PATHOLOGY

Medical Committee
The University of Jordan

■ SHEET

□ SIIDE

Lecture Number: 19

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بسم الله الرحمن الرحيم

You will find this lecturesomehow a rigid topic, so while studying this sheet be patient(بدها طولة بال). Enjoy!

Cardiac Cancer (rare)

Hypothesis explaining **rarity**:

- Heart doesn't have much fat so obesity will be a huge risk factor for having cardiac cancer, as we can see lipoma or liposarcoma in the fat surrounding the heart.
- Our bodies don't consist of that much cardiac tissue, it is only found in your heart.
- Primary heart cancer (sarcomas) arises from the heart itself while Secondary heart cancer (metastasized cancer) arises from another region in the body, typically in secondary cancer type the patient would be already dead considering that its extensively spread in the body and we didn't even get the chance to detect it.
- Cardiac damage (severe) from a primary tumor the patient would be already dead before diagnosing it. The patient dies because there is no heart function.
- Angiogenesis (when blood vessels split into several branches) it is called intssusceptive angiogenesis (this kind of angiogenesis is more commonly found in embryogenesis where we need creation of blood vessels without any neoplastic proliferation.

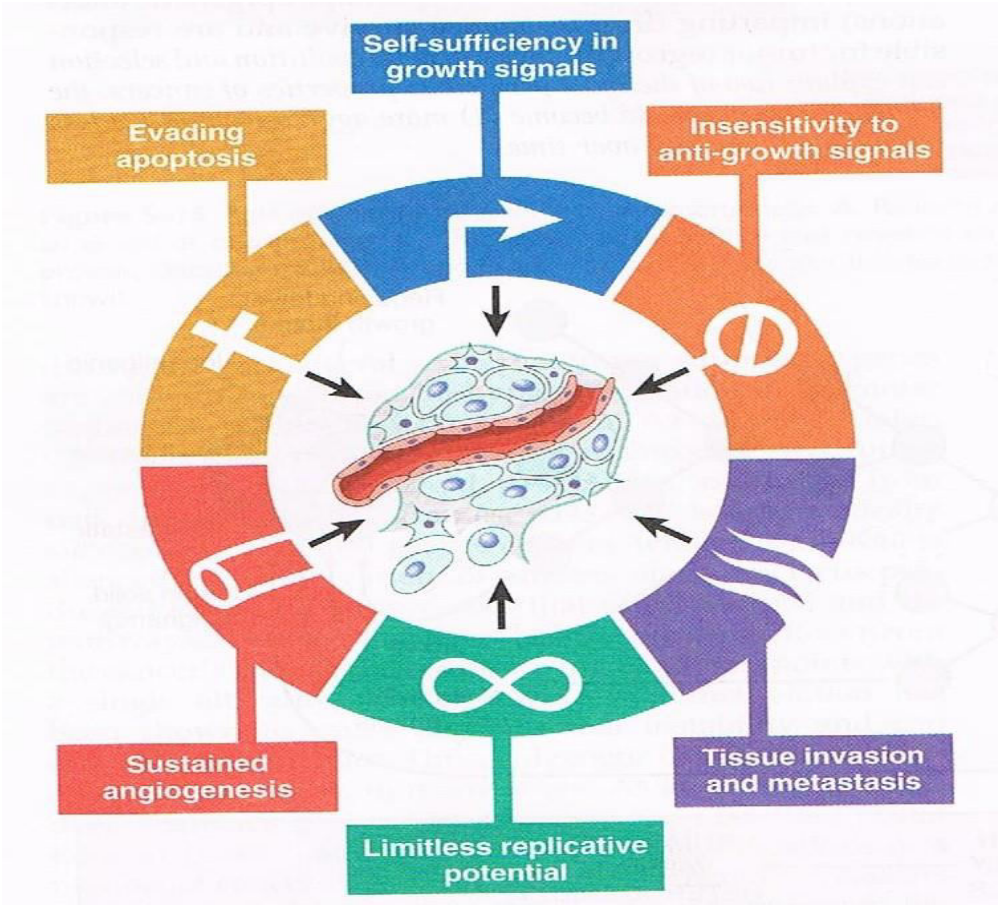
For your own information , not required in our final

Written by Sara Al- Ibrahim

Hallmarks of Cancer

Review of cell signaling

- Receptors signaling outside the cell where the signal molecule is picked up by a receptor "specific receptor", then there will be transient signal through cascade including molecules and proteins(transduction factors) till eventually reaching the nucleus to turn on or off a certain genes.
- There is variation in location of that receptor could be inside the cell or in the surface membrane.
- Receptor activation is called transient receptor activation because normally when you send a signal by a ligand ,this ligand will bind to the receptor and this will send a signal inside the cell , this signal is not permanent its usually turned off by negative feed-back mechanism .



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***you should put in mind that one hallmark of cancer isn't enough to give a full ability and amount of proliferation ,almost all of them are needed to have complete cancerous tumor ....and this is why while treating cancer we need multiple therapies for each pathway that is participating in cancer proliferation .

#FIRST HALMARK :Self-sufficiency in growth signals .

The mechanisms that endow cancer cells with the ability to proliferate are grouped according to their role in the growth factor- induced signal transduction cascade and cell cycle regulation:

1. Growth factors
2. Growth factor receptors
3. Downstream signal-transducing proteins
4. Nuclear transcription factors
5. Cyclins and cyclin-dependent kinases

1) Growth Factors :

- Typically growth factors are secreted in a paracrine secretion method where one cell secret s the growth factor and adjacent cells pick it up (at normal conditions) .
- In cancer this could be abnormal where tumor cells" not necessarily just tumor cells " send signals to stromal cell to produce more growth factors, also alternatively cancer cells could also gain the ability to produce growth factors and its receptors, giving an autocrine positive feedback loop for stimulation of proliferation and more growth factors.

Examples:

- Many glioblastomas (tumor in the brain) secrete platelet-derived growth factor and express the PDGF receptor
- Many sarcomas make both Transforming Growth Factor –α (TGFα) and its receptor.

2)Growth Factors Receptors :

There are two ways to have the receptor activated:

1. Having a mutated growth factor receptor, which means it is independently active sending continuous mitotic signals to cells ,even in the absence of the growth factor in the environment .

Example: colon and lung cancer

2. Over expressed growth factor receptor (more common) which trigger amplification.

Example:

- ERBB1 the EGF receptor(Epidermal growth factor receptor), is overexpressed in 80% of squamous cell carcinomas of the lung.

-The gene encoding a related receptor HER2/NEU(ERBB2), is amplified in 25% to 30% of breast cancers and adenocarcinomas of the lung, ovary, and salivary glands.

so one ligand can bind to several receptors leading to increased signaling.

*In cancers where we have over expression of certain growth factor receptor they had a poor prognosis previously but nowadays because we have antibodies that block the receptors outside and they stop signaling these cancers became highly treatable .

Note : pathways and mechanisms are really important to understand because these pathways could give branches to several other pathways that lead to some complications to a specific patient . Then this patient will need to go to the doctor, and typically the doctor will give him a standard therapy but the patient wont response due to having a branched pathway of the original pathway that need another therapy specific to that pathway , so we should be as doctors more accurate.

3)Downstream signal-transducing proteins:

Signal transducers which are proteins on molecules that are responsible for taking the signal from the receptor to the nucleus.

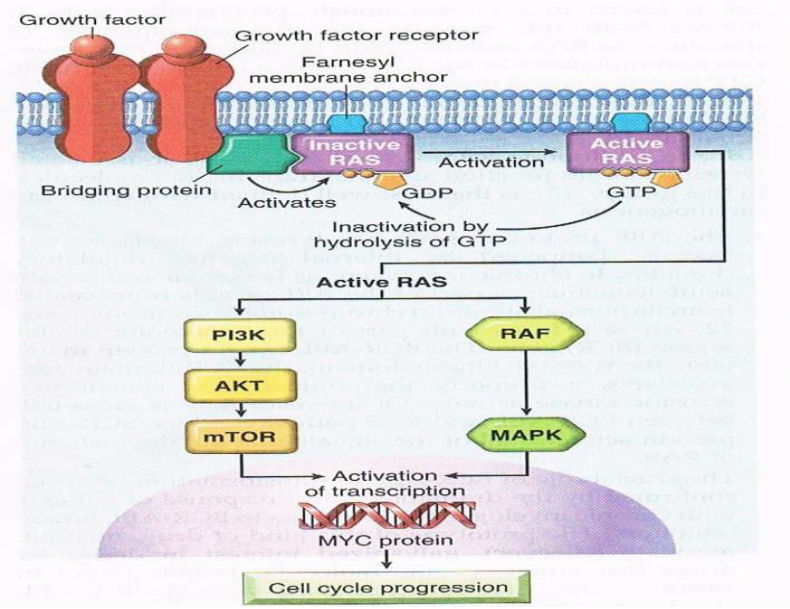
- One of the proteins that were mentioned before is (RAS) its named RAS because it was discovered in RAT sarcomas , its is a member of a family of small G proteins which is bond to the members and to certain growth factor receptors like (EGF) receptors and (PDGF) receptors
- G proteins as you remember are proteins that exchange (GTP) and (GDP) depending on whether they have (GTP) bound to them or (GDP) "it's similar to phosphorylation because (GTP) and (GDP) are only a one phosphate difference".
- Keep in mind that RAS doesn't represent the trimeric G-protein (the one composed of α ,β ,γ) it's a small G-protein .

1. Inactive RAS have (GDP) bound to it.
2. When growth factor is bond to the receptor it stimulates the exchange of (GDP) to (GTP).
3. Now RAS is active when (GTP) is bound to it.
4. Normally once the RAS is bound to GTP this binding is short lived (transient) because it has an internal GTP-ase activity of which will hydrolyze GTP to GDP by releasing phosphate group out from GTP and RAS will be in "GDP state " the inactive state .
5. GAPs which are a family of **G**TP-ase **a**ctivating **p**roteins, they favor the hydrolysis of GTP back to GDP and they help to prevent uncontrolled RAS activation ,when RAS is activated by binding to GTP the signal is transmitted, leading to activation of downstream regulators, through two possible pathways, until reaching the nucleus to activate the transcription producing MYC proteins, which will lead to cell cycle progression.

*the two possible pathways are:

1. BRAF so-called RAF/ERK/MAP kinase pathway.

2. PI3 Kinase/AKT pathway.



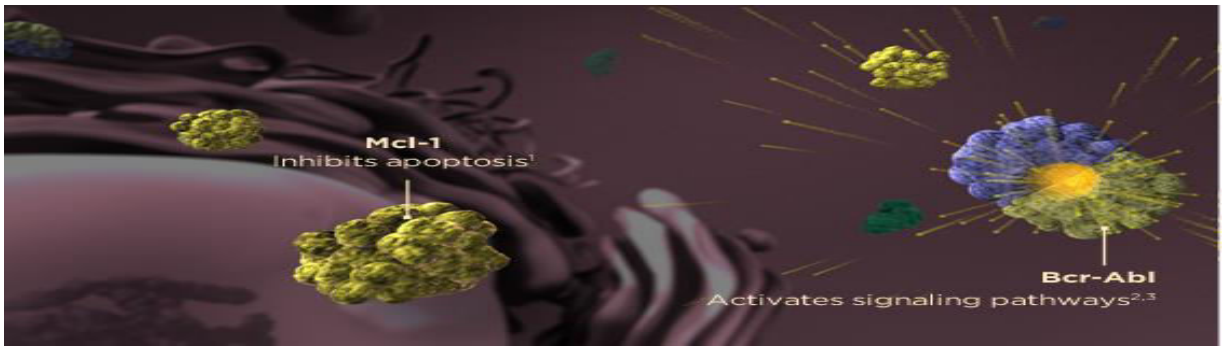
Possible mutations:

1. Mutations of **GTP-ase activity** in activated RAS (RAS constantly bound to GTP), which means RAS is turned on all the time preventing any new transient signaling through that receptor.
2. Mutation in **GAPs** will do the same thing having a constantly activated RAS or <u>prolonged time is needed to deactivate RAS</u> because of GAPs are mutated RAS GTPase activity will not be accelerated which will lead to prolonged activation of RAS .
3. Mutation in **NF1**(Neurofibromin 1) cause a disease called (**Neurofibromentosis** type 1) which can be detected when it causes effects to the iris and the skin .
   NF1 actually is a GAP protein when mutated RAS will be constantly turned on(inappropriate RAS activity).
4. Mutation in the **upstream** regulators (growth factor receptors).
5. **Downstream** mutation, when we have mutations in the regulator proteins of the two possible pathways, such as, mutation in RAF or IP3 kinase.

Number 3 + 4 these mutations mimic RAS mutations

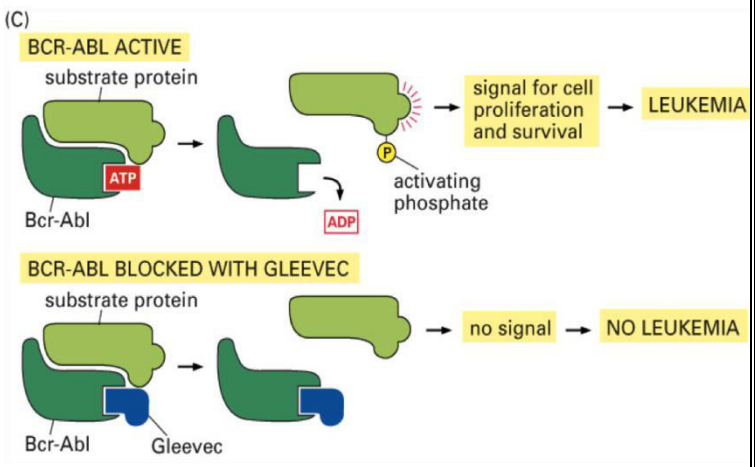
*<u>RAS is the most commonly mutated proto-oncogen in human tumors (specifically in pancreatic adenocarcinoma) because if you look at any tumor it either has a mutation in RAS GTPase activity site or it has a mutation in the upstream or downstream pathway of RAS activation</u> *

BCR-ABL Fusion protein(activated tyrosine kinase):



- ABL has several domains most important one and the one that we need to consider is the kinase domain, which is responsible of phosphorylation of other proteins.
- There are other two domains and a cap which are considered the **regulatory** domains; those regulatory domains make sure that the protein is turned off when there is no outside signal.
- If BCR remove the cap or any other regulatory domain, it will no longer act as a regulatory domain, so the activity of the protein will be constantly turned on becoming a very potent kinase.
- There is cross talk between BCR-ABL protein and RAS pathway, since the BCR-ABL activate the signals that are downstream of RAS pathway.

- We can inhibit the BCR-ABL fusion protein through a drug called **Gleevec** (an antibody for the BCR-ABL complex which blocks ATP binding site on BCR-ABL and prevents phosphorylation of the singaling molecules , resistance to this antibody can develop by having mutation in BCR-ABL complex located at the site where the antibody attach to it .



- BCR-ABL is an example of the concept of **oncogene addiction** it's a term that describes a condition when the cell completely depends on a single signaling pathway for its growth and proliferation .In chronic Myelogenous leukemia the cells almost entirely depends on the BCR-ABL proteins for its proliferation that's

why BCR-ABL is the target protein for cancer treatment therapy because BCR-ABL signaling can be seen as the central **lodgepole** (lodgepole as if you think of it, it looks like a circus tent which have its important piece the central pole, which without it the tent will collapse). The cells that surround are addicted in signaling out from BCR-ABL fusion protein, so we treat cancer by targeting BCR-ABL proteins through specific anti-bodies, because they are sensitive to them. So subsequently when inhibiting BCR-ABL kinase the lodgepole is removed so the structure will collapse, curing the patient out of cancer.

4) Nuclear transcription factors:

*we will depend on and understand the mechanism of MYC transcription factor only because in our book several examples of these transcription factors are mentioned, such as, MYB/JUN/FOS/REL, BUT THOSE ARE NOT REQUIRED.

- MYC transcription factor have two kinds of targeting:
1. Direct targeting: when MYC binds to a promoter and stimulates the transcription of a certain gene.
2. Indirect targeting: when MYC binds to a promoter of another gene and that gene happens to be a transcription factor which will activate other genes .

- MYC can activate genes that will affect the cell cycle, apoptosis, metabolism, dynamics, energy metabolism and much more by truing on(up regulation) or off(down regulation) of certain genes.
- Example of a gene activated by MYC to be turned on is Cyclin Dependent Kinases (CDK)s which are responsible of progression through the cell cycle.
- Example of a gene activated by MYC to be turned off is Cyclin Dependent Kinases inhibiters (CDKI)s. so when we turn off or inhibit the CDKIs we activate the cell cycle (because inhibition the inhibitor means activation).
-MYC over expression through translocation between chromosome 8 and chromosome 14 (gene codes for productions of immunoglobulin's) and the promoter here (Immunoglobulin gene) is highly active and by translocation you get MYC gene close to an overexpressed gene which will cause over-expression of MYC and over production of immunoglobulin's.

5) Cyclins and cyclin-dependent kinases:

Cell Cycle:

It's a very controlled process involves large numbers of molecules and proteins acting either outside or inside the cell.

1. **Inside** the cell: we achieve DNA replication and division through sequence of events: G0-phase (were cells have not entered the cell cycle), G1-phase (presynthetic phase), S-phase (DNA synthesis), G2-phase (premitotic-phase),M-phase (mitotic phase)

- The cell cycle has multiple checkpoints(transition states):

(G0-G1): where Quiescent cells which are cells that are not actively replicating found in the G0 phase can emerge to G1 phase by stimulation of certain growth factors making the Quiescent cells actively replicating , some cells doesn't undergo G0 phase they just enter G1 phase after completing mitosis (continuously replicating cells).
*** we can find those Quiescent cells which are inactive then activated for replication in the liver.

(G1-S) transition point known as the restriction point or the rate limiting step , in which we need to be sure that our DNA is corrected and repaired during G1 phase, because in the S phase we are going to replicate the DNA without being damaged. This point is governed by retinoblastoma and guarded by the protein P53

(G2-M) checkpoint it is a transition state between G2 and M phase where we want to check and make sure that the replicated DNA is not mutated, there is no any major abnormalities and that the chromosomes are aligned correctly before splitting.
****SO the (G1-S) transition point monitors the integrity of DNA before DNA replication, whereas (G2-M) checkpoint checks DNA after the replication and monitors whether the cell can safely enter mitosis.

****when DNA damage is sensed by the cell it will triggers **DNArepair** through p53 protein, but if the damage is severe the cell will trigger **apoptosis**.

2. **Outside**the cell: the cell cycle and its check points are regulated by proteins outside the cell

-these proteins called cyclins (so-called because of the cyclic nature of their production and degradation)

-other proteins (enzymes) called CDKs cyclin dependent kinases require cyclins for their activity , so when they bind together the enzyme is activated forming a complex , these complexes are important for regulation and progression of the cell cycle phases and transition state.

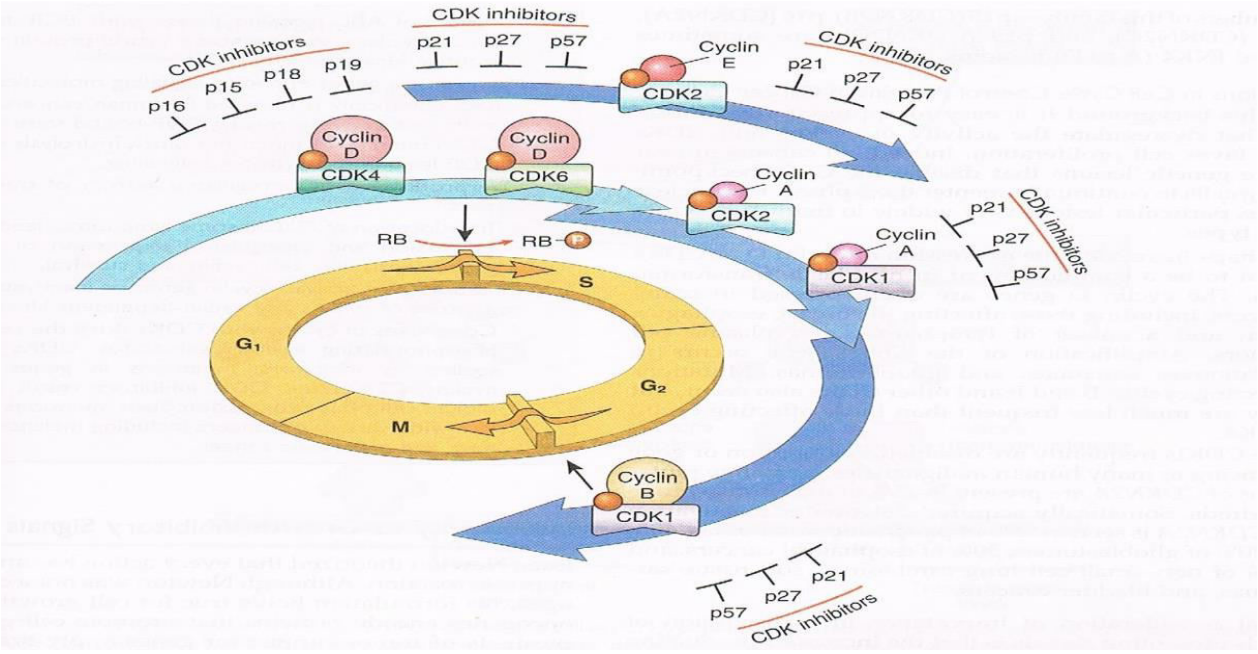
- cyclinD-CDK4, cyclinD-CDK6, cyclinE-CDK2 regulate the G0-S transition point by phosphorylation of RB protein .

- cyclinA-CDK2 , cyclinA-CDK1 are active in the S phase .

- cyclinB-CDK1 is essential for to the G2-M transition state .

- There is also two families of CDKIs (Inhibitors) that block the activity of the complexes and the progression of cell cycle

1. **INK4** inhibitors (composed of **p16, p15, p18** and **p19** respectively) acting on cyclinD-CDK4 and cyclinD-CDK6
2. the other family or three inhibitors (composed of **p21,p27** and **p57**) which are general inhibitors that can inhibit all CDKs.



***The increasing or the overexpression of cyclin D and CDK4 is more frequent than any other complexes at neoplastic transformation because they control a particular checkpoint **(G1-S) checkpoint** by controlling the phosphorylation of RB protein (retinoblastoma protein the governor of cell cycle)

- cyclin D is frequently overexpressed in multiple cancers including those affecting the breast, esophagus, liver ,and subsets of lymphomas and plasma cells cancers
- CDK4 is amplified also in melanomas, sarcomas, and glioblastomas.

#SECOND HALLMARK: Insensitivity of growth inhibitory signals.

- When you inhibit a growth inhibitor this lead to activation of growth, where the cell cycle is inappropriately activated .This Hallmark is about inactivation of Tumor suppressor genes .
- When this happen the surrounding cells can detect there is something wrong or abnormal, so they can stimulate the replicating cells (senescent cells) to go back to a non replicatory cells (quiescent cells) and so on stopping the progression of cell cycle. Also, there are some stimulated proteins (p53) proteins which induce apoptosis of the cell so it won't continue growing or replicating.

RB Gene : Governor of cell cycle

- Retinoblastoma gene (RB) is the first tumor suppressor to be discovered
- Retinoblastoma was an uncommon (rare disease) childhood tumor, because the discovery of the tumor suppressor gene was accomplished by the study of a rare disease.
- 60% of retinoblastoma are **Sporadic** (non hereditary) due to somatic mutation.
- Whereas 40% of retinoblastoma are **Familial** (hereditary)

***FAMILAIL transmission follows an autosomal dominant inheritance pattern (At a GENETIC level) **why**?

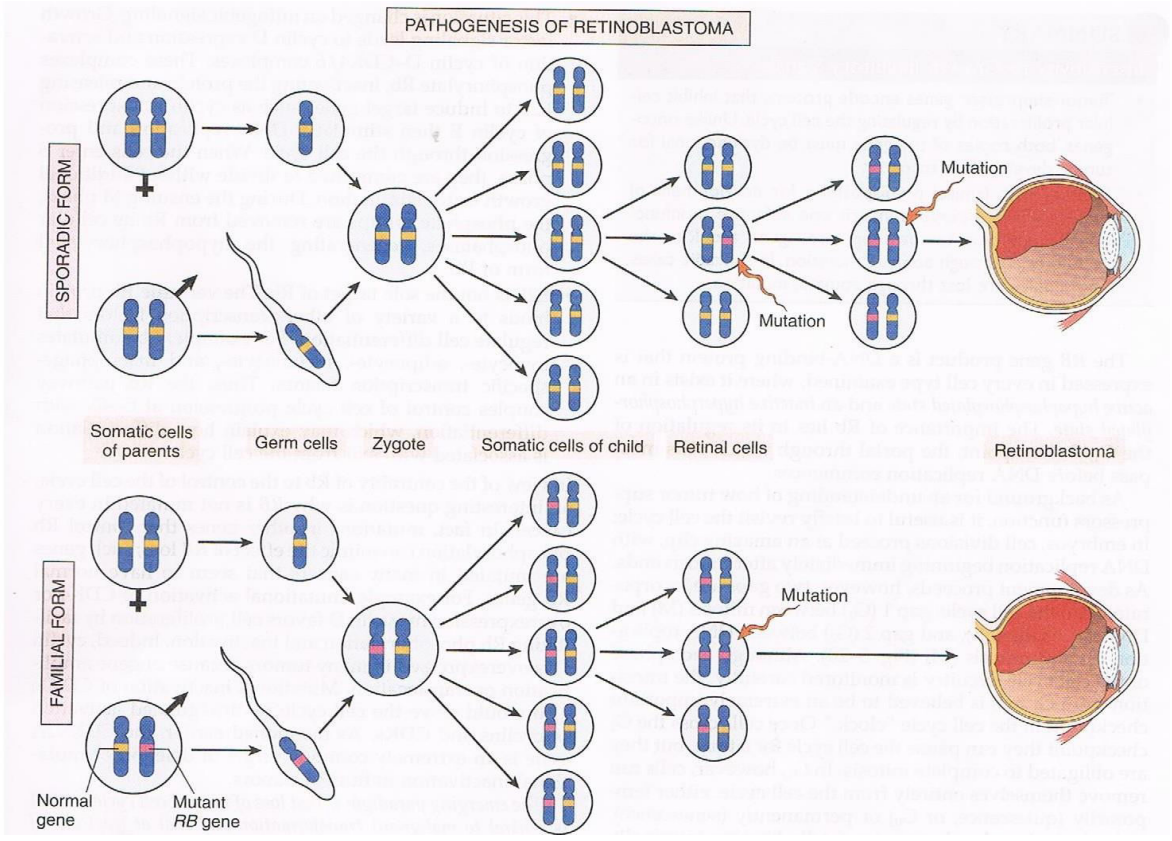
Because in a **familial** case all cells in the body have a one mutated allele in RB gene due to inheritance of it by families, so a **one** single somatic mutation where the normal RB gene "not the inheritably mutated one" is lost in retinoblast as a result of somatic mutation therefor it is considered as **Dominant**.

***SPORADIC case follows an autosomal recessive pattern (At a **CELLULAR** level) **why**?

Because here both RB alleles are normal alleles not mutated (this why we look at it as a matter of cellular level "in general"),so to get the disease we need to lose the normal alleles "mutated" due to somatic mutation of **both** alleles, here we need two somatic mutations to get the cancer this is why we called it **Recessive** .The end result is the same, a retinobalst (retinal cell) has lost both of the normal copies of the RB gene so it become cancerous .

***SO as a result of what we mentioned:

- **Heterozigosity** of RB gene (meaning one allele is mutated and the other is normal) then the RB locus is not neoplastic (cancerous).
(Tumors develop when the cell loses its normal RB gene copy and thus become**homozygous** for the mutant allele)

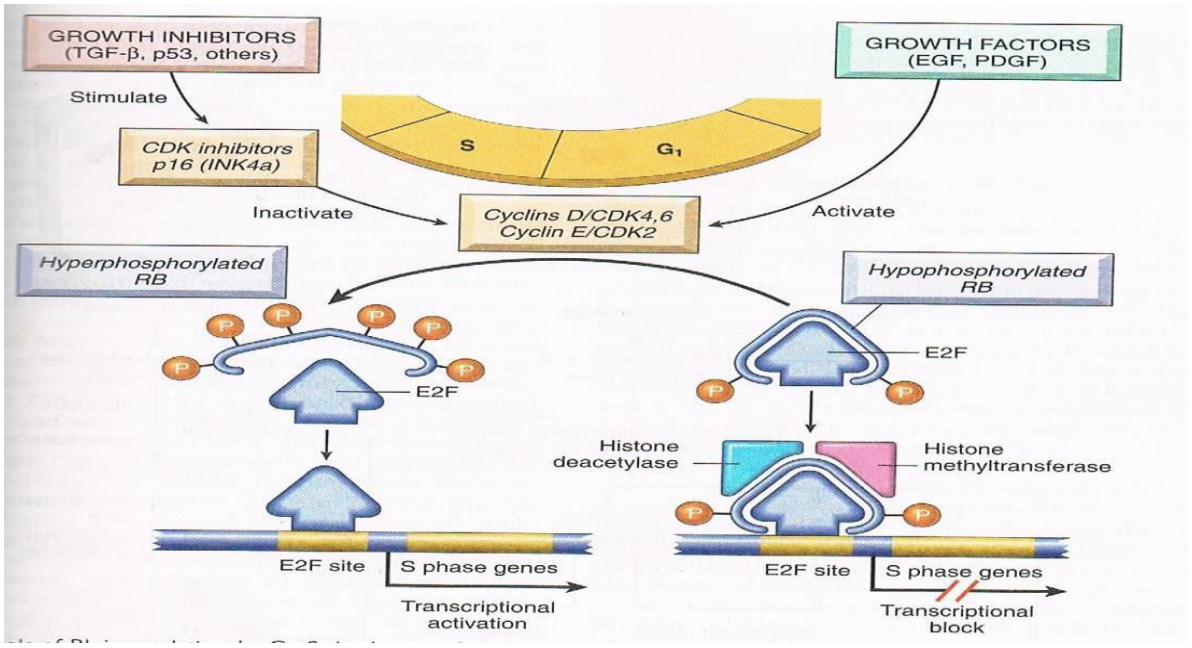


*****Now we want to explain and understand the mechanism that enforces G1-S transition through the RB protein, which is the governor of cell cycle and specifically at G1-S checkpoint:**

- As we mentioned before to initiate the replication phase **S-phase** it 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 hypophosphorylated 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. Enzymesmodify 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 (mitogenicsignaling) 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-β, 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.



***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.

  *** 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) **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.

Review Test

1-the proto-oncogene that is activated by point mutation and result in Pancreatic Adenocarcinoma is :

A. oncogen on chromosome 13
B. NMYC
C. RAS
D. RB

2- A 70 year old female with 4 months history of weight loss and increasing generalized icterus .An abdominal CT-scan revealed a mass in the head of the pancrease .Molecular analysis revealed that the neoplastic cells showed continuous activation of cytoplasmic kinases because a mutation greatly reduced the ability to hydrolyze GTP after growth factor stimulation .Which of the following oncogenes is most likely to be involved in this process ?

A. MYC
B. RAS
C. NEU
D. ABL

3 . One of the following is wrong about the Familal form mutation of RB gene :-

A. It's inherited
B. It has an increasing risk of developing Retinoblastoma
C. All the Retinoblast cells have at least one mutated allele of the RB gene
D. It's similar to haploinsufficiency

4.About BCR-ABL fusion protein one of the following is wrong :

A. It result from Philadelphia chromosome translocation
B. It has inhibited Tyrosin kinase activity
C. It's an example about oncogene addiction
D. Gleevec drug inhibits it's activity

5 . A 52-year-old woman feels a lump in her right breast and goes to her physician. On physical examination there is a 3 cm right breast mass fixed to the chest. This mass is biopsied and on microscopic examination shows nests of cells with marked

hyperchromatism and pleomorphism. These cells are estrogen receptor positive. Flow cytometry is performed.Compared with surrounding non-neoplastic stromal cells , the neoplastic cells are more likely to be in which of the following phases of cell cycle ?

A. G0
B. G1
C. M
D. S

6 . Cell cycle is regulated by all the following except :

A. CDK
B. Cyclins
C. Tumor suppressor genes
D. MYC protein

1	2	3	4	5	6
C	B	D	B	D	D

3 . Haploinsufficiency is different from the familial form of mutation , haploinsufficiency indicates that a tumor suppressor gene becomes inhibited after mutation of 1 allele which means the cell becomes cancerous but in the familial from one allele is inheritably mutated but the cell is not cancerous yet it needs another mutation (hit) on the other normal allele to become cancerous

5 . S-phase is highly seen in cancerous cells and Hyperchromatism indicates active DNA synthesis which occurs in the S-phase

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THE END

***please refer to the slides to check the photos so you can understand more .

"Do it with **Passion** or not at all " ˜ʚ˜

Written by Sara Al- Ibrahim