NEOPLASIA

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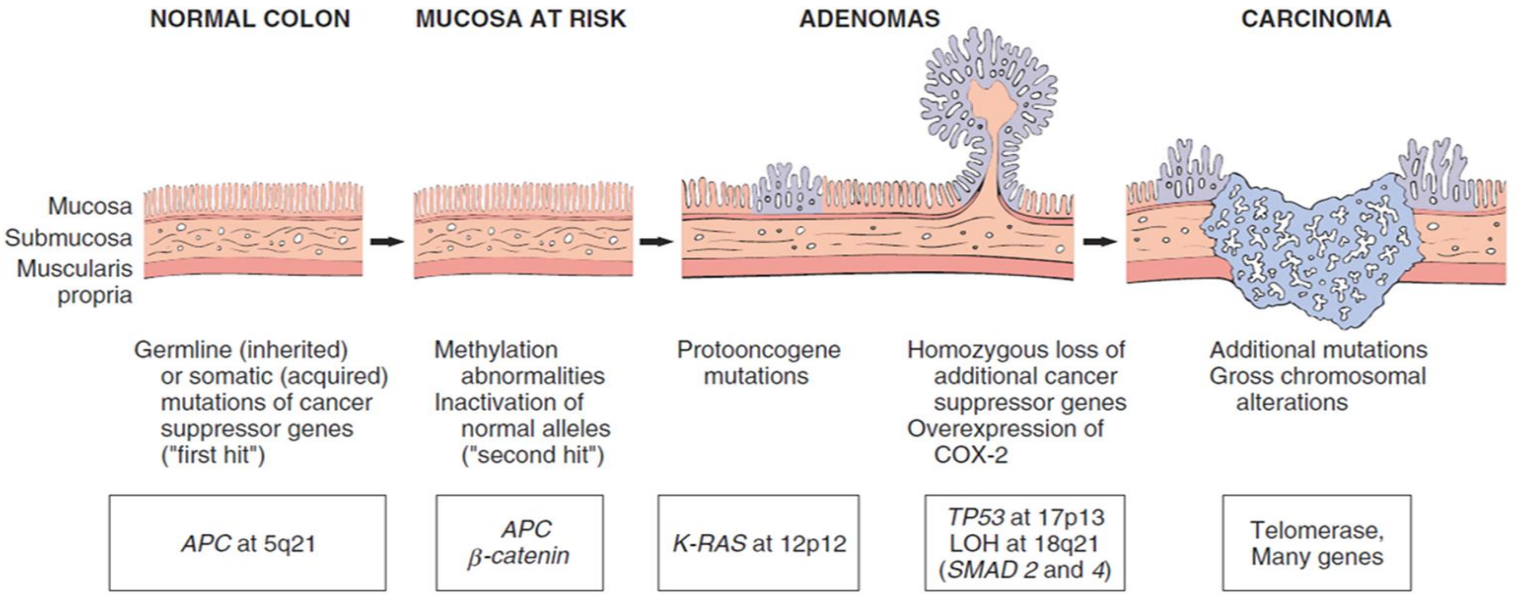
Carcinogenesis

- Carcinogenesis is a multistep process resulting from the accumulation of multiple genetic alterations that collectively give rise to the transformed phenotype and all of its associated hallmarks.

In most cases, no single mutation is sufficient to transform a normal cell into a cancer cell.



Tumor Progression:

This is the stepwise accumulation of mutations resulting in increasing features of malignancy:

- More Aggressive
- Less responsive to therapy

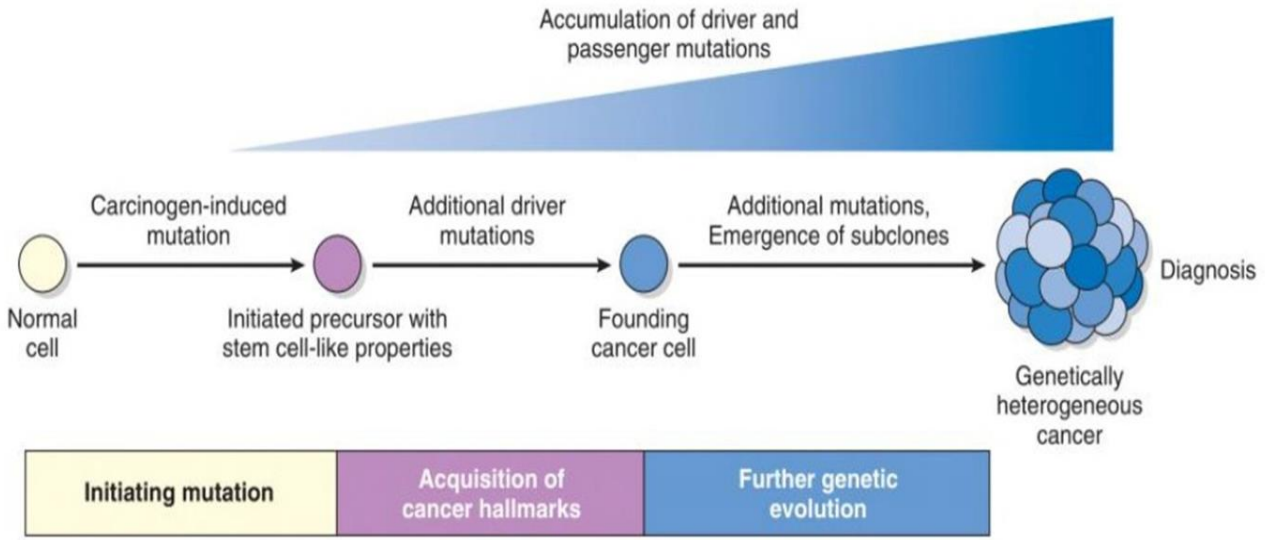


Fig. 6.16 Development of cancer through stepwise accumulation of complementary driver mutations. The order in which various driver mutations occur is usually unknown and may vary from tumor to tumor.

Hallmarks of Cancer

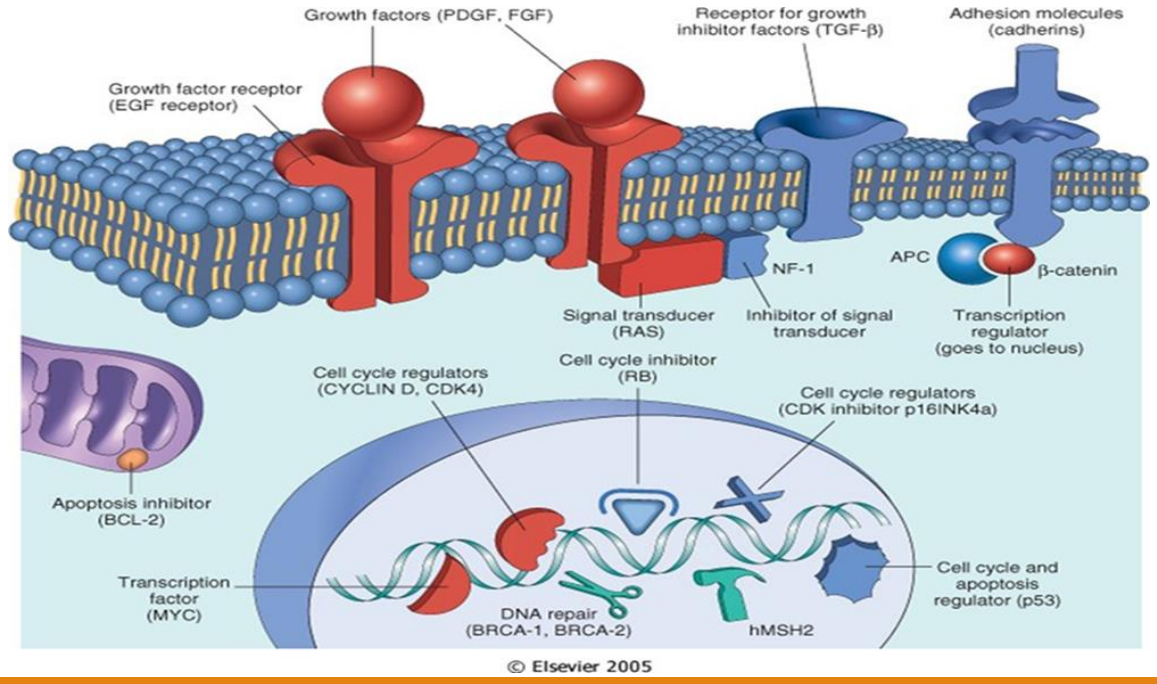
- All cancers appear to display eight fundamental changes in cell physiology and two enabling factors (genomic instability and tumor-promoting inflammation) that promote cellular transformation and subsequent tumor progression.

1. Self-sufficiency in growth signals
2. Insensitivity to growth-inhibitory signals
3. Altered cellular metabolism
4. Evasion of apoptosis
5. Limitless replicative potential (Immortality)
6. Sustained angiogenesis
7. Ability to invade & metastasize
8. Evasion of immune surveillance

1. Self-sufficiency in growth signals

- Gain of function mutation in Genes coding for growth: Classified by the site of action

- Proto-oncogenes: Normal.
- Oncogenes: Mutant/overexpressed
- oncogenes ➡ oncoproteins (promote cell growth, even without normal growth-promoting Signals).
- They include genes coding:
  1. Growth factors
  2. Cell surface receptors
  3. Signal transduction proteins
  4. Nuclear transcription factors
  5. Cell cycle proteins
  6. Inhibitors of apoptosis



1- Oncogenes coding Growth Factors

- Some cancers acquire the ability to synthesize the same growth factors (GF) to which they are responsive (autocrine) or send signals to activate normal cells in the supporting stroma to synthesize the same GF to which they are responsive (paracrine).
  - Platelet-derived growth factor (PDGF) seen in glioblastomas
  - Transforming Growth Factor (TGF- α) in sarcomas
- Products of other oncogenes (e.g. RAS) may cause overexpression of GF.

2- Oncogenes coding Growth Factor Receptors

- **Many of the growth factor receptors function as oncoproteins when they:**

- Mutant receptor ⟶ continuous signals even in the absence of GF…

Or Normal but overexpressed ⟹ hypersensitive to GF…

- Epidermal GF receptor family:

ERBB1 overexpressed in sq. CA lung

ERBB2 (Her2) amplified in breast Ca

- Increase = POOR PROGNOSIS

3- Oncogenes in signal transduction

- The signals are transmitted to the nucleus through various signal transduction molecules.

- Two important oncoproteins in the category of signaling molecules:

1. RAS

2.ABL

1. RAS

- RAS proteins **are inactive when bound to GDP**
- Stimulation of cells by growth factors: exchange of GDP for GTP and generate active RAS.
- Intrinsic guanosine triphosphatase (GTPase) of RAS hydrolyzes GTP to GDP, releasing a phosphate group and returning RAS to its quiescent GDP-bound state.
- The GTPase protein is magnified dramatically by a family of GTPase-activating proteins (GAPs).

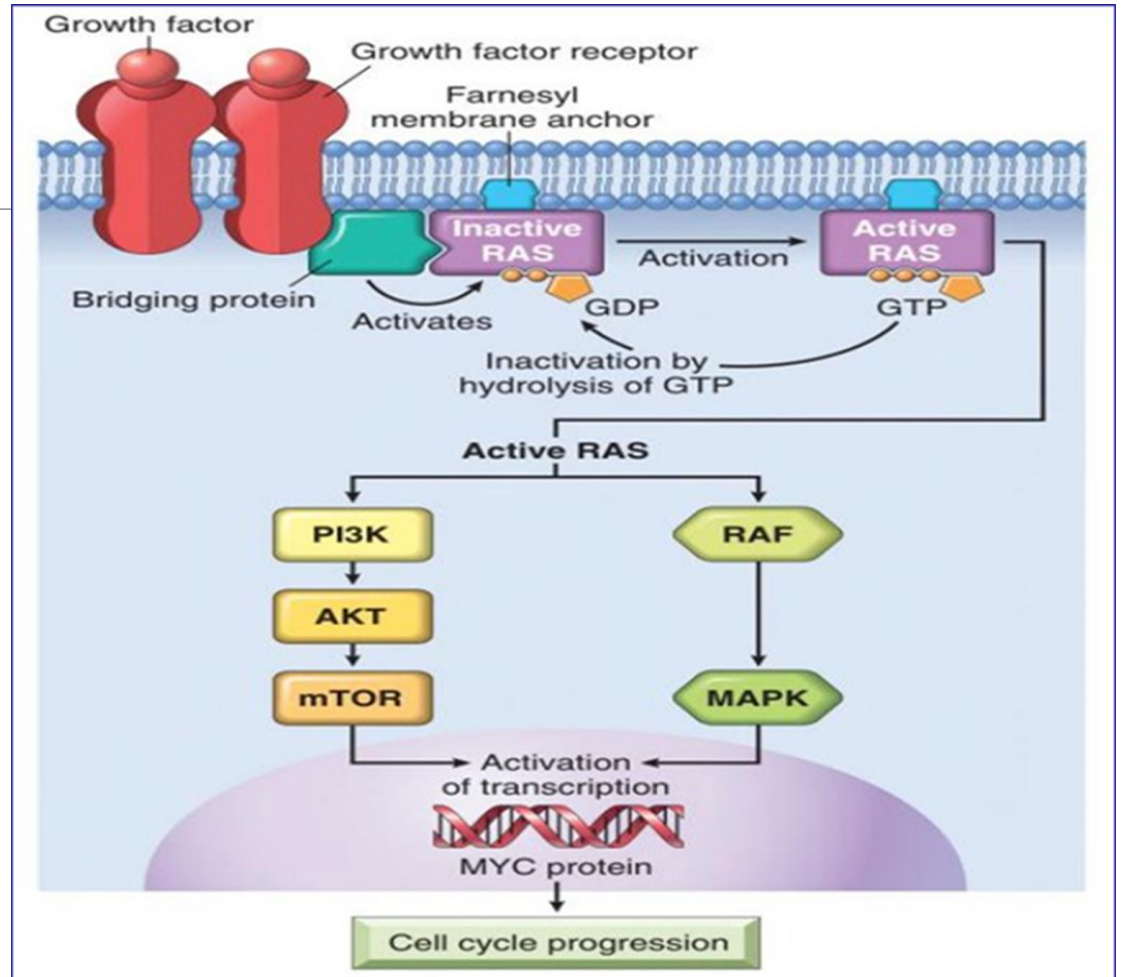


Fig. 6.18 Model for action of RAS. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS transduces proliferative signals to the nucleus along two pathways: the so-called "RAF/ERK/MAP kinase pathway" and the *PI3 kinase/AKT pathway*. *GDP*, Guanosine diphosphate; *GTP*, guanosine triphosphate; *MAP*, mitogen-activated protein; *PI3*, phosphatidylinositol-3.

- Active RAS → Signal transduction (RAF/MAP-K or PI3-K/AKT pathways) → transcription activation

- RAS most commonly is activated by point mutations in amino acid residues that are either within the GTP binding pocket or in the enzymatic region that carries out GTP hydrolysis

-Activated RAS stimulates downstream regulators of proliferation by several interconnected pathways.

- Commonest oncogene mutation in human tumors.

- Point mutations in codons 12, 13 are present in 30% of cancers, especially CA pancreas & Colon.

2. ABL:

- Non-receptor tyrosine kinase function as signal transduction molecule
- The ABL proto- oncogene has tyrosine kinase activity dampened by internal negative regulatory domain

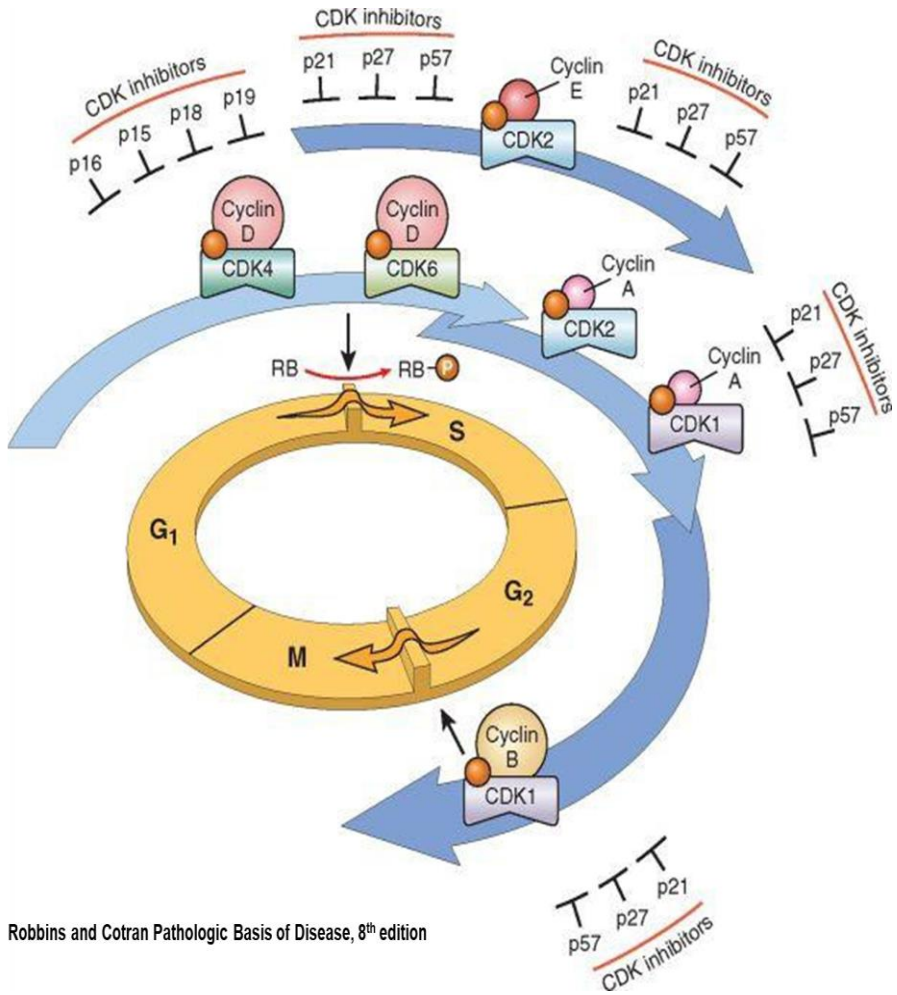
- Chronic myeloid leukemia: t(9;22) ⟹ BCR-ABL hybrid gene
- This new gene protein is retained in the cytoplasm where it has tyrosine kinase activity activates all of the signals downstream of RAS ⟹ cell proliferation

4- Nuclear Transcription Factors:

- DNA transcription regulated by genes e.g. MYC, JUN,….etc. that regulate the expression of growth-promoting genes, such as cyclins ⟶ initiation of cell cycle
- MYC mutation ⟹ sustained activation
- Examples:
  - Dysregulation of *MYC* in Burkitt lymphoma

5- Cyclins & Cyclin Dependent – Kinases regulate cell cycle phases

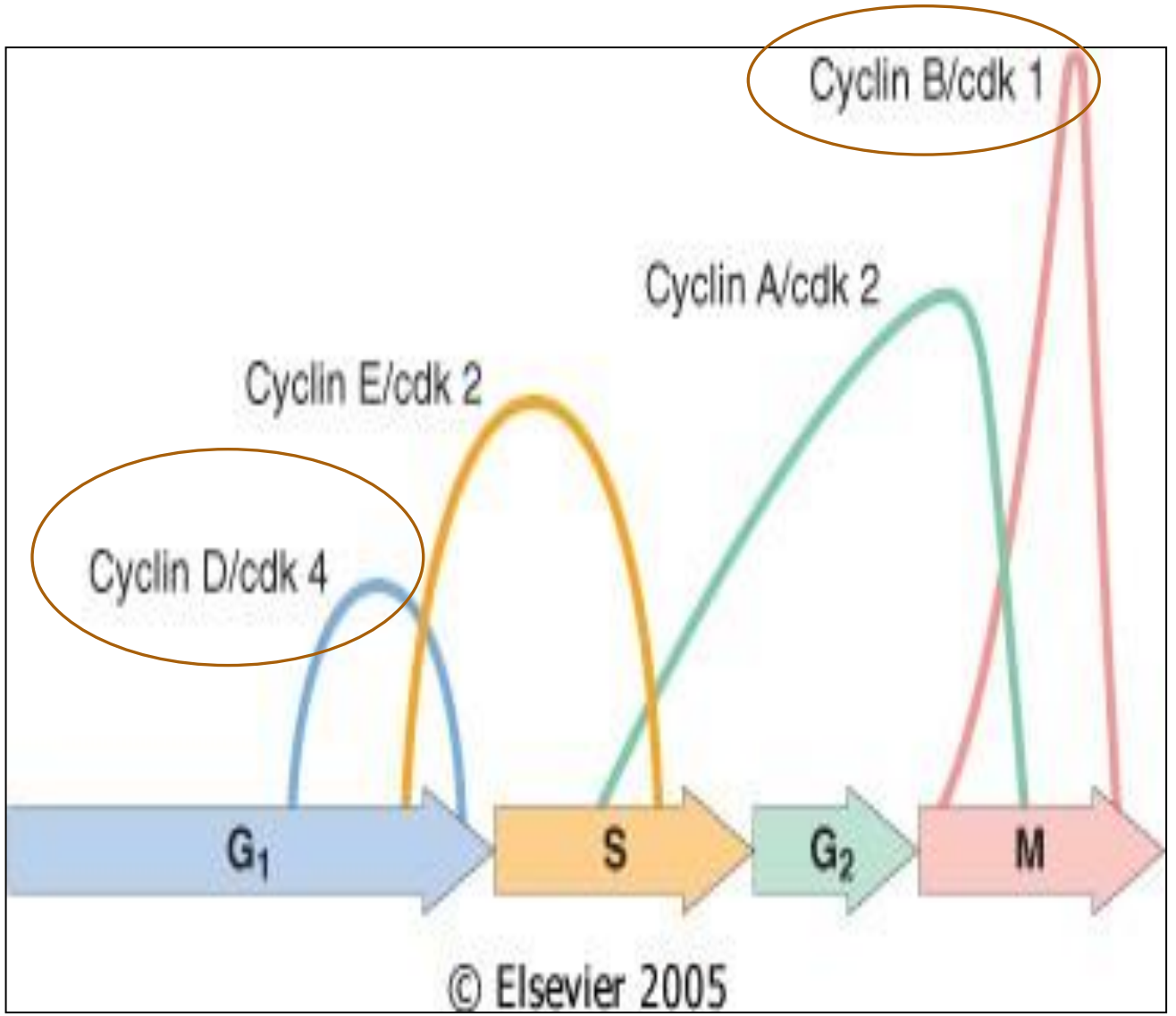
- Family of proteins that control entry of the cells at specific phases of cell cycle (D, E, A, B….etc.)
- Level of a specific cyclin increases at a specific phase, then decreases rapidly after the cell departs that phase
- Function by **phosphorylating certain proteins (e.g. RB protein)**
- Cyclins bind to CDKs, activating them



Robbins and Cotran Pathologic Basis of Disease, 8th edition

Two important checkpoints, each of which is tightly regulated by a balance of growth-promoting and growth-suppressing factors, as well as by sensors of DNA damage:

- **G1 - S phase checkpoint:**
Cyclin D family---CDK4 & CDK6
- **G2 - M transition:** Cyclin B-CDK1



- CDK inhibitors regulate the activity of CDK/ Cyclin.

- Selective or nonselective inhibition.

- Examples: p21, p27 & p57 inhibit all CDKs while INK4 Inhibitors (p15, p16, p18 & p19) inhibit CDK4 & CDK6.

- The tumor suppressor protein p53 controls expression of p21.

Mutations that dysregulate activity of cyclins & CDKs → Gain-of-function mutations and cell proliferation:

e.g.: Cyclin D is overexpressed in breast

Loss-of-function mutations involving CDKIs ⟶ cell proliferation:

- Disabling mutations of CDKN2A (encoding p16): germline (in melanoma)
- Acquired deletion or inactivation of *CDKN2A* is seen in pancreatic carcinomas

A final consideration :

- The increased production of oncoproteins does not by itself lead to a sustained proliferation of cancer cells.

- There are two built-in mechanisms, **cell senescence and apoptosis**, that oppose oncogene-mediated cell growth.

- Therefore, genes that regulate these two braking mechanisms must be disabled to allow the action of oncogenes to proceed unopposed.

2.Insensitivity to growth-inhibitory signals

- **Disruption in Cancer Suppressor Genes**

- Growth inhibitory pathway by:

RB gene: Regulate cell cycle

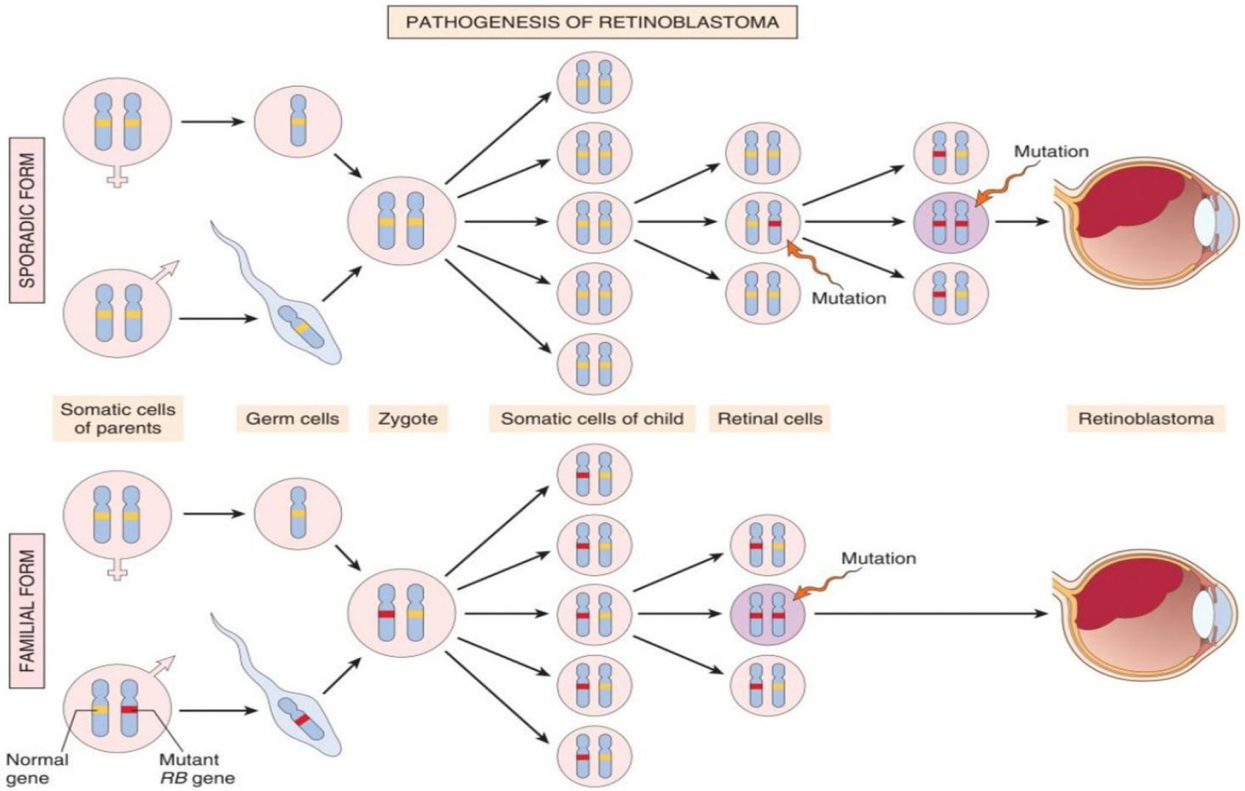
TP53 gene: Regulate cycle & apoptosis

TGF- β: Block GF signals

APC gene: regulates β –catenin

1- *RB* gene (Governor of cell cycle):

- The first tumor **suppressor** gene to be discovered
- First studied in Retinoblastoma
- *RB* gene ⟹ RB protein
- Both copies of the gene must be lost for neoplastic transformation to occur
- This is called loss of heterozygosity
- Familial (*RB* ⇨ *RB*) or
- Sporadic (*RB* ⇨ *RB* ⇨ *RB*)



Mode of action of *RB* gene:

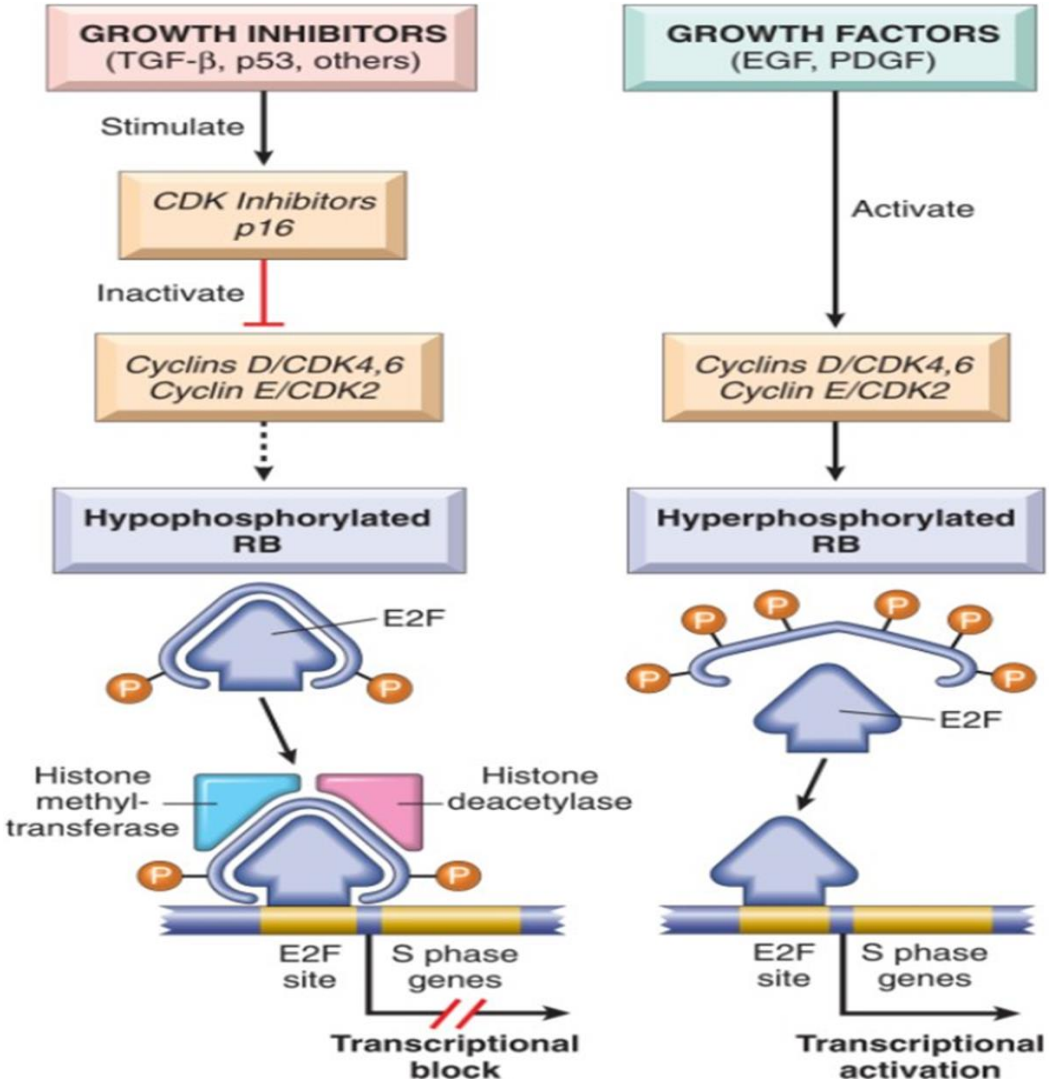
- **The function of the RB protein is to regulate the G1/S** checkpoint, the portal through which cells must pass before DNA replication commences.

- G1 – S transition and DNA replication require the activity of cyclin E/CDK2

- Cyclin E is dependent on the E2F family of transcription factors

- **Active hypophosphorylated RB** binds to & inhibits the E2F family of transcription factors --- NO TRANSCRIPTION of cyclin E

Growth factor signaling leads to cyclin D expression and activation of cyclin D- CDK4/6 complexes, which ⟹ phosphorylate RB, inactivating the protein and releasing E2F ⟹ induce target genes such as cyclin E. TRANSCRIPTION (G1 -- S phase)



Retinoblastoma:

- Sporadic in 60% of cases
- Familial (40%), AD
- In familial form, patients carry one mutation in their genome, followed by a second mutation in retinal cells
- No tumor develops unless two alleles in chr. 13 become mutant (two hit theory)
- Familial form: ↑ incidence of bilateral ret., osteosarcoma, and other tumors

2- TP53 (Guardian of Genome)

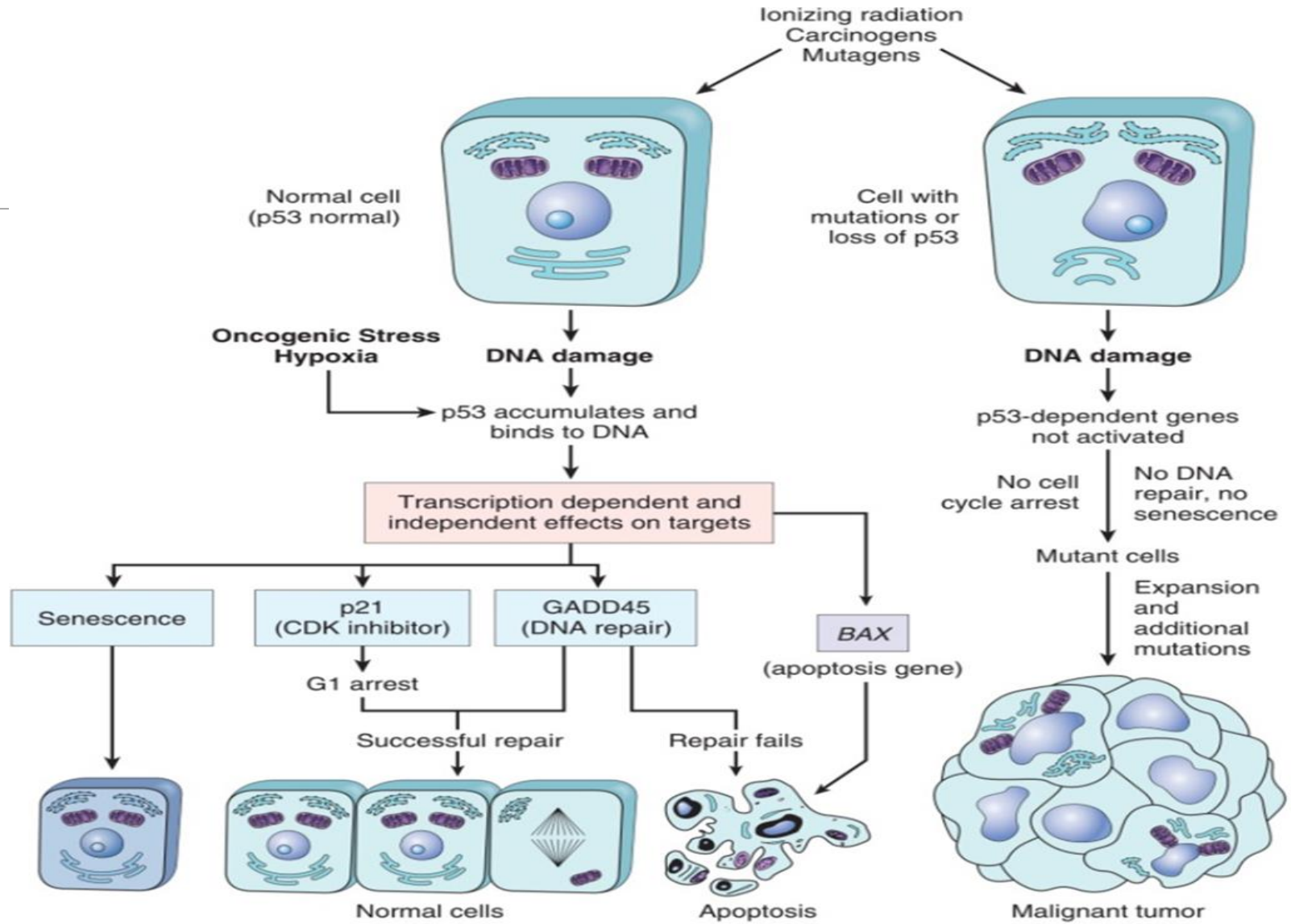
- **The most commonly mutated gene (and suppressor gene) in human cancer.**
- Homozygous loss in 70% of cancers
- *TP53* is a negative regulator of the cell cycle (protein product is p53).
- 'Guardian of the Genome' OR (Policeman) preventing genetically damaged cells from progressing through a new cycle.
- p53 is inactivated by MDM2.
- Upon DNA damage or other stresses, various pathways will dissociate the p53 and MDM2 complex.

Mode of activation & action:

- p53 senses DNA damage or other stresses through various sensors, like protein kinases e.g. ATM protein
- p53 released from MDM2 & activated with longer half-life →
  - Transcription of CDKI gene (p21) → cell cycle arrest at G1 (Quiescence) ---Result: more time for repair --- Normal

OR

- If repair fails ----Senescence (permanent cell cycle arrest) or Apoptosis (p53 is a positive regulator of apoptosis)
- **OR** *Fixed mutation* --- ***NEOPLASIA***


Ionizing radiation
Carcinogens
Mutagens
Normal cell
(p53 normal)
Cell with
mutations or
loss of p53
Oncogenic Stress
Hypoxia
DNA damage
DNA damage
p53 accumulates and
binds to DNA
p53-dependent genes
not activated
No cell
cycle arrest
No DNA
repair, no
senescence
Transcription dependent and
independent effects on targets
Mutant cells
Expansion
and
additional
mutations
Senescence
p21
(CDK inhibitor)
GADD45
(DNA repair)
BAX
(apoptosis gene)
G1 arrest
Successful repair
Repair fails
Normal cells
Apoptosis
Malignant tumor

❑ Significance of TP53 mutation:

- Acquired mutation in many cancers

  e.g. colon, lung, …etc

- Inherited mutation in one allele---

  Li-Fraumeni syndrome – 25-fold ---malignancy: sarcoma, breast carcinoma….. etc

3- Transforming Growth Factor-β (TGF-B):

A potent inhibitor of proliferation (Antiproliferative activity): -

Act by binding to a complex composed of TGF-β receptors I and II, resulting in the transcriptional activation of **CDKIs** with growth-suppressing activity and **repression** of growth-promoting genes.

- Mutations may alter the **type II TGF-β receptor.**

-Mutational inactivation of TGF-β components seen in a very high percent of pancreatic carcinoma

4- Contact Inhibition, APC:

- Cell–cell contacts in many tissues are mediated by homodimeric interactions between transmembrane proteins called **cadherins**.

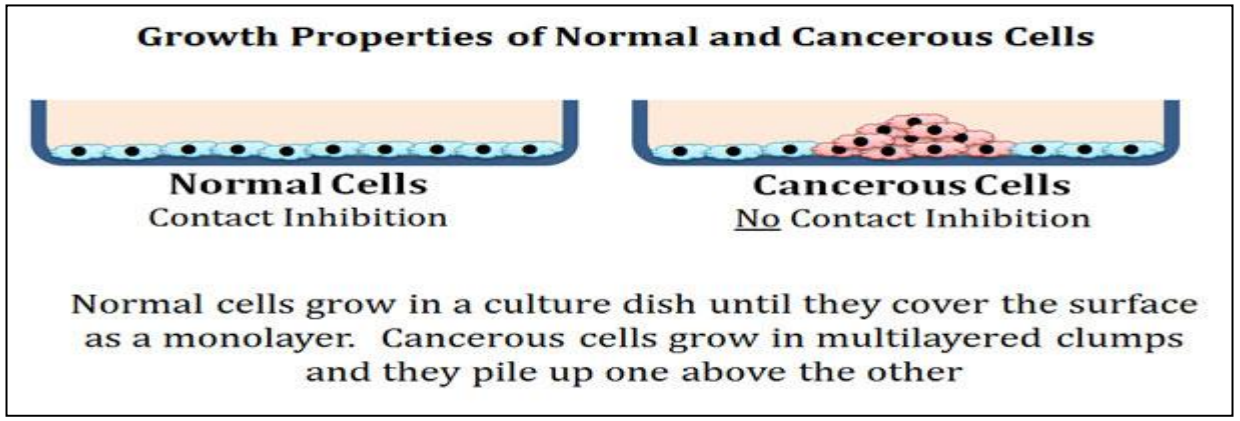
  - **E-cadherin** (E for epithelial) mediates cell–cell contact in epithelial layers.

- **Contact inhibition** enables noncancerous cells to cease proliferation and growth when they form confluent monolayers and **contact** each other.

- **Two mechanisms have been proposed to explain how E-cadherin maintains contact inhibition:**

1-Tumor suppressor gene *NF2*

2- **APC** gene and **B catenin** (**a key component of the WNT signaling pathway**).



-This characteristic is lost when cells undergo malignant transformation, leading to uncontrolled proliferation and solid tumor formation.

By:

-E-cadherins are reduced in many cancers.

- Mutant APC.

ACTION OF APC GENE:

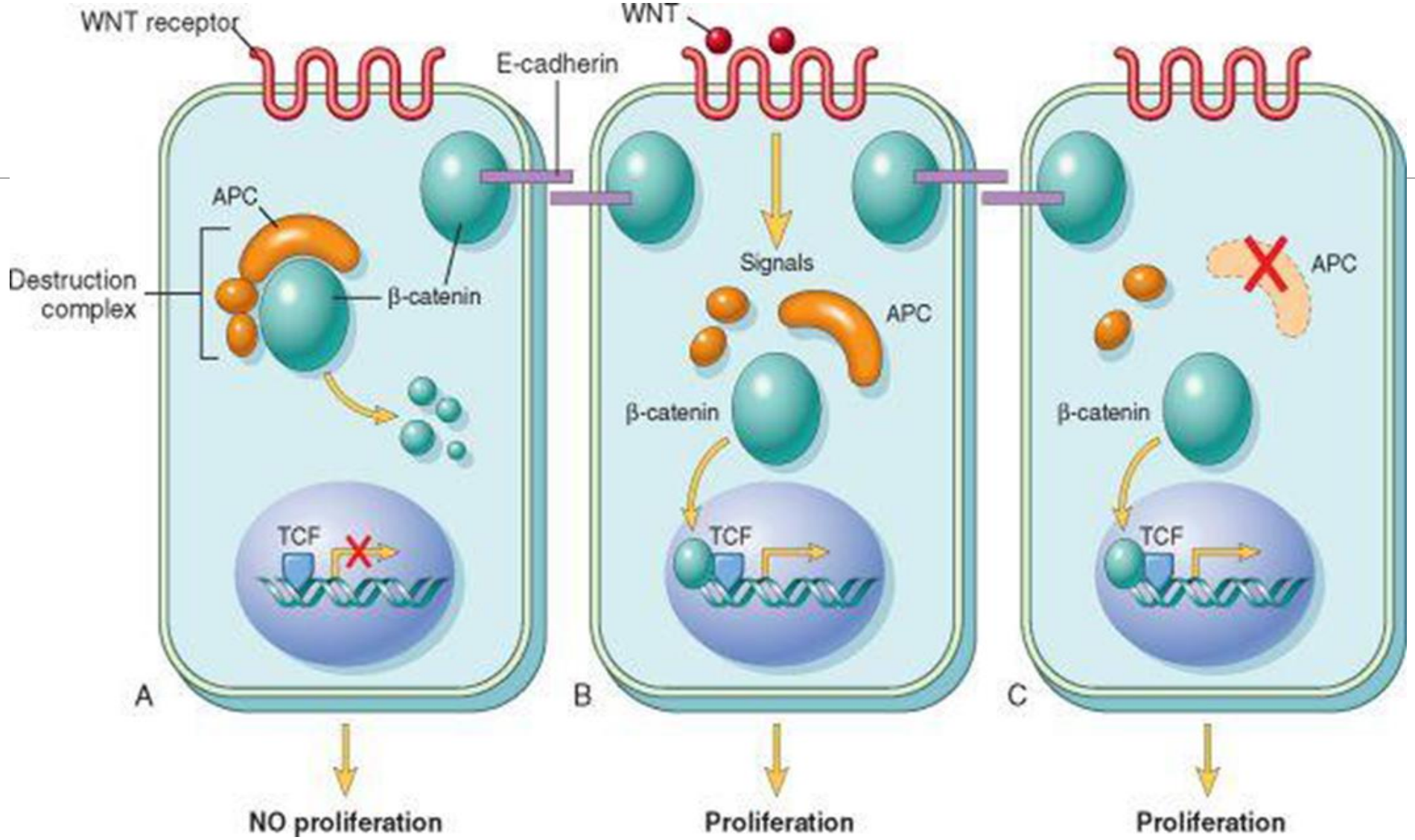
- Gene product is a cytoplasmic protein that acts in adhesion by regulating the destruction of **β-catenin** in the cytoplasm.

- In quiescent cells that have not been exposed to WNT, cytoplasmic β-catenin is degraded by a *destruction complex, so no proliferation of cells occur.*

E-Cadherin+ APC + β-catenin → **Destruction Complex**

- With the loss of APC (in malignant cells), β-catenin degradation is prevented, and the WNT signaling response is inappropriately activated in the absence of

WNT ⟶ transcription of growth-promoting genes as well as transcriptional regulators that repress E-cadherin expression and thus reduce contact inhibition and proliferation.


WNT receptor
E-cadherin
WNT
APC
Destruction complex
β-catenin
Signals
APC
APC
β-catenin
β-catenin
TCF
TCF
TCF
A
B
C
NO proliferation
Proliferation
Proliferation

Clinical significance of APC ⟶

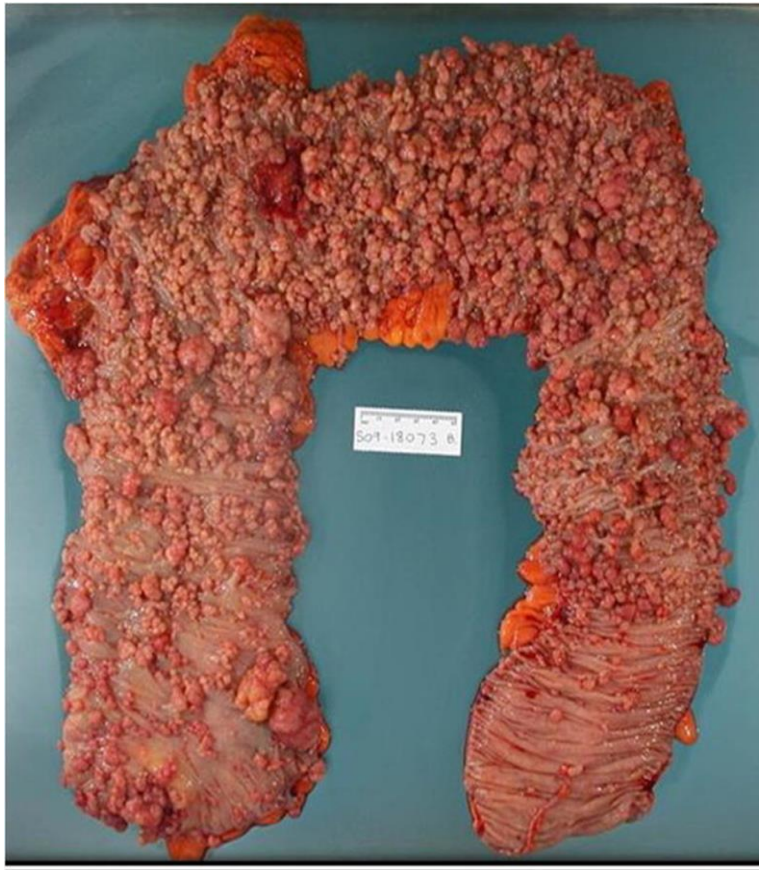
Familial Adenomatous Polyposis Coli (FAP)

- **AD** syndrome.
-Individuals with inherited one mutant allele of APC develop 100s to 1000s of adenomatous polyps by their teens or twenties.

-Additional mutations → colonic carcinoma (100% ↑↑ risk).

-70-80% of sporadic colonic carcinoma show mutant APC.

-Colonic cancers with normal APC have activating mutations of **β-catenin.**



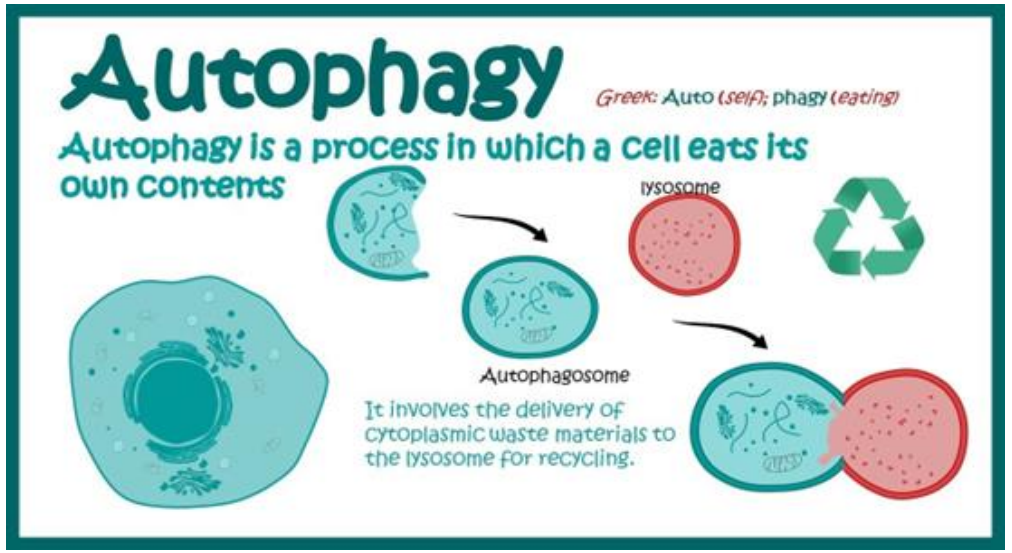
3. Altered cellular metabolism

AUTOPHAGY:

- A state of severe nutrient deficiency in which cells arrest their growth and cannibalize their own organelles, proteins, and membranes (into lysosomes) as carbon sources for energy production.

-Cancer cells may accumulate mutations avoiding autophagy OR alter the process making it inefficient.

Result: Prolonged cell life!,



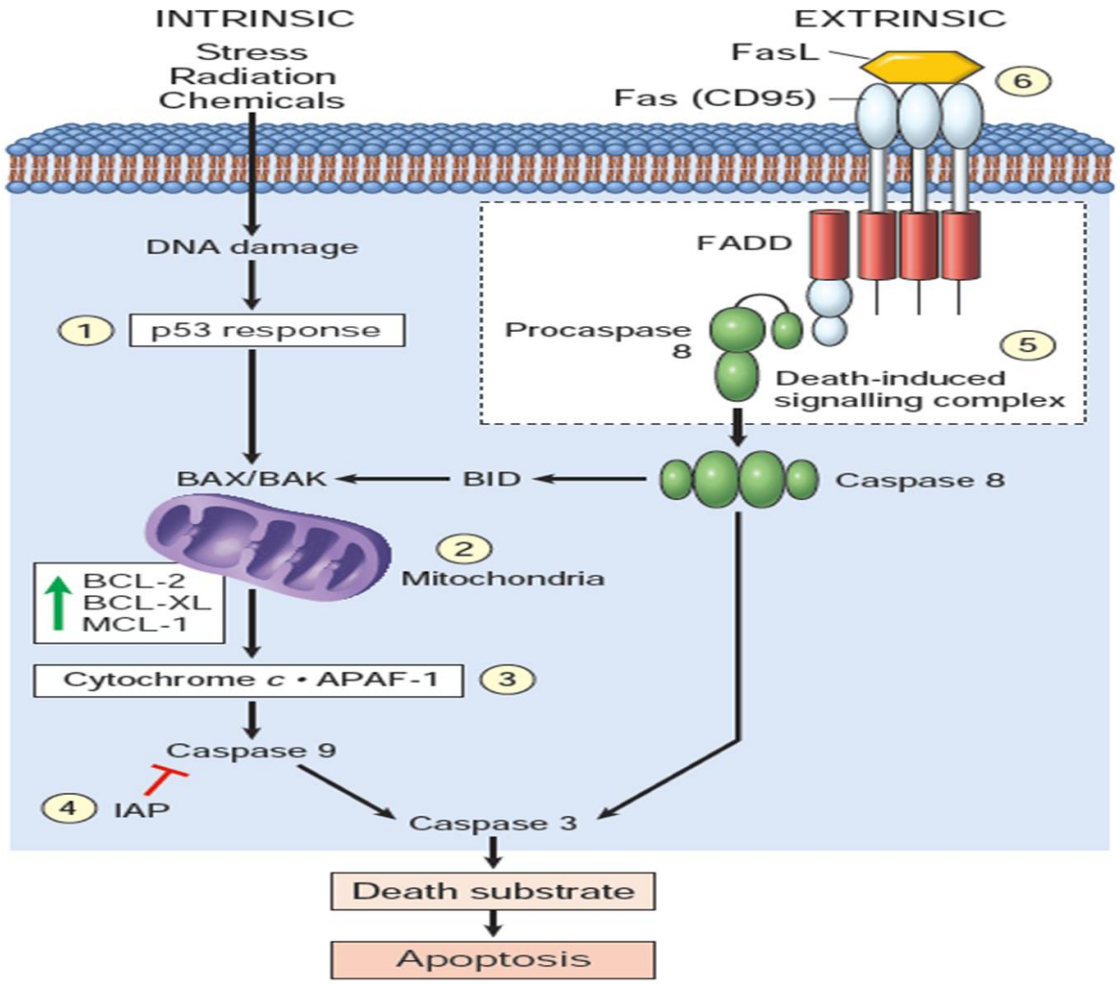
4- Evasion of apoptosis:

BCL-2 Family:

BAX, BAK : Pro-apoptotic

BCL-2 , BCL-XL, MCL1: Anti-apoptotic

BH3-only proteins regulate the balance



- Cancer cells are subject to several intrinsic stresses that can initiate apoptosis, particularly DNA damage.

- Tumor cells frequently contain mutations in genes that regulate apoptosis, making the cells **resistant to cell death.**

Apoptosis:

Extrinsic pathway: - Some tumors have **↓levels of CD95** → ↓ Apoptosis

Intrinsic pathway (*mitochondrial pathway*):

1. **loss of p53 function**, either by way of TP53 mutations or overexpression MDM2.

2. **overexpression of anti-apoptotic members of the BCL2** family, which protect cells from the action of the pro-apoptotic members of the BCL2 family.

5.Limitless replicative potential (immortality):

- Most normal cells have a capacity of at most 70 doublings. Thereafter, the cells lose the ability to divide and enter replicative senescence due to the progressive shortening of telomeres at the ends of chromosomes.

Telomeres Are specialized structures at the end of chromosomes that are shortened after each division and may play a role in determining the life of individual cells.

- Shortening is prevented by TELOMERASE (Active in stem cells, not in somatic cells).

- Tumor cells, unlike normal cells, are capable of limitless replication.

- In many cancers, telomerase is reactivated.

6- Sustained Angiogenesis :

- Tumors remain small or in situ (< 1-2 mm,Diameter) without angiogenesis.

1- Supplies needed nutrients and oxygen.

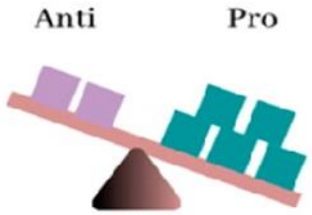
2- Newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors.

-The resulting tumor vasculature is effective at delivering nutrients and removing wastes, it is not entirely normal; the vessels are leaky and dilated.

-The molecular basis of the angiogenic switch involves increased production of angiogenic factors and/ or loss of angiogenic inhibitors.

Angiogenesis ≈ Antiangiogenesis

Angiogenic Switch



- These factors may be produced by the tumor cells or by inflammatory

cells (e.g., macrophages) or resident stromal cells (e.g. fibroblasts).

Angiogenic factors:

- Controlled by HYPOXIA which induces angiogenic factors by tumor cells ➡ Hypoxia-Inducible Factor **(HIF-1α)**→**VEGF** ⟶ stimulates the proliferation of endothelial cells and guides the growth of new vessels toward the tumor.

- Gain-of-function mutations in *RAS* or *MYC* upregulate the production of VEGF:↑ VEGF

- Proteases from tumor or stroma can release the basic angiogenic **FGF** stored in the ECM

❖ Anti- angiogenesis:

1. **Thrombospondin1**(TSP-1) induced by P53

- Thus, loss of p53 in tumor cells provides a more permissive environment for angiogenesis

2. **VHL** protein destroys HIF-1 α → No VEGF

- Germline mutation of VHL → von Hippel-Lindau Syndrome → hereditary renal CA, CNS hemangiomas.

3. Angiogenesis inhibitors:

Angiostatin, Endostatin, Vasculostatin from stromal cells in ECM.

↑ vascular density = Poor prognosis

7- Ability to invade & metastasize:

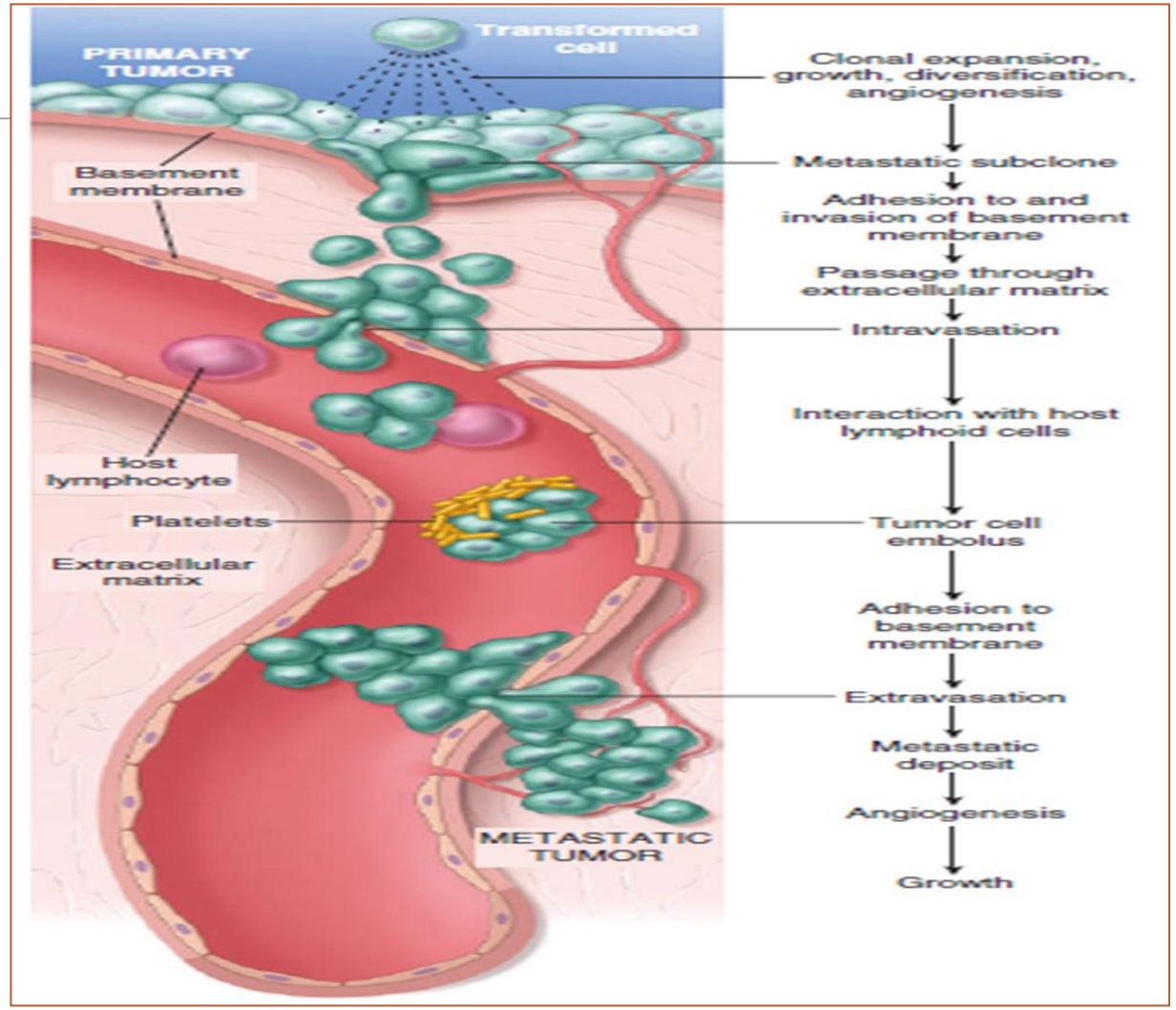
-Tumors may generate clones and accumulate mutations, leading increased rate of growth, Invasion, Metastases …

Metastasis occurs in two phases:

1- Invasion of extracellular matrix

- Composed of collagens, glycoproteins & proteoglycans.

2- Vascular dissemination and homing of tumor cells



1- Mechanism Of Invasion Of ECM:

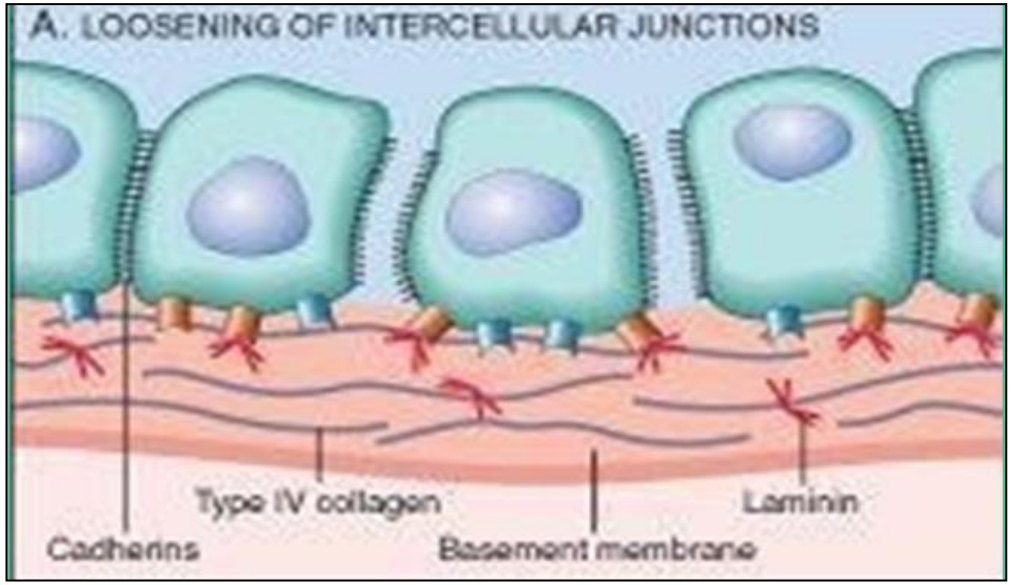
1- Loosening of intercellular connections between tumor cells and Detachment:

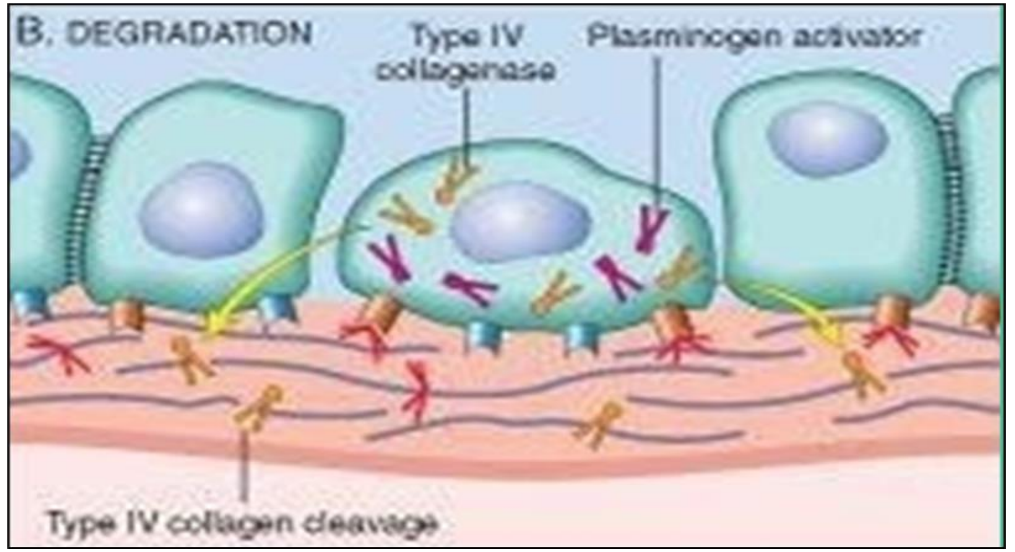
- Inactivation of E-Cadherin OR activation of β catenin→ detachment of cells.

2- Degradation of ECM by proteases:

e.g. Matrix Metalloproteinase (MMPs), Cathepsin D, Type IV collagenase

Result of digestion of ECM → Cleavage products have chemotactic activity for more tumor cells



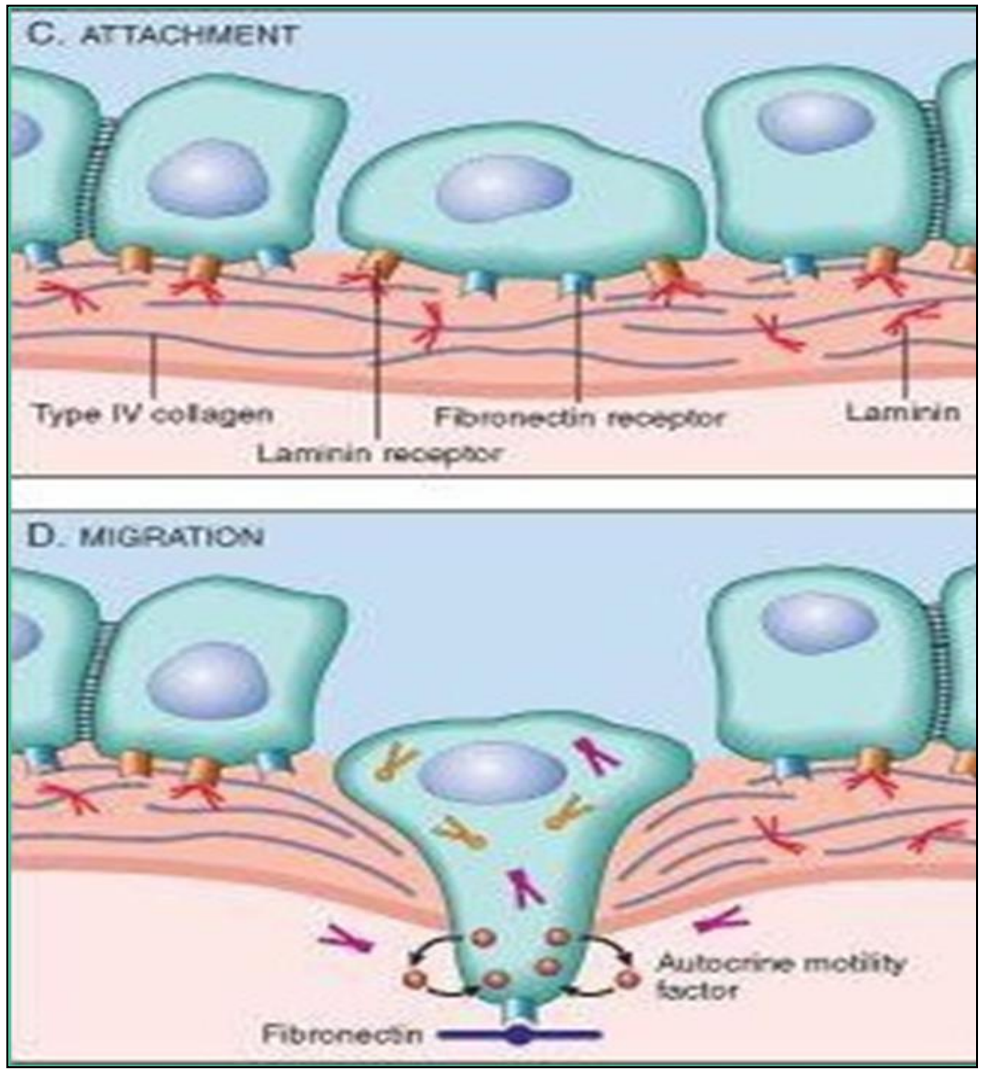


3- Attachment of tumor cells to matrix components

4- Migration of tumor cells (*Locomotion)*:

-Propelling tumor cells through the degraded basement membranes and zones of matrix

- <u>Such movement seems to be directed by</u>
  - Tumor-derived cytokines
  - Motility factor from Stromal cell



2- Vascular dissemination:

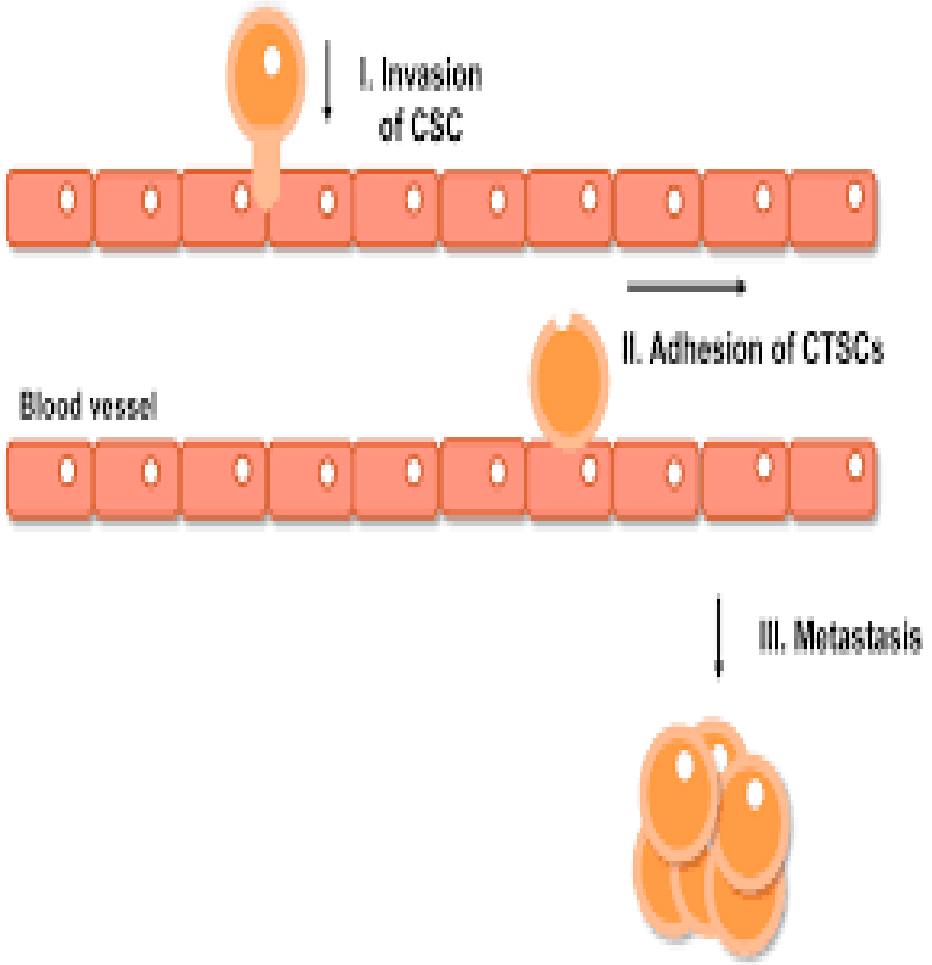
1- Invasion of the circulation:

Adhesion to endothelium→ retraction of endothelium → vessel

2- Attack by NK cells, some escape by formation of a thrombus/embolus

3- Escape from circulation:

Adhesion to endothelium→ retraction of endothelium → escape to tissue



What influences site of metastases ?

- **Anatomical Location and vascular drainage of the primary tumor.**
- Complimentary adhesion molecule between tumor cells & target organs
- Chemoattractants liberated by target organs
- Protease inhibitors present in certain tissues

EXAMPLES OF TROPISM (HOMING)

Lung Carcinoma → Adrenals & Brain
Neuroblastoma → Liver & Bone

- Less common sites of metastases: muscle, skin, thyroid, heart …etc.

- Spleen & Cartilage are almost never involved by metastatic tumors.

8. Evasion of Immune Surveillance

TUMOR IMMUNITY: Host Defense Against Tumors:

-Normal immunity present to protect against the development of tumors
-Tumors have ANTIGENS counteracted by ANTIBODIES in the body

Evidence?

- The direct demonstration of tumor-specific T cells and antibodies in patients
- When there is no immunity (immunosuppressed patients) → More Cancers
- Patients with congenital **immune deficiency** have **↑risk of cancer**

Host defenses

- CTLs (CD8+ T-Cells)
- NK cells
- T helper cells
- Macrophages
- Humoral (Antibodies)

Types of tumor antigens:

1- Products of mutant oncogenes & tumor suppressor genes

2- Mutant proteins in chemical and radiation-induced tumors

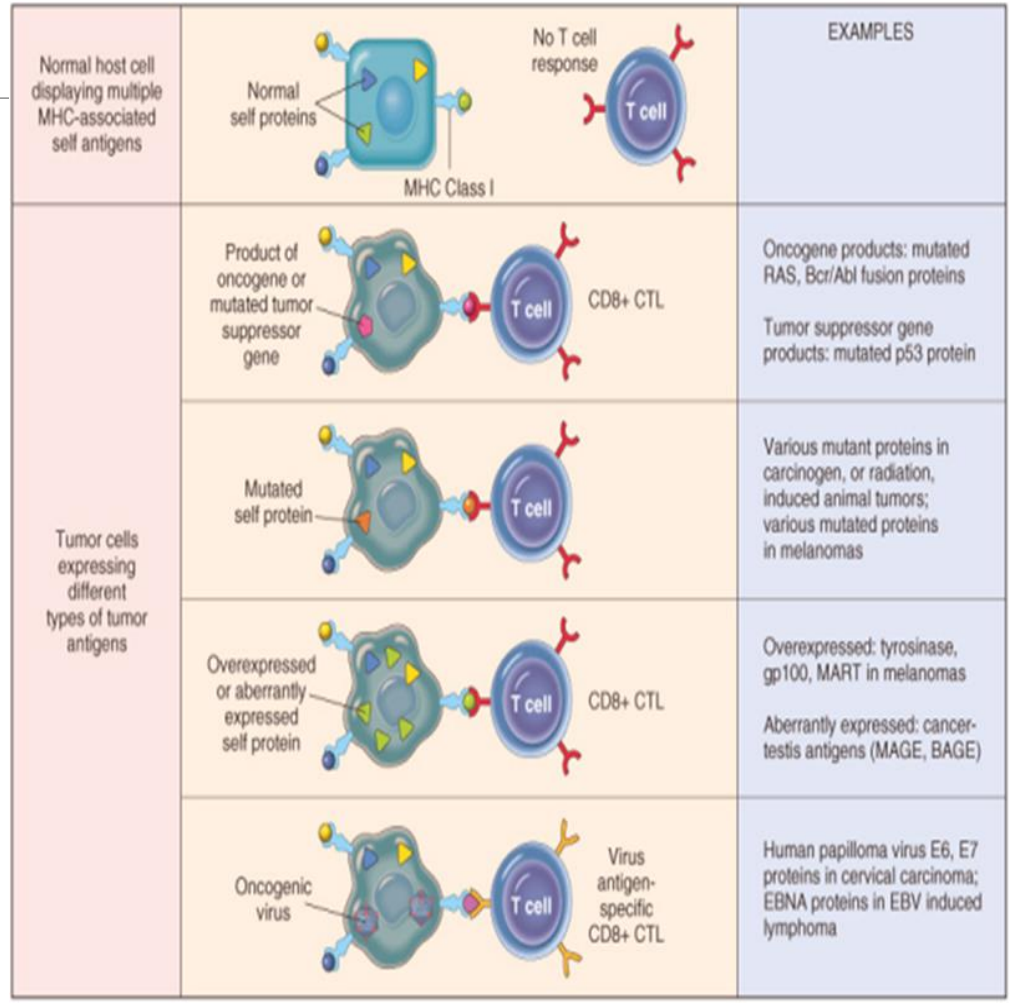
3- Overexpressed or aberrantly expressed cellular proteins.

e.g.: **Tyrosinase in melanoma**

4- Tumor AG produced by oncogenic viruses in HPV (E6,E7)

5- Oncofetal AG: CEA and α fetoprotein

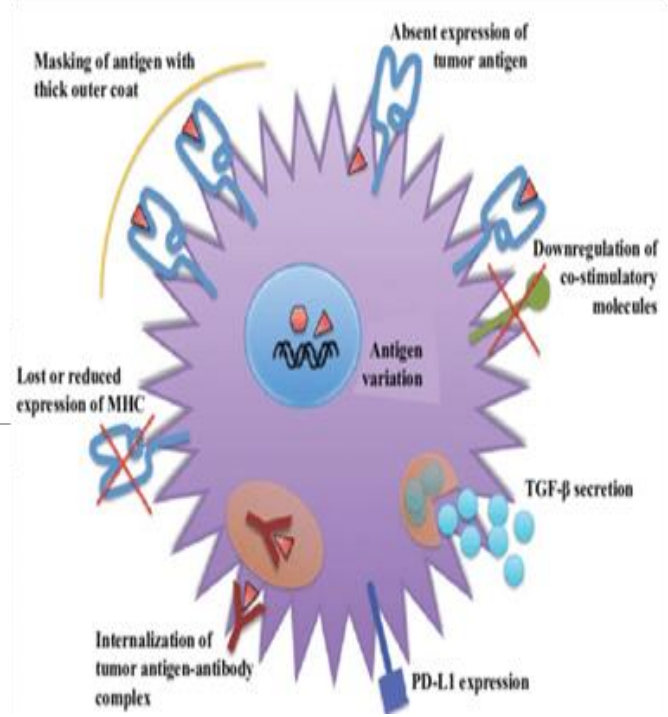
6- Several mucins MUC-1



How do tumor cells escape immune surveillance?

-In immunocompetent patients, tumors may avoid the immune system by :

- Selective outgrowth of antigen-**negative** variants.
- **Loss or reduced expression** of MHC molecules on tumor cell surface
- **Immunosuppression** mediated by expression of certain factors (e.g. Pd-1 ligands) by the tumor cells.
- **Antigen masking**
- **Downregulation of co-stimulatory molecules** (sensitization of T- cells requires costimulatory molecules).



Genomic Instability as an Enabler of Malignancy:

- Individuals born with inherited **defects in DNA repair genes** are at greatly **increased risk for the development of cancer.**

- Includes:

- **Mismatch repair**
- **Nucleotide excision repair**
- **Recombination repair**

1- Mismatch repair genes:

- These repair errors in the pairing of nucleotides during cell division (Spell Checkers) e.g. G+T instead of A+T.

- Defective **in (HNPCC – Hereditary Nonpolyposis Colonic Ca. syndrome):**

  - This syndrome accounts for 2-4% of all **colonic ca, AD.**

- Carcinomas of the colon affecting predominantly the cecum and proximal colon (right colon)

- A characteristic finding in the genome of patients with mismatch repair defects is microsatellite instability (MSI).

2- Nucleotide excision repair genes

- **Defective in Xeroderma Pigmentosum:**
  - Autosomal recessive disorder.
  - Increased risk for cancers arising in sun-exposed skin.

-UV rays in sunlight cause cross-linking of pyrimidine residues. The nucleotide excision repair system repairs such DNA damage.

- Several proteins are involved in nucleotide excision repair, and the inherited loss of any one of these can give rise to xeroderma pigmentosum.

3. DNA Repair by Homologous Recombination:

- A group of AR disorders comprising **Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia** is characterized by hypersensitivity to DNA-damaging agents.

Theses have defects in DNA Repair by Homologous Recombination

BRCA-1 & BRCA-2:

- Cells with a defective version of these genes develop chromosomal breaks and severe aneuploidy. Both genes seem to function, at least in part, in the **homologous recombination DNA repair pathway**

- **50% of familial breast cancers & ovarian CA**

- Rarely inactivated in sporadic cases.


THANK
YOU