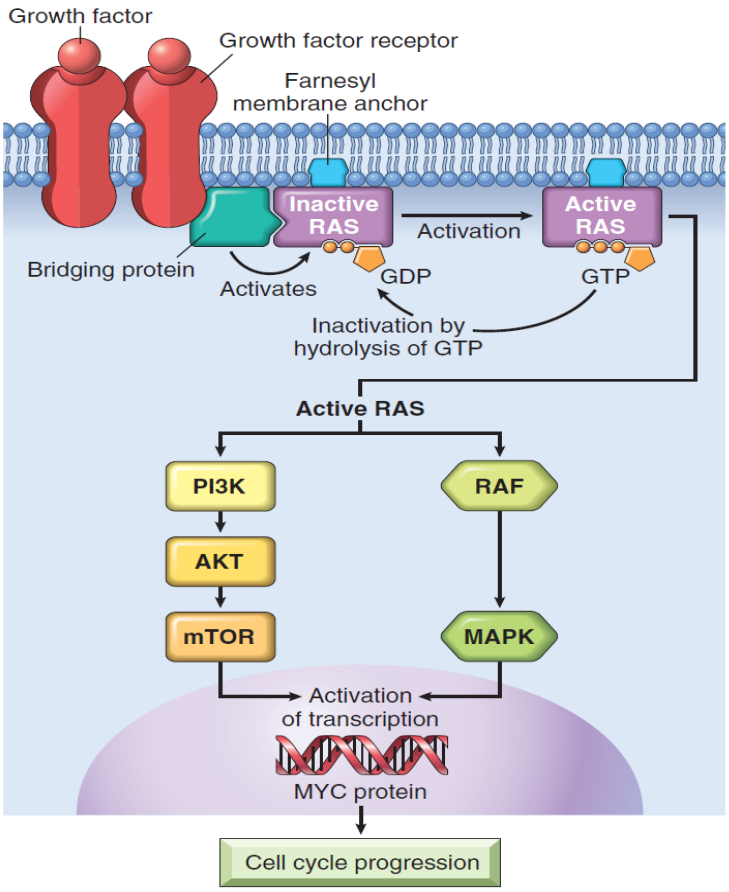
A) RAS : small G protein, it is a receptor associated signal transducer, active when bound to GTP, and inactive when bound to GDP *(from GTP to GDP (inactivation) by the action of GTPase).*
Activating RAS will activate downstream pathways (not to memorize), cause change in the transcription of certain genes, Particularly; MYC, which stimulates cell cycle progression (proliferation).

**so if you want it be more active where you predict the mutations?

1- Point mutation in GTP binding pocket make RAS always bound to GTP and constantly active.

2- Point mutation in hydrolytic pocket make it off, so GTP remains bound.

3-GAPs: “G protein associated proteins” they are responsible for the enhancement of the GTPase activity of RAS and prevention of uncontrolled RAS activity “hydrolyze GTP to GDP” (remember NF1 is one of them we took in 1st lecture) so they must be inhibited by some mutations to induce MYC to induce proliferation.
4- Mutations may occur at the downstream that related to RAS, RAF, and PI3K.
,,,most commonly proto-oncogenes mutated in all tumors is RAS.



The result of all these mutations is:

Continuous activation of RAS

↓

continuous expression of MYC

↓

continuous proliferation

- This continuous proliferation means that the cell is self-sufficient in growth signals, which is a hallmark of cancer.

**does turning on proliferation make tumor??
NO, it needs a lot of mutations to occur like mutation in tumor suppressor and turning off cell death….. (One mutation doesn't produce cancer).

B) ABL:

-It's a non-receptor associated tyrosine kinase, which can function as a signal transduction molecule.
-In contrast, RAS is associated with a receptor, but ABL can activate the downstream RAS.
**ABL proto-oncogene has tyrosine kinase activity, which is inhibited by a regulatory domain…….. A translocation of the ABL gene from its normal position on chromosome 9 to 22, where it fuses with RCB gene……. Now ABL has no regulatory domain, and the RCB-ABL fusion protein causes Constitutive tyrosine kinase activity.*
**The cross-talk between RCB-ABL and RAS is that RCB-ABL protein activates all of the signals that are downstream of RAS.*

Because it's a new fusion protein; we can produce a drug that targets tyrosine kinase; which is Imatinib (Gleevec) ….. *(RCB-ABL kinase inhibitor)*

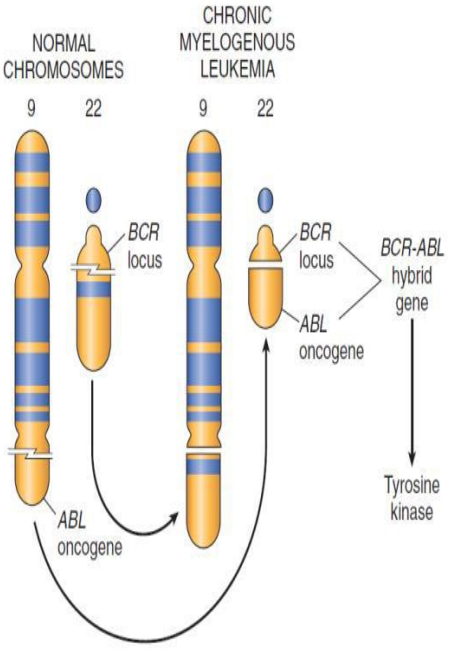
> Antibody for the BCR-ABL complex which blocks ATP binding site on BCR-ABL and prevents phosphorylation of the signaling molecules.

in the past not all respond ideally to this drug because one mutation can't produce a tumor, all tumor addicted to oncogenic pathways it's like circus tent kinase present at the middle (it's the supportive one, it must by mutated in the majority of tumor but also not enough to cause a tumor alone) and at

the periphery the supportive mutations like evade apoptosis and immune system … etc(they needed to cause tumor but alone can't induce a cancer) There are a percentage of patients who have ABL mutation after treatment recurrence occur and a resistant because when they treated a selective pressure added so some mutation escape and causes resistant by targeting the binding area of the drug on ABL/BCR protein (I think you know this by now)

**so ABL/BCR is an example of the concept oncogene addiction

-Oncogene addiction means that the tumor is profoundly dependent on a single signaling molecule to proliferate.
- In chronic myelogenous leukemia, although the development of cancer requires many mutations but the transformed cell continues to depend on the BCR-ABL proteins to proliferate and to survive.



The picture above show how the mutation (translocation, review last lecture) occur to produce tyrosine kinase.

The doctor asked a question (just mental storm question):

Why we shutdown pathways to get cancer?

1-because not all tumor be maximally addicted to one major mutation.

2-if you give a drug at its maximal dose you except side effect and if not respond you give more drugs so more side effects (that's a way for cancer to survive through activate multi pathways so multi drugs and multi side effect)

4-Transcription factors:

Factors that turn on/off transcriptional genes, each factor has set of targets. The one we will discuss is MYC:

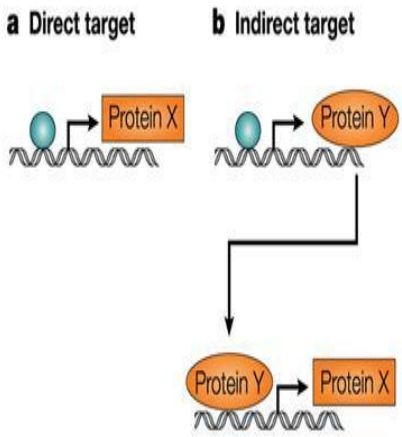
MYC: it can activate OR repress genes that are involved in cell cycle.
1-it can activate cyclins and cyclins dependent kinases (CDKs), inhibits some cyclins dependent kinase inhibitors (CDKIs), all of these result in proliferation.

2-it can affect DNA metabolism and energy metabolism.

3-its turn on aerobic glycolysis  what glycolysis??!! Is the cancer cell needs pyruvate to go through TCA and ETC to produce energy!! The answer is NO, cancer cells replicate so they need carbon-backbone to build themselves, that glycolysis provides (it looks like the mechanism that embryo uses to have a fast growth from one cell)

- *MYC transcription factor have two kinds of targeting:*
[A] - Direct targeting: when MYC binds to a promoter and stimulates the transcription of a certain gene.
[B] - Indirect targeting: when MYC binds to a promoter of a gene,

producing a protein that will act as a transcription factor targeting another gene.



Remember: MYC can be over expressed Burkitt lymphoma through a translocation between chromosomes 8 and 14. This occurs because in chromosome 14, we have the immunoglobulin heavy chain gene that has an active promoter. And when you place the MYC gene in front of that very active promoter, MYC will be over expressed.

- MYC is also amplified in breast, colon, lung and many other cancers.
- NMYC and LMYC genes are amplified in neuroblastomas and small cell cancers of the lung, respectively.

5- Cyclin and cyclin dependent kinase:

We will start our discussion with the normal cell cycle, then we will see how a mutation in one of cell-cycle-regulating proteins will lead to insufficiency of growth signals (which is the hallmark of cancer that we are talking about).
- The cell cycle consists of the following phases: G0 / G1 / S / G2 / M.
What tells the cell when to proceed from one phase into another?
(Mainly) Growth factors (G0 to G1).

The cell cycle has multiple checkpoints:
Why does the cell cycle have such checkpoints?
Because of its important role in maintaining tissue homeostasis and regulating physiological growth processes, such as regeneration and

repair. Also, any problem in the cell cycle can have disastrous effects (e.g.: malignant transformation), so the cell cycle must be tightly and strictly controlled. - The purpose of these checkpoints is to make sure that cells with damaged DNA or chromosomes don't complete replication.

- There's a checkpoint between each two successive phases:
[1] How the cell entering the cell cycle "enter G1 phase?
- Cells can enter the G1 phase:
→After completing the M phase (continuously replicating cells).
→From the G0 phase (quiescent cells).

A. **quiescent cells** (not actively replicating) are in the G0 phase. These cells can emerge to the G1 phase by:
1)) stimulation of certain growth factors→ making the quiescent cells actively replicating. Example: Hepatocytes.

2)) Signaling from ECM components through integrins→ Quiescent cells can also be induced to proliferate by signaling from ECM components through integrins. This is very important for healing and repair, because unless this type of interaction with integrins is present, you won't be able to fill the gap when you are injured.

B- **Continuously replicating cells:** cells don't go into a G0 phase, instead, they just enter the G1 phase after completing mitosis.

[2] - (G1-S): Cells in G1 progress through the cell cycle and reach a critical stage at the G1-S transition, known as a restriction point, a rate-limiting step for replication. On passing this restriction point, normal cells become irreversibly committed to DNA replication.

- The restriction point is a very critical stage. Why?
1- (G1-S) after passing from the G1 phase into the S phase, the cell will

replicate its DNA. So, if there's any damage in the DNA that's not repaired before the S phase, this damage will be heritable and mutations will emerge. In order to avoid this, **Rb governs** this transition and **p53 guards** it – "the mechanism will be explained in a while".

- P53 detects DNA damage. (If there is a mutation, P53 accumulates, stops cell cycle, and tries to fix it, then continue the cell cycle).
- Rb is phosphorylated by CDK causing DNA replication by activation of S phase genes transcription. (This will be explained later on).

2- (G2-M): it is a transition state between G2 and M phase where we want to check and make sure that: **a-** the replicated DNA is not mutated. **b-**There is no abnormalities in the chromosomes. **c-** The chromosomes are aligned correctly before splitting and are all attached to the spindle properly.

**This is all to get a healthy daughter cell with the correct number of chromosomes and normal DNA. Otherwise, we may end up with DNA mutations (if the DNA damage persists) or aneuploidy (if the daughter cells didn't have the normal number of chromosomes).

$$cyclin attach to CDK (to activate them).

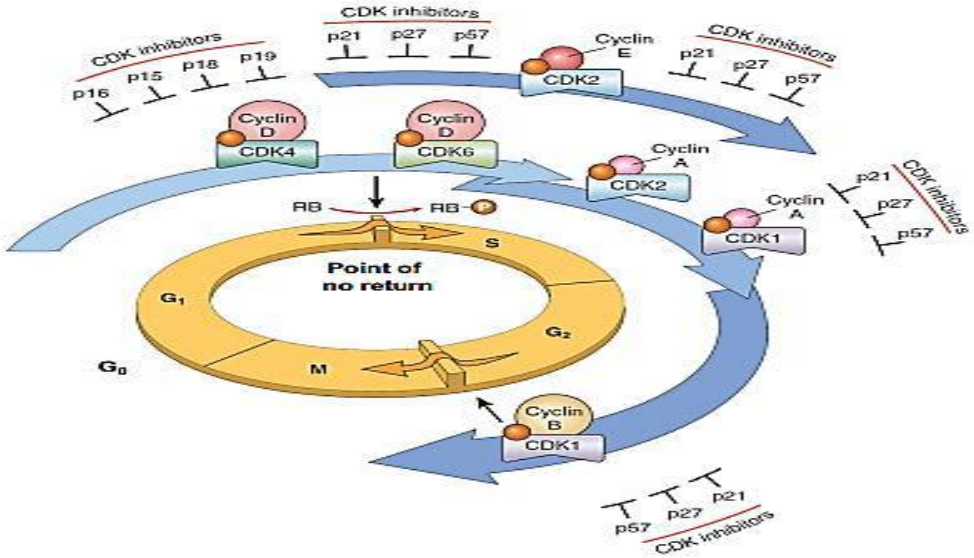
$$ CDKs: are present inside the cell all the time (always expressed) but their activity depends on the presence of cyclins, as the name implies (i.e unless cyclins are produced, CDKs are not going to be active).

CDKs phosphorylate other proteins that have roles in the cell cycle. ex: CDKs phosphorylate Rb (Retinoblastoma) protein. And only when Rb is phosphorylated, the cell can go from G1 to S phase. (Cyclin D binds to CDK4 or CDK6 and they phosphorylate Rb).

$$ another layer of control; *cyclin dependent kinase inhibitors*. they are two group:

1- P21, P27, P57: they occur through all the phases of the cycle, they are non-specific (inhibit all CDKs).
2- The specific ones: P16, P15, P18, P19, also known as *INK4 (A to D),* they affect cyclin dependent kinase 4.

> Remember: p16 is hypermethylated when this happen it turns off CDKI that act on CDK 4 allow the cycle to move on.



Keep in mind: the check point from G1 to S phase is controlled by both Rb and P53.

<u>So what are the mutations that affect Rb?</u>

1-Over expression of cyclin D, that lead to more activation, in (breast, esophagus, liver lymphomas and plasma cell tumors)

2- CDK4 amplification or over-expression ‘’the most common in CDK “ results in: melanomas, sarcomas, and glioblastomas.

3- Mutations in the CDKIs lead to turn them off, how?
Can be targets of genetic and epigenetic changes leading to cancer. Logically, if a loss-of-function mutation occurs in a CDKI, cyclin-CDK complexes will continue functioning leading to more proliferation. *(Look at the table below)*

**Germline mutations of CDKN2A (p16).	-present in 25% of melanoma-prone kindreds
**Somatically acquired deletion or inactivation of CDKN2A (P16)	a- pancreatic carcinomas. b- glioblastomas. c- esophageal cancers. d- non–small cell lung carcinomas. e- soft tissue sarcomas. f- bladder cancers.

**because it's a universal mechanism (cell cycle); many cancer can get this mutation.

Remember: genes can be silenced by DNA methylation and histone modification. Refer to epigenetic modification and cancer (page 175), or the previous sheet.

Note that glioblastoma is an example of both, somatic mutation of CDK2A and amplification of CDK4.

NOW we done with the 1st hallmark of cancer 

Second hallmark of cancer: Insensitivity to Growth Inhibitory Signals

(Evading growth suppressors))

- For a cancer to develop, it's not enough to activate growth-promoting genes, because in the normal cells there are brakes that prevent further growth. These are called tumor suppressor genes.
- So, if you inhibit the inhibitor, you will lead to growth activation (a characteristic of cancer).

A) **RB protein**:

-Tumor suppressor.

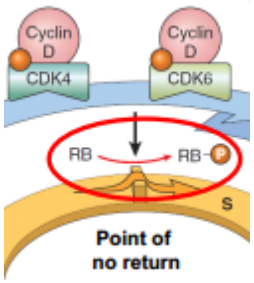
-Governor of the Cell cycle.

- RB one of the four genes that are almost always mutated in all types of cancer.

- Retinoblastoma is a autosomal dominant syndrome, if one copy inherited you will have higher risk of the disease (the more aggressive the earlier you get),,but at molecular it behave as autosomal recessive.
- Retinoblastoma gene (RB) is the first tumor suppressor gene to be discovered and a rare disease. It's an uncommon childhood tumor Approximately 60% of retinoblastomas are sporadic, and the remaining ones are familial.
- The discovery of tumor suppressor genes was accomplished by studying this rare

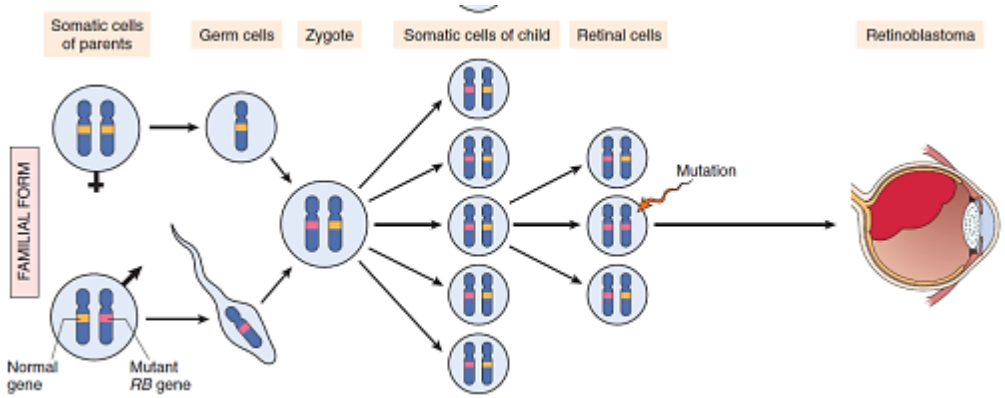
- A mutation in Rb will not only lead to retinoblastoma but to a multitude of tumors. Why? Because all cells at some points of their lives will go into the cell cycle, so Rb is common to all cells and a mutation in it will cause cancer in any cell.

-so you need two defective copies to get the disease (keep in mind it so difficult to lose two copies)



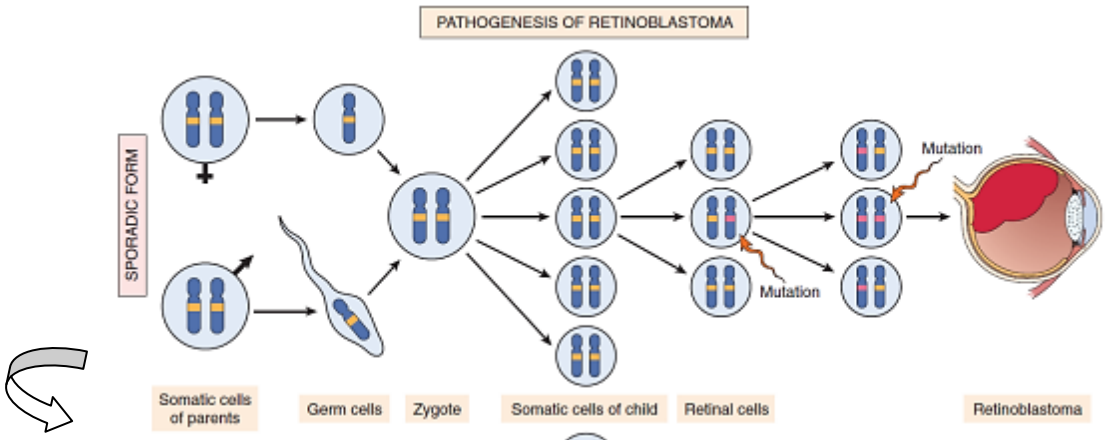
- Knudson Hypothesis: (two-hit) hypothesis: loss of two alleles is required to produce retinoblastoma.

- Retinoblastoma can be familial or sporadic→



In the familial transmission, it follows an autosomal dominant pattern. One defective copy is inherited from parents and the other is lost during life as a result of somatic mutations.

- Patients with familial retinoblastoma are at a higher risk for development of certain cancers. Why? Because one of the alleles is already lost, so there's a higher chance of losing both alleles during life.
- The other allele will be lost by a somatic mutation.



In sporadic cases, both normal RB alleles are lost by somatic mutations in one of the retinoblastoma.

A storm mind question, we said that to lose 2 alleles is very hard, so why the sporadic is more common?
It's all about epidemiology (some Math) to understand lets explain it with example:

Let's assume we have two populations one is very large with million people and we give it the symbol A, and the other population is small with thousand people we give it the symbol B... Assume that the percentage of retinoblastoma is 10% of the population so:
In A population NO. of people get retinoblastoma is 100 thousands
& in B population NO. Of people get retinoblastoma is 100
As we see if we look at A it's a huge number but because of the high number of population it's not seen at all consider as rare …
But in B it's a small number but because it's a small population it consider as common disease and that's how you consider a disease common or not.
I hope that I explain it well

*****Now we want to explain and understand the mechanism that enforces G1-S transition through the RB protein, which is the governor of cell cycle and specifically at G1-S checkpoint:**

- The Initiation of DNA replication (S-phase) requires the activity of cyclinE/CDK2 complexes.
- CyclinE is expressed and dependent on E2F family of transcription factors (E2F family normally or during the G1 phase is bounded to a hypo-phosphorylated RB, where here RB when bounds to E2F family inhibits it, preventing the transcription of cyclin E and therefor no progression in cell cycle until DNA is completely repaired).

-hypophosphorylated RB Blocks E2F in at least two ways:
1. it sequesters or surrounds the E2F family preventing it from interacting with transcription activator.
2. Enzymes modify chromatin at the promoters (promoters of the transcription of cyclinE)to make their DNA make up insensitive to transcription factors. →Those enzymes (Chromatin remodeling proteins: Histone deacetelyases and Histone methyltransferases) are recruited or proclaimed by RB.

- When we have a signaling growth factor (mitogenic signaling) this leads to:

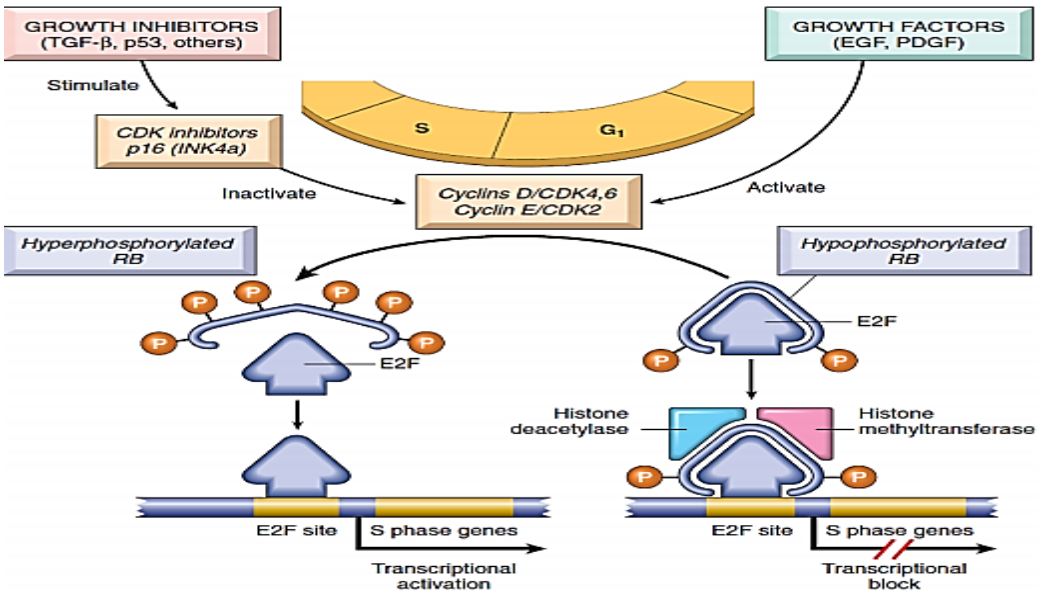
1. CyclinD expression and activation of cyclinD-CDK4/6 complexes these complexes phosphorylate RB (producing hyperphosphorylated RB) and release E2F to induce target genes such as cyclinE.
2. CyclinE expression through E2F family stimulates DNA replication and progression through the cell cycle.

- During M phase (mitotic phase) the phosphate groups are removed from RB by cellular phosphatases, thus regenerating the hypophosphorylated RB.
- The phosphorylation of RB is inhibited by CDKIs because they inactivate the CDK complexes: first there will be growth inhibitors (such as TGF- α, p53), these growth inhibitors stimulate CDK Inhibitors (such

as p16 "INK4a") ,these inhibitors inactivate cyclinE/CDK2 and cyclinD/CDK4,6 complexes, thus inhibition of phosphorylation subsequently no cell cycle progression.

**Conclusion:
- G1 phase → Rb is hypophosphorylated.
- S phase →Rb is hyperphosphorylated (because only here we want E2F to work).
- M phase → Rb is hypophosphorylated again

***Important Question: Why RB is not mutated in every cancer??
- Well a mutation in other genes that control Rb phosphorylation can mimic the effect of RB loss (mutation), such as mutational activation of CDK4 or overexpressing of cyclinD, and mutational inactivation of CDKIs, also would drive the cell cycle.



Actually, not all cancers have Rb mutations. However, mutations in other genes that control Rb phosphorylation can mimic the effect of RB

loss; such genes are mutated in many cancers that seem to have normal RB genes.
Examples:
1- Mutational activation of CDK4 or overexpression of cyclin D favors cell proliferation by facilitating Rb phosphorylation and inactivation. - Indeed, cyclin D is overexpressed in many tumors because of gene amplification or translocation.
2- Mutational inactivation of CDKIs also would drive the cell cycle by unregulated activation of cyclins and CDKs. - CDKN2A gene is an extremely common target of deletion or mutational inactivation in human tumors.

*** We can have inhibition or loss of sensitivity of growth inhibitors through the transforming proteins of several oncogenic human DNA viruses. For example HPV virus (Human Papilloma Virus which is related to cervical cancer) produces E7 protein that bind to the hypophosphorylated RB, preventing it from inhibiting the E2F transcription factor, thus RB is not functioning leading to uncontrolled growth.

As a conclusion, Rb mimicking mutations can be:
a- Activation of CDK4 (mutation).
b- Over-expression of cyclin D (translocation/ amplification).
c- Inactivation of CDKI (e.g. CDKN2A) (mutation/ deletion/epigenetics).

I'm sorry for that long sheet ^^ and I hope you all the high marks.

(ليس هناك أي شي ضروري لتحقيق نجاح من أي نوع أكثر من المثابرة، لأنه يتخطى كل شيء)