

# Neoplasia 18 lecture 5

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# ILOS

- 1. understand that insensitivity to growth inhibition is an important cancer hallmark.
- 2. list the tumor suppressor genes that if mutated result in this insensitivity.
- 3. understand the role of RB and TP53 in carcinogenesis.

# Intro

- The balance between tumor suppressor genes and proto-oncogenes in normal cells allows us to renew cells when needed in a controlled, regulated manner.
- Transformed cancer cells lose this balance. They keep proliferating.
- This continuous proliferation is achieved via 1. gaining the ability to “synthesize” their own growth signals (the first hallmark we already discussed) and 2. bypassing the negative regulatory mechanisms ( the hallmark we will discuss in this lecture )

# Second hallmark of cancer: insensitivity to growth inhibitors

- Normally growth inhibition is achieved by tumor suppressor genes. Loss or decreased functions of tumor suppressor genes allows cancer cells to proliferate without being affected by inhibitory growth signals.
- Main genes/ pathways mutated to cause insensitivity to growth inhibition:
  1. RB gene
  2. TP53 gene
  3. TGF beta pathway
  4. Contact inhibition
  5. APC gene

# RB gene

- RB gene= retinoblastoma gene = governor of cell cycle
- RB is a key negative regulator of the cell cycle, it is directly or indirectly inactivated in most human cancers
- The function of the RB protein is to **regulate the G1/S checkpoint**, the portal through which cells must pass before DNA replication starts.

# How does RB act ? The concept:

- Like all our genes RB is translated to a protein , which is the RB protein.
- RB protein acts by binding to DNA.
- Specifically, RB protein binds to a family of transcription genes called **E2F** genes.
- E2F genes are responsible for the transcription of **cyclin E**.
- The binding of RB to E2F inactivates the E2F.. So there is no transcription of cyclin E.
- As you know from last lecture, cell cycle progression depends on cyclins and CDKs.
- So: **lack of cyclin E prevents progression of the cell cycle.**

# How does RB act? .. The details!

- RB is active at the beginning of G1 phase.
- This activity of RB depends on its phosphorylation state. Strangely RB is **active when it's hypo-phosphorylated**. *Note that most of our proteins are activated by gaining phosphate groups. RB is an exception to this rule.*
- So: at the beginning of G1 phase, active, hypo-phosphorylated RB binds to E2F transcription factor family, preventing cyclin E formation.
- Cyclin E/ CDK2 complex is important for initiation of DNA replication ( for entering the S phase)
- **As long as cyclin E/CDK2 complex is not formed , cells are trapped in the G1 phase and cannot move to the S phase.. In other words they cannot cross the G1/S checkpoint.**
- Some cells leave cell cycle to G<sub>0</sub> or go into senescence at this stage.. But if they cross the G1/S they are committed to undergo mitosis without the need of any extra growth signals. That's why this checkpoint is important.

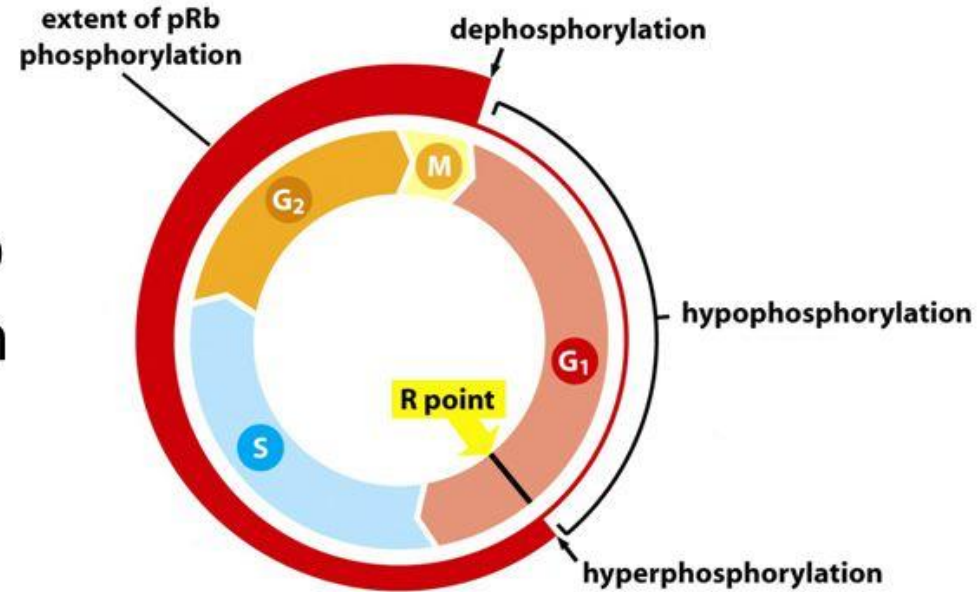
# How does RB inactivate E2F?

- This inactivation occurs via 2 mechanisms
- 1. RB **sequesters** ( = يَحْبِ ) E2F which prevents E2F from interacting with other transcription factors.
- 2. RB recruits proteins that bind to and **inhibit E2F promotor**. This makes E2F gene insensitive to transcription factors
- The net result is preventing transcription of E2F.



## RB regulates progression through G1 phase

**Un- and Hypo-phosphorylated pRb** inhibits the cell from entering a new cell cycle



pRb = RB protein

# Importance of RB function

- RB inactivation of cell cycle allows the cell to have time to check the cell size, protein content and configuration.
- This pause is important because cells that cross the G1/S are committed to DNA replication.. We don't want this to happen unless cells have normal protein content and are really ready to divide.
- However, this inactivation cannot continue forever.. We need to deactivate RB at a certain point to allow cells to enter the S phase.

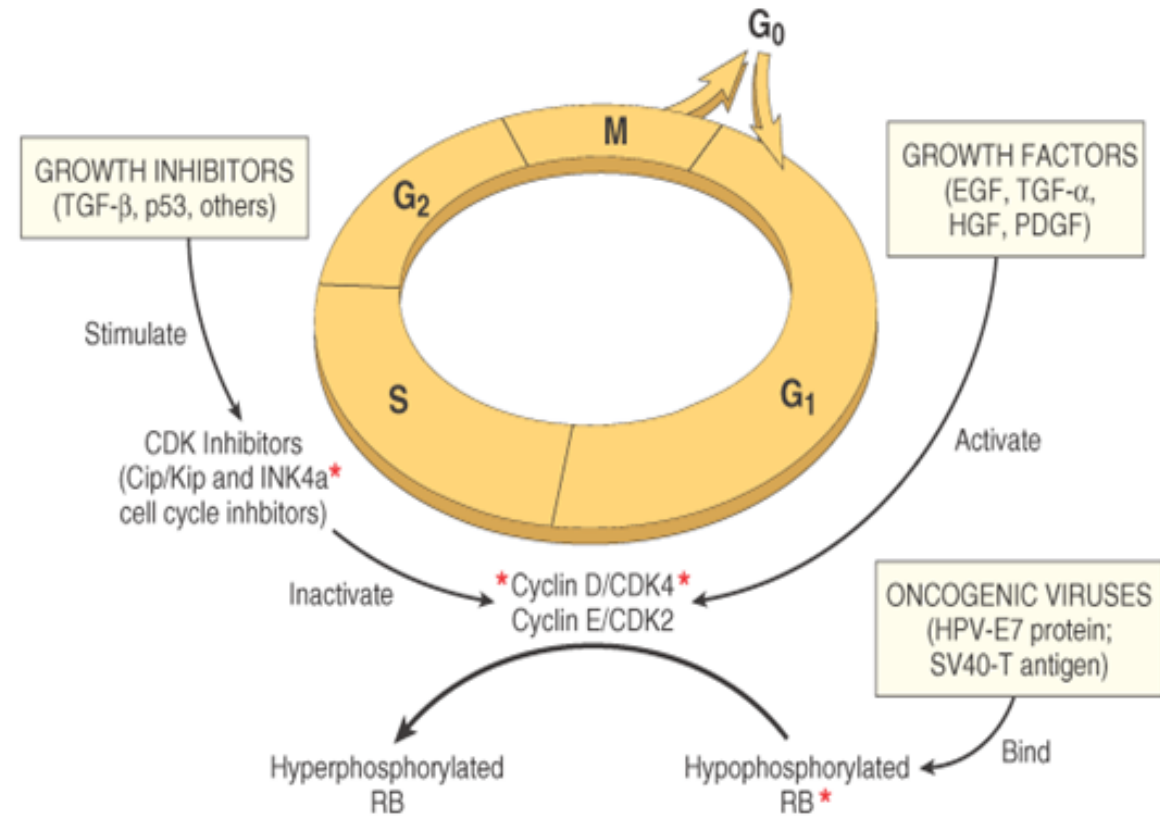
# How RB is deactivated/ How cells can cross the G1/S checkpoint?

- RB is deactivated by phosphorylation.
- This happens when cells are subject to mitogenic ( growth) signals.
- Growth signals cause cyclin D expression.
- Cyclin D complexes with CDKs... these complexes phosphorylate and inactivate RB.
- Once RB is deactivated, E2F genes can be transcribed, resulting in cyclin E formation.
- Cyclin E/ CDK complexes can start the S phase.
- Remember the DEAB sequence we talked about last time.. Cyclin D is the first needed, then E.. Makes sense now?? I hope so!

Note that in the presence of growth inhibitory signals, RB is active and cells cannot divide

With growth stimulatory signals RB is deactivated via cyclin D/ CDKs complexes

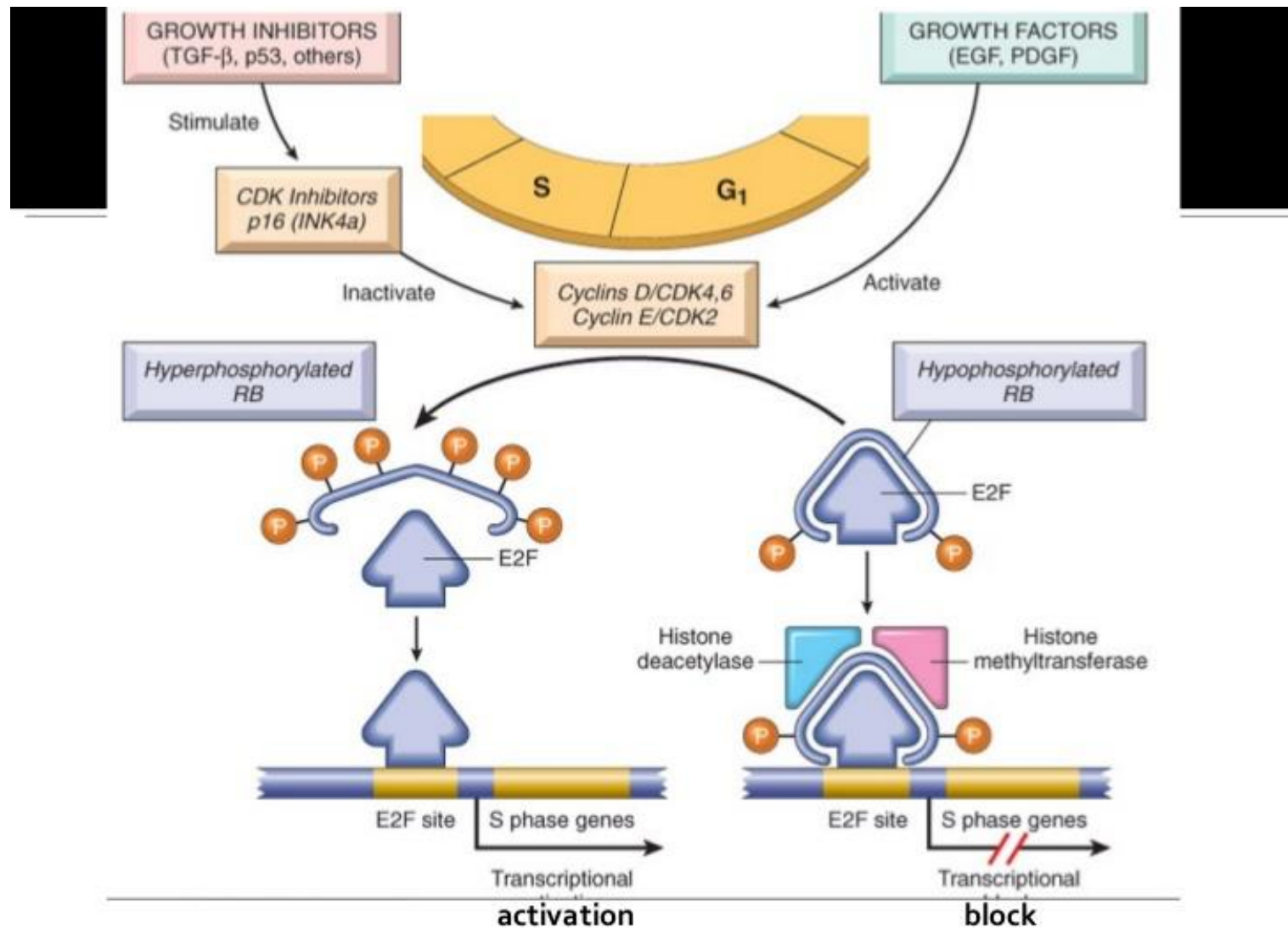
Note that certain viruses can deactivate RB by hyper-phosphorylating it.. This is how these viruses cause cancer ( become oncogenic)



Role of RB as a cell cycle regulator

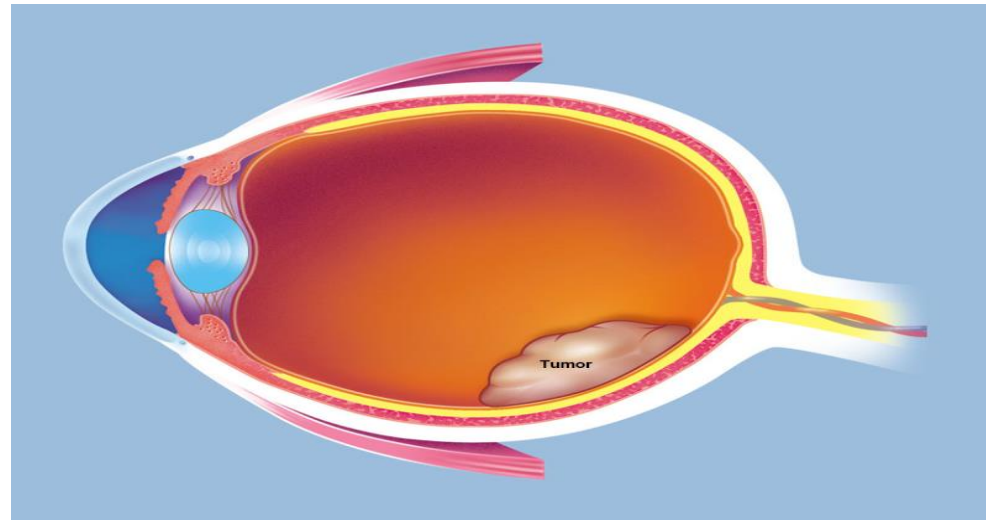
# note

- Once in S phase cells are committed to division.. They don't need additional growth signals
- In M phase phosphate removed from RB ,so it goes back to its inactive state .



# RB is named after a tumor called: Retinoblastoma

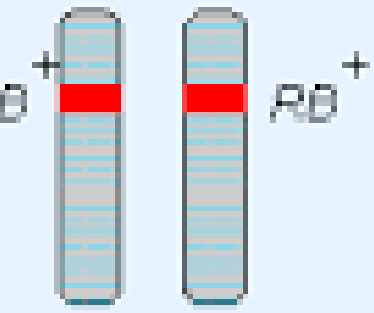
- Retinoblastoma is a rare childhood tumor affecting the eye (retino)
- RB gene was first discovered in this tumor and it's named after it
- However, RB is mutated in most human cancers, not just retinoblastoma tumor.



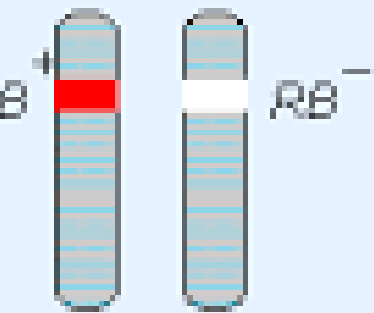
# Retinoblastoma

- 60 % of cases are sporadic, 40% familial
- In familial cases the predisposition to develop the tumor is inherited as an autosomal dominant trait
- However, to develop retinoblastoma: we need both copies of the RB gene to be mutated ( remember that tumor suppressor genes are recessive)
- Loss of the two genes is called the two hit hypothesis

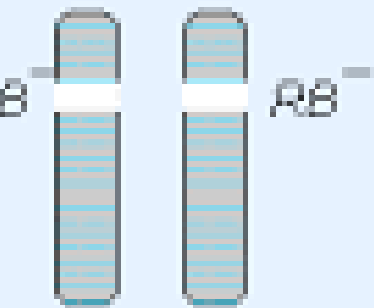




Normal individual  
has 2  $RB^+$  alleles



Loss of one allele in  
somatic cells has no  
effect; loss of one allele  
in germ cells creates  
carrier with wild  
phenotype



Loss of second allele in  
somatic cells induces  
tumor formation

# Two hit hypothesis

- There are two alleles for RB
- BOTH must be deleted or functionally deactivated before developing a tumor.
- In familial cases there is an inherited, germ line mutation in one of the alleles. The second allele is mutated or deleted later in somatic cells.
- In sporadic cases both alleles need to be mutated and/or deleted to have cancer.

# Two hit hypothesis

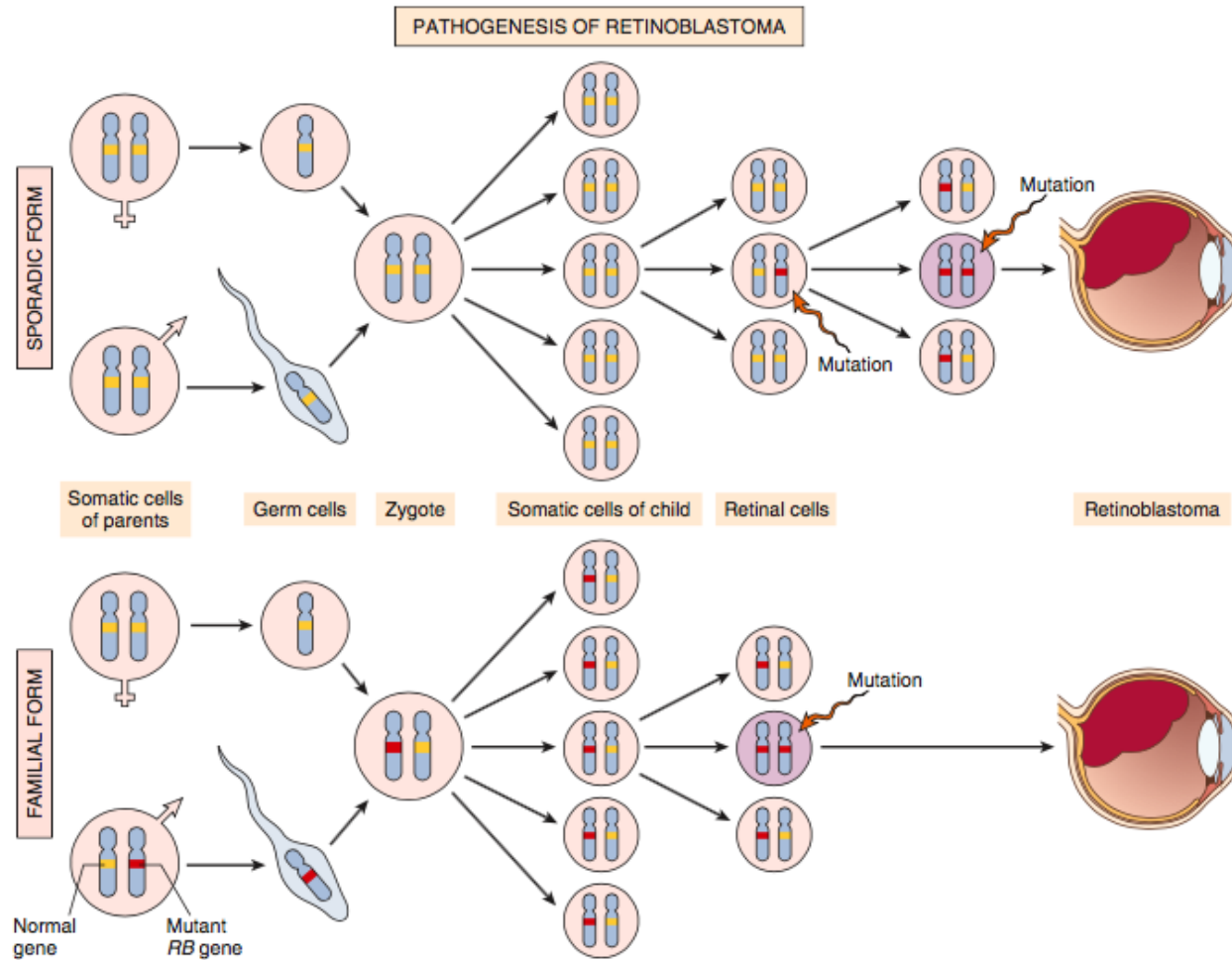


Fig. 6.19 Pathogenesis of retinoblastoma. Two mutations of the *RB* chromosomal locus, on 13q14, lead to neoplastic proliferation of the retinal cells. In the sporadic form, both *RB* mutations in the tumor-founding retinal cell are acquired. In the familial form, all somatic cells inherit one mutant *RB* gene from a carrier parent, and as a result only one additional *RB* mutation in a retinal cell is required for complete loss of *RB* function.

# Two hit hypothesis

- Two mutations (hits) required to develop retinoblastoma
- The 2 mutations involve the RB gene on chromosome 13 (13q14) locus
- **Both copies of RB gene need to be deactivated to develop retinoblastoma**
- In familial cases, one hit is inherited (germ line mutation)  
the other is acquired
- In sporadic cases, both mutations are acquired

# NOTE

- People with inherited RB have increased risk of other cancers.. Mainly osteosarcomas and soft tissue sarcomas

# Mode of inheritance of RB

- Let's come back to this point: we said retinoblastoma is inherited as an autosomal dominant trait  
In familial cases
- However, we know that RB gene is recessive
- Puzzling? It is.. But there is an explanation!
- In familial cases affected individuals inherit one abnormal allele, this mutation is enough to transmit the risk of malignant transformation because almost all those born with one abnormal gene will acquire a second somatic mutation. This means **all those born with one abnormal chromosome will have the disease.**
- In other words, **one abnormal inherited mutation will result in the phenotype** ( increased risk of developing cancer).. By definition, this is a dominant trait.
- **But we know that the phenotype needs a second mutation.. Because the gene is recessive.**
- This concept applies to ALL inherited syndromes that cause tumors by tumor suppressor genes.. We will discuss these in the relevant lectures.

# Don't be lost in terminology!!!

- **Homozygous loss** (both copies lost) of RB gene can be seen in many cancers like breast, bladder...
- **A cell heterozygous in RB locus is not neoplastic ( one normal and one abnormal allele)**
- The two hits are essential for neoplastic transformation.. This makes both copies abnormal, and this is called homozygous loss.
- Note that people who inherit a germline mutation are born with a heterozygous RB ( one normal copy and one mutated). When they acquire another mutation, both copies become abnormal.. They lose the heterozygosity! This is called **loss of heterozygosity (LOH)**
- So LOH means: homozygous abnormality; both copies abnormal

# Important note

- Rb is important for tumorigenesis but it is not mutated in all cancers
- If it is not mutated, other gene mutations mimicking RB mutation must play a role
- Mutations in genes affecting RB phosphorylation: Mutational activation of cdk4 or overexpression of cyclin D favor cell proliferation by inactivating RB

# NOTE

- Loss of normal cell cycle control is found in all tumors through mutations of RB, cyclin D, CDK4 or CDKN2A (which is a CDKI)



- Some viruses like HPV have protein (E7) which binds to the hypophosphorylated RB and prevents it from inhibiting E2F
- So RB is functionally deleted

# TP53

- This is also an important regulator of cell cycle.

# TP53 gene: the guardian of the genome

- Tp53 is one of the most commonly mutated genes in cancer
- It encodes p53 protein

P53 causes growth inhibition by three mechanisms

1. Temporary cell cycle arrest: quiescence
2. permanent cell cycle arrest: senescence
3. Triggering apoptosis

- P53 monitors internal stress whereas RB senses external signals
- P53 is triggered by several stresses: anoxia, inappropriate oncogene activity (MYC or RAS) or DNA damage.

# HOW p53 works?

- In non-stressed healthy cells, p53 is short lived: 20 minutes because it binds MDM2 which is a protein that targets it for destruction via ubiquitin proteasome pathway.
- When cells are stressed ..sensors that include protein kinases are activated (ATM is one of these kinases)
- These activated kinases catalyze post translational modifications of p53 and release it from MDM2
- Now p53 has longer life span and can drive transcription of certain genes.. hundreds of them

Once activated p53 causes transcription of many genes including:

- 1. genes mediating cell cycle arrest, like p21.
- 2. DNA repair genes.
- 3. genes involved in senescence like CDKI
- 4. pro-apoptotic genes including BAX and PUMA

# Cell cycle arrest

- 1. genes mediating cell cycle arrest. This occurs late in G1. caused by p53 dependent transcription of CDK1 gene= p21.
- P21 protein inhibit cyclin/CDK complexes and prevents phosphorylation of RB
- So cell is arrested in G1
- Pause to repair any DNA damage

- 2. expression of DNA damage repair genes
- If DNA is repaired successfully ,p53 upregulates transcription of MDM2.. Destruction of p53.. Removal of the block on cell cycle.
- If DNA not repaired p53 makes cells enter apoptosis or senescence



# Senescence by p53

- 3. Senescence needs activation of p53 and or RB and expression of their mediators like CDKI
- Mechanisms of senescence unclear but seem to involve global chromatin change, with permanent change gene expression

## 4. P53 induced apoptosis

- Induced by pro-apoptotic genes including BAX and PUMA

- P53 also **represses** proliferative and anti-apoptotic genes (bcl2)
- ? P53 is a transcriptional activator so how could it repress certain gene expression
- Answer by **miRNAs..** P53 stimulates expression of micrRNAs that result in inactivation of certain genes, like the example above: bcl2.

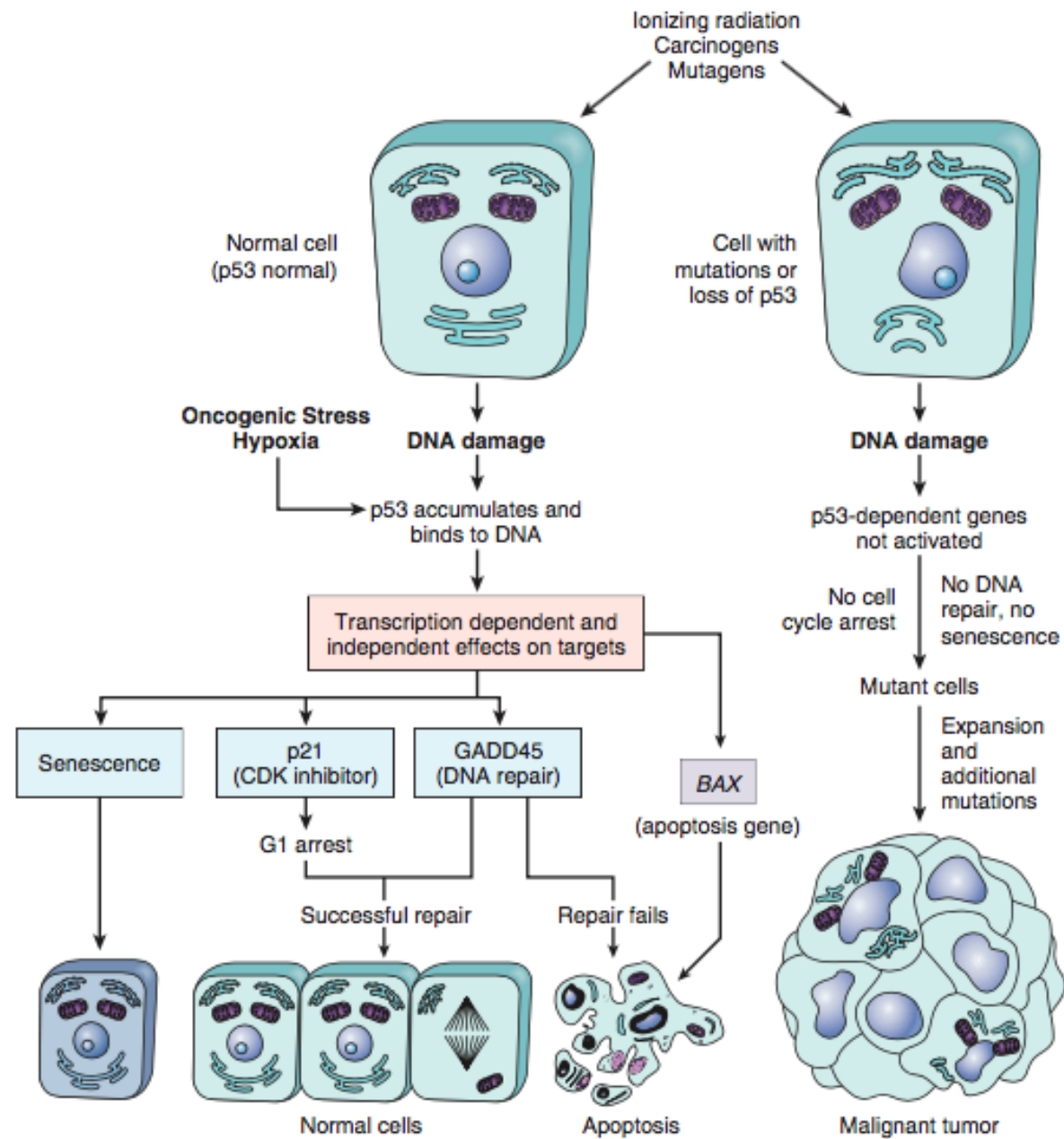


Fig. 6.21 The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G1 and induction of DNA repair, by transcriptional upregulation of the cyclin-dependent kinase inhibitor CDKN1A (p21) and the GADD45 genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of TP53, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.

- More than 70% of human cancers have mutated TP53
- Both copies of the gene need to be lost for cancer to develop
- Mostly somatic
- Rare li Fraumeni syndrome: inherit defect in one allele.. More predisposition to cancer (Sarcoma, breast carcinoma , leukemia and brain tumor)

- P53 can become nonfunctional by some DNA viruses
- HPV, Hep B, EBV.. Proteins can bind to p53 and deactivate it
- Note p53 activated by phosphorylation

# Summary 1/2

- Insensitivity to inhibitory growth signals is an important cancer hallmark.
- This insensitivity is achieved via inhibiting both copies of tumor suppressor genes.
- Among others, RB and Tp53 are the most important tumor suppressor genes deactivated in human cancer.
- RB was discovered in familial retinoblastoma cases. Patients inherit a defective allele copy in an autosomal dominant fashion, and acquire a second mutation or deletion resulting in LOH and tumor formation. These two mutations are called the two hit hypothesis.
- Sporadic cases of retinoblastoma result from two acquired hits.
- RB is mutated in other neoplasms as well.
- RB inhibits cell proliferation by deactivating transcription of F2F genes resulting in decreased cyclin E. Decreased cyclin E prevents cells from entering the S phase.
- RB is deactivated normally by phosphorylation achieved from interaction with cyclin D/ CDK4 complexes formed in response to growth signals.
- Loss of normal cell cycle control is found in all tumors through mutations of RB, cyclin D, CDK4 or CDKN2A (which is a CDKI)

# Summary 2/2

- Tp53 is one of the most commonly mutated genes in cancer
- P53 protein causes growth inhibition by arresting the cell cycle temporarily (quiescence) or permanently (senescence) or triggering apoptosis
- normally p53 has a life span of 20 minutes , it is destroyed by ubiquitin proteasome pathway through the MDM2 protein that targets it for destruction.
- When cells are stressed kinases like ATM kinase catalyze post translational modifications of p53 and release it from MDM2. this activates the p53 which stimulates transcription of hundreds of genes including those mediating cell cycle arrest.
- This arrest allows cells to pause to repair any DNA damage.
- If repairing the damage fails, p53 stimulates apoptosis or senescence.
- If mutated, cells will proliferate regardless of DNA damage present and cells will bypass apoptotic and senescence signals.



*Thank  
you*

