Neoplasia
lect 5

Insensitivity to Growth-Inhibitory Signals

- **The signal of tumor suppressor genes have anti-growth signals that can prevent cell proliferation in different mechanisms:**

1. May cause dividing cells to enter G0 (quiescence)

2. May cause the re-entery of cells in a postmitotic, differentiated pool and lose replicative potential.

Retinoblastoma gene (RB): Governor of the Cell Cycle

- **RB gene is a key negative regulator of the cell cycle, is directly or indirectly inactivated in most human cancers.**
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- **Retinoblastoma is an uncommon childhood tumor.**
- **Approximately 60% of retinoblastomas are sporadic, and 40% are familial.**
- **The predisposition to develop the tumor being transmitted as an autosomal dominant trait.**

- **To account for the sporadic and familial occurrence of an identical tumor, Knudson, in 1974, proposed his now famous *two-hit* hypothesis.**

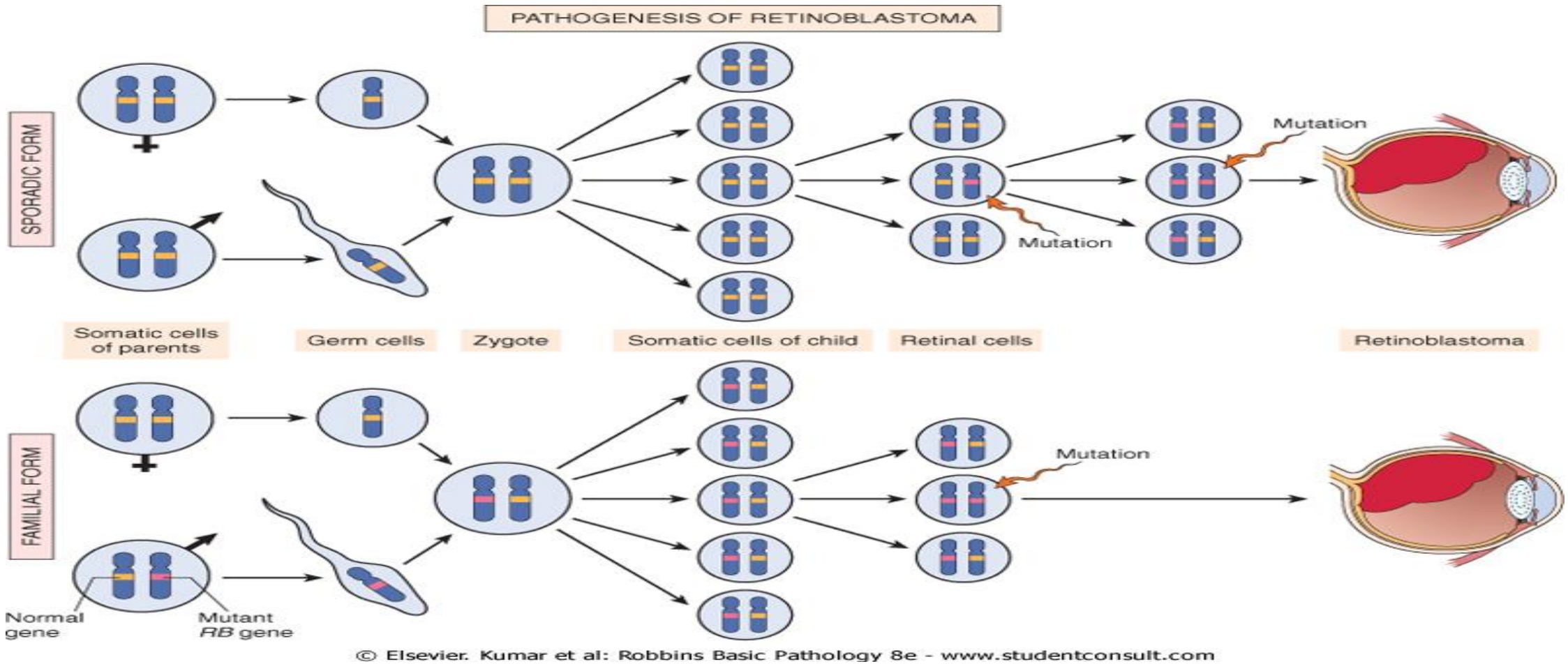
- **Two mutations *(hits)* are required to produce retinoblastoma.**
- **These involve the *RB* gene, located on chromosome 13q14.**
- **Both of the normal alleles of the *RB* locus must be inactivated (two hits) for the development of retinoblastoma.**

- **In familial cases, children inherit one defective copy of the *RB* gene in the germ line while the other copy is normal.**
- **Retinoblastoma develops when the normal *RB* gene is lost in retinoblasts as a result of somatic mutation.**

- **In retinoblastoma families only a single somatic mutation is required for expression of the disease**
- **The familial transmission follows an autosomal dominant inheritance pattern.**
- **In sporadic cases, both normal *RB* alleles are lost by somatic mutation in one of the retinoblasts.**
- **A retinal cell that has lost both of the normal copies of the *RB* gene becomes cancerous**

- **Although the loss of normal *RB* genes was discovered initially in retinoblastomas, it is now evident that homozygous loss of this gene is a fairly common event in several tumors including :**
- **Breast cancer**
- **Small-cell cancer of the lung**
- **Bladder cancer.**

- **Patients with familial retinoblastoma also are at greatly increased risk of developing osteosarcomas and some soft tissue sarcomas.**



- **The function of the RB protein is to regulate the G1/S checkpoint, the portal through which cells must pass before DNA replication commences.**
- **The transition from G1 to S is an extremely important checkpoint in the cell cycle**

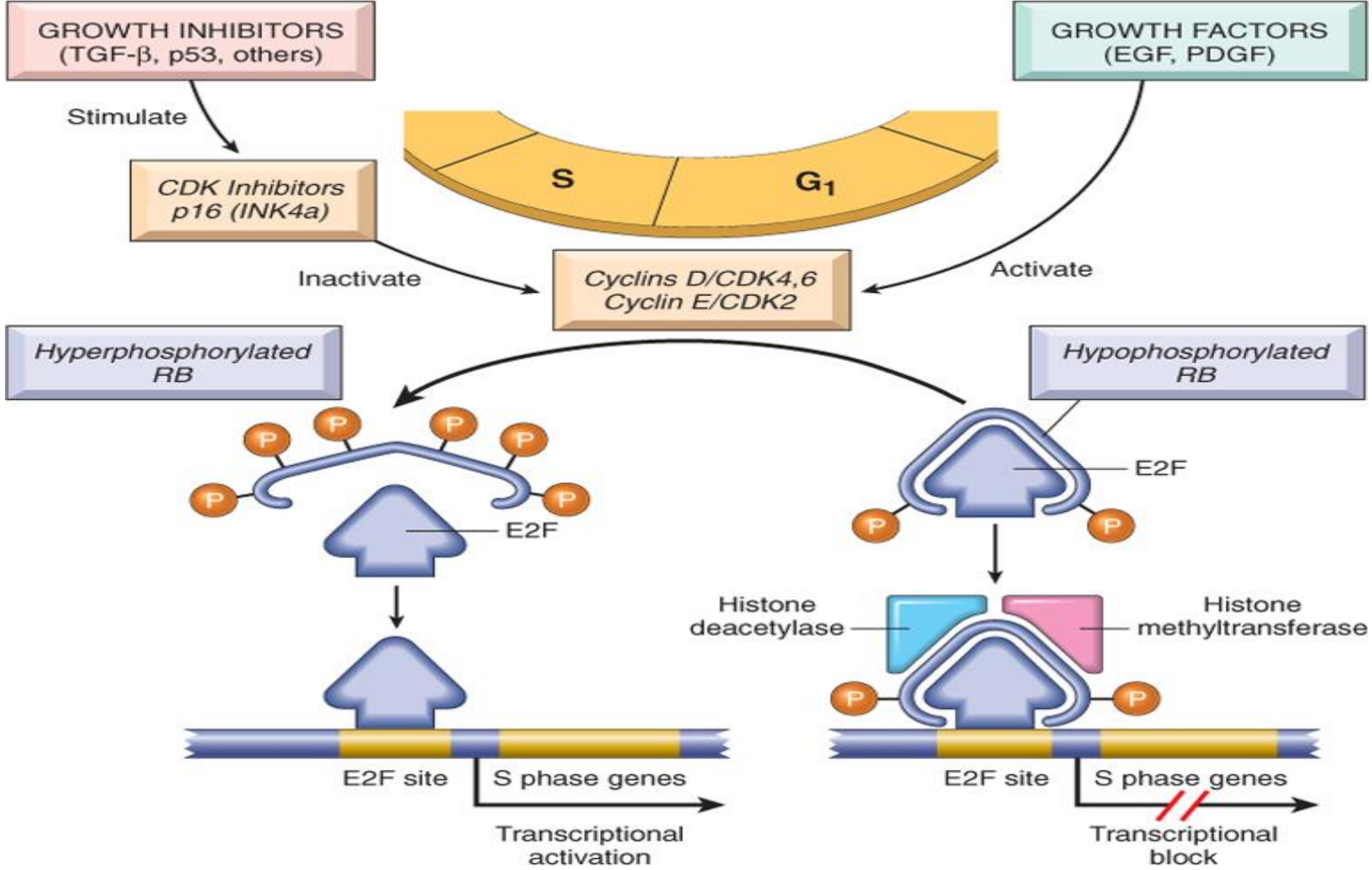
RB Gene and Cell Cycle

- **The *RB* gene product is a DNA-binding protein that is expressed in every cell type examined**
- **It exists in an *active hypophosphorylated* and an inactive hyperphosphorylated state.**
- **The importance of RB lies in its enforcement of G_1, or the gap between mitosis (M) and DNA replication (S).**

- **In embryos, cell divisions proceed with DNA replication beginning immediately after mitosis ends.**
- **However, as development proceeds, two gaps are incorporated into the cell cycle:**
- **1-Gap 1 (G_1) : between mitosis (M) and DNA replication (S)**
- **2-Gap 2 (G_2) : between DNA replication (S) and mitosis (M)**

- **Although each phase of the cell cycle circuitry is monitored carefully**
- **the transition from G_1 to S is believed to be an extremely important checkpoint in the cell cycle clock.**
- **Once cells cross the G_1 checkpoint they can pause the cell cycle for a time, but they are obligated to complete mitosis.**

- **In G_1 cells can exit the cell cycle :**
- **1- Temporarily, called quiescence**
- **2- Permanently, called senescence.**
- **In G_1, therefore, diverse signals are integrated to determine whether the cell should enter the cell cycle, exit the cell cycle and differentiate, or die.**
- **RB is a key factor in this decision process.**



- **The initiation of DNA replication requires the activity of cyclin E/CDK2 complexes, and expression of cyclin E is dependent on the E2F family of transcription factors.**
- **Early in G_1, RB is in its hypophosphorylated active form and it binds to and inhibits the E2F family of transcription factors preventing transcription of cyclin E.**

- **Hypophosphorylated RB blocks E2F-mediated transcription in at least two ways :**
- **1- it sequesters E2F, preventing it from interacting with other transcriptional activators.**
- **2- RB recruits chromatin remodeling proteins, such as histone deacetylases and histone methyltransferases, which bind to the promoters of E2F-responsive genes such as cyclin E.**
- **These enzymes modify chromatin at the promoters to make DNA insensitive to transcription factors.**

- **This situation is changed upon mitogenic signaling.**
- **Growth factor signaling leads to cyclin D expression and activation of cyclin D-CDK4/6 complexes.**
- **These complexes phosphorylate RB inactivating the protein and releasing E2F to induce target genes such as cyclin E.**
- **Expression of cyclin E then stimulates DNA replication and progression through the cell cycle.**

- **When the cells enter S phase, they are committed to divide without additional growth factor stimulation.**
- **During the ensuing M phase, the phosphate groups are removed from RB by cellular phosphatases, regenerating the hypophosphorylated (active) form of RB.**

- **E2F is not the sole target of RB.**
- **The versatile RB protein has been shown to bind to a variety of other transcription factors that regulate cell differentiation.**
- **E.g**

  RB stimulates myocyte-, adipocyte-, melanocyte-, and macrophage-specific transcription factors.

- **RB pathway is important to :**

1- Control of cell cycle progression at G_1

2- Induce cell differentiation

3- Induce senescence

- **Mutations in other genes that control RB phosphorylation can mimic the effect of *RB* loss**
- **Genes are mutated in many cancers that seem to have normal *RB* genes.**

- **E.g**
- **Mutational activation of CDK4 or overexpression of cyclin D would favor cell proliferation by facilitating RB phosphorylation and inactivation.**
- **Cyclin D is overexpressed in many tumors because of gene amplification or translocation.**
- **Mutational inactivation of CDKIs also would drive the cell cycle by unregulated activation of cyclins and CDKs.**

- **Simian virus 40 and polyomavirus large-T antigens, adenovirus EIA protein, and human papillomavirus (HPV) E7 protein all bind to the hypophosphorylated form of RB.**
- **The RB protein, unable to bind to the E2F transcription factors, is functionally deleted, and the cells lose the ability to be inhibited by antigrowth signals.**

p53 Gene: Guardian of the Genome

- **The *p53* tumor suppressor gene is one of the most commonly mutated genes in human cancers.**
- ***P53 induces neoplastic transformation by three interlocking mechanisms:***

1-Activation of temporary cell cycle arrest (termed quiescence),

2-Induction of permanent cell cycle arrest (termed senescence),

3-Triggering of programmed cell death (termed apoptosis).

- ***p53*** **can be viewed as a central monitor of stress, directing the stressed cells toward an appropriate response.**
- **A variety of stresses can trigger the *p53* response pathways including :**

1-Anoxia

2-Inappropriate oncogene expression (e.g., *MYC* or *RAS*),

3-Damage to the integrity of DNA.

- **In nonstressed healthy cells p53 has a short half-life (20 minutes) because of its association with MDM2, a protein that targets it for destruction.**
- **When the cell is stressed for example by an assault on its DNA p53 undergoes post-transcriptional modifications that release it from MDM2 and increase its half-life.**
- **During the process of being unshackled from MDM2, p53 also becomes activated as a transcription factor.**

- **Dozens of genes whose transcription is triggered by p53 have been found.**
- **They can be grouped into two broad categories:**
- **1-those that cause cell cycle arrest**
- **2-those that cause apoptosis.**

- **If DNA damage can be repaired during cell cycle arrest, the cell reverts to a normal state; if the repair fails, p53 induces apoptosis or senescence.**

- **The manner in which p53 senses DNA damage and determines the adequacy of DNA repair are not completely understood.**
- **The key initiators of the DNA-damage pathway are two related protein kinases:**
- ***1-ataxia-telangiectasia mutated (ATM).***
- ***2-ataxia-telangiectasia mutated related (ATR).***

- **Patients with this disease, which is characterized by an inability to repair certain kinds of DNA damage, suffer from an increased incidence of cancer.**
- **The types of damage sensed by ATM and ATR are different, but the down-stream pathways they activate are similar.**
- **Once triggered, both ATM and ATR phosphorylate a variety of targets, including p53 and DNA repair proteins.**
- **Phosphorylation of these two targets leads to a pause in the cell cycle and stimulation of DNA repair pathways respectively.**

- ***p53-mediated cell cycle arrest may be considered the primordial response to DNA damage*** **.**
- **It occurs late in the G_1 phase and is caused mainly by *p53*-dependent transcription of the CDKI *CDKN1A (p21).***
- **The *CDKN1A* gene inhibits cyclin-CDK complexes and prevents phosphorylation of RB essential for cells to enter G_1 phase.**
- **Such a pause in cell cycling gives the cells time to repair DNA damage.**

- **p53 also helps the process by inducing certain proteins, such as GADD45 (growth arrest and DNA damage), that help in DNA repair.**
- **If DNA damage is repaired successfully, p53 up-regulates transcription of MDM2, leading to destruction of p53 and relief of the cell cycle block.**
- **If the damage cannot be repaired, the cell may enter p53-induced senescence or undergo p53-directed apoptosis**

- **more than 70% of human cancers have a defect in this gene, and the remaining malignant neoplasms have defects in genes up-stream or down-stream of *p53.***
- **Homozygous loss of the *p53* gene is found in virtually every type of cancer, including :**
- **1-carcinomas of the lung,**
- **2-carcinoma of colon,**
- **3-carcinoma of breast .**

- **Less commonly, some individuals inherit a mutant *p53* allele; this disease is called the *Li-Fraumeni syndrome*.**
- **inheritance of one mutant allele predisposes individuals to develop malignant tumors because only one additional hit is needed to inactivate the second, normal allele.**

- **Patients with the *Li-Fraumeni syndrome* have a 25 X greater chance of developing a malignant tumor by age 50 compared with the general population.**
- **In contrast to patients who inherit a mutant *RB* allele, the spectrum of tumors that develop in patients with the Li-Fraumeni syndrome is varied**
- **The most common types of tumors are: sarcomas, breast cancer, leukemia, brain tumors, and carcinomas of the adrenal cortex.**