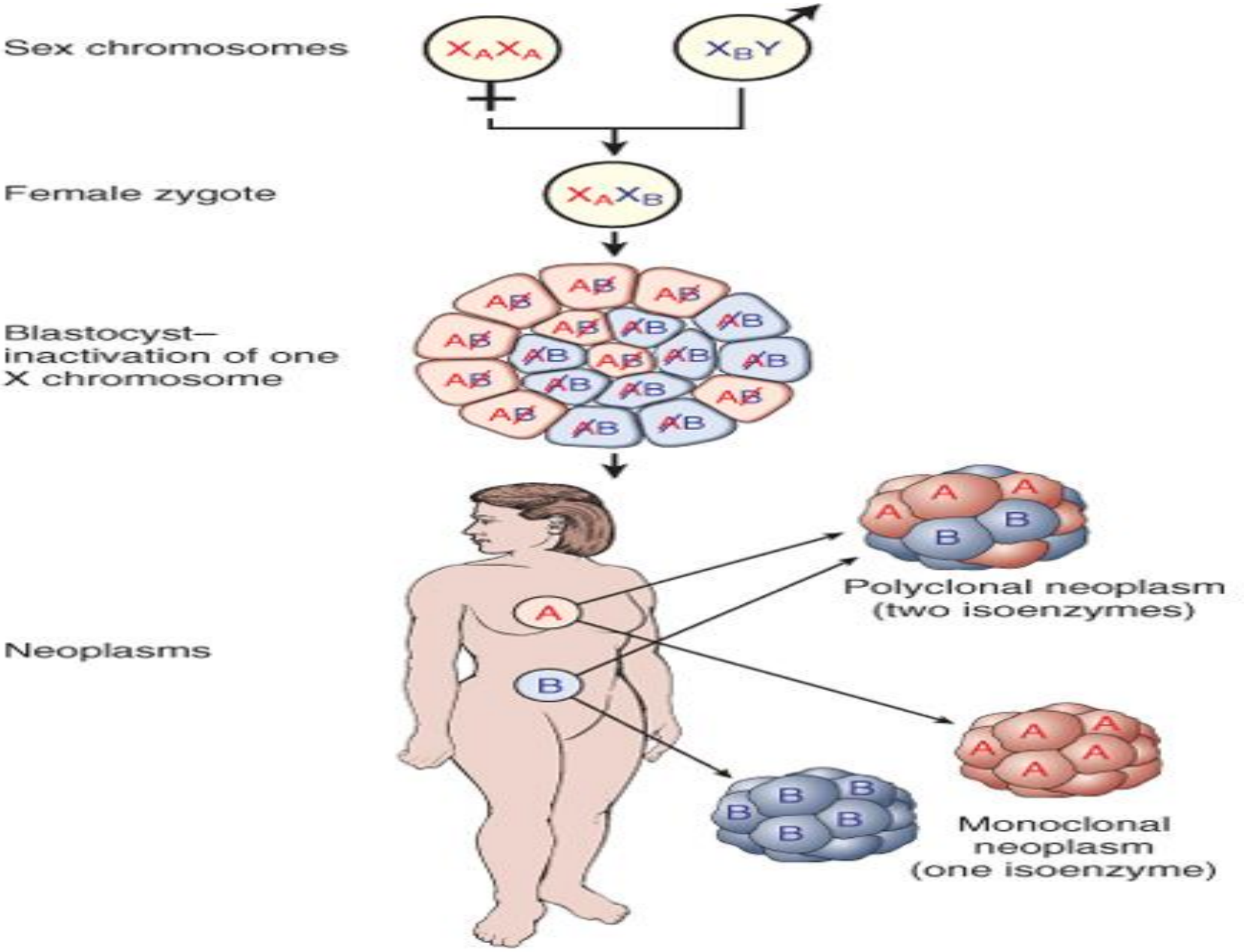
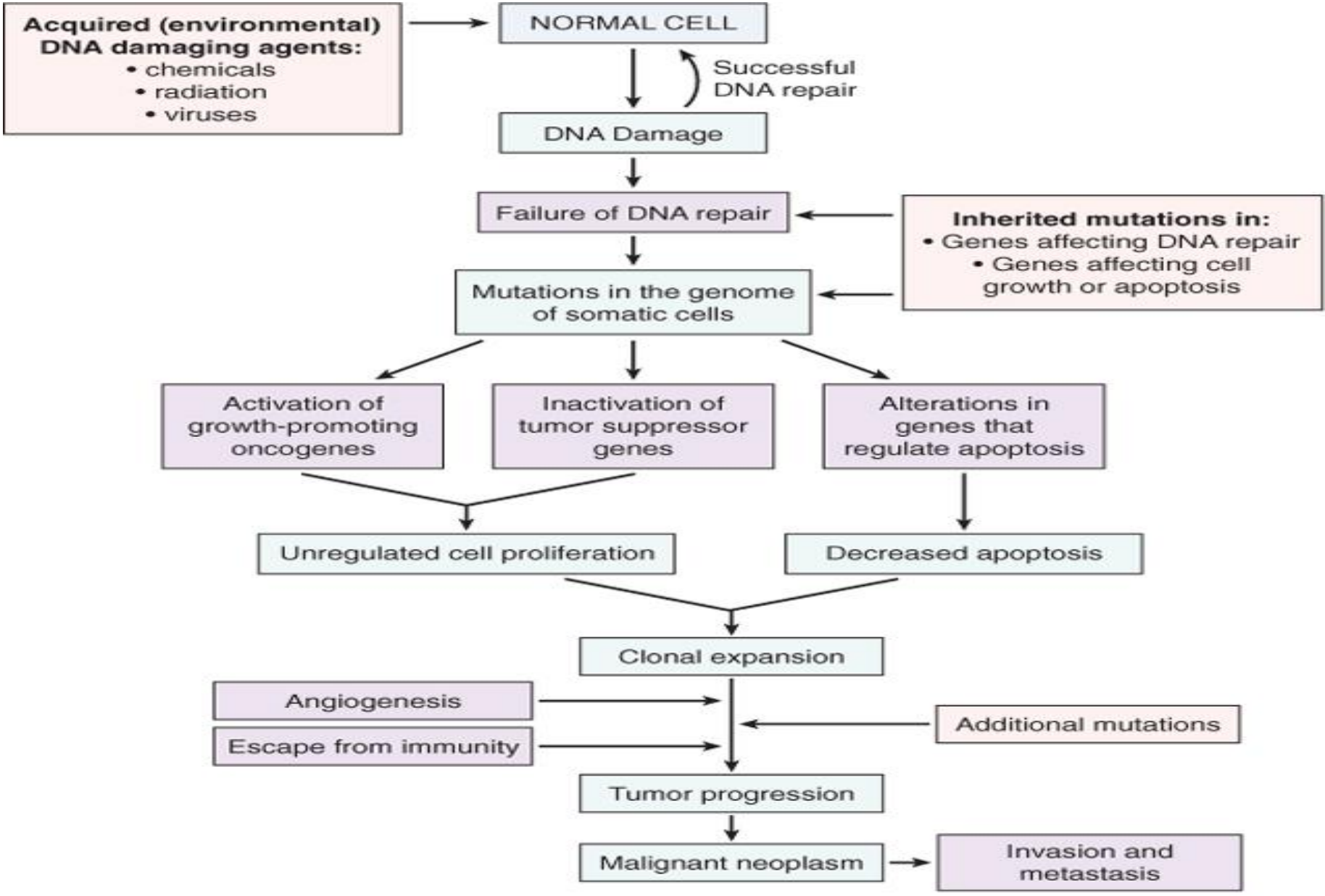
CARCINOGENESIS: THE MOLECULAR BASIS OF CANCER

- *Nonlethal genetic damage lies at the heart of carcinogenesis.*
- Mutation) may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line.

- **The genetic hypothesis of cancer implies that a tumor mass results from the clonal expansion of a single progenitor cell that has incurred genetic damage (i.e., tumors are monoclonal).**
- **Clonality of tumors is assessed readily in women who are heterozygous for polymorphic X-linked markers, such as the enzyme glucose-6-phosphate dehydrogenase or X-linked restriction-fragment-length polymorphisms.**



- ***Four classes of normal regulatory genes are involved :***
- ***1-growth-promoting proto-oncogenes,***
- ***2-growth-inhibiting tumor suppressor genes,***
- ***3-genes that regulate apoptosis***
- ***4-genes involved in DNA***



- **Mutant alleles of proto-oncogenes are called oncogenes.**
- **They are considered dominant because mutation of a single allele can lead to cellular transformation.**
- **Both normal alleles of tumor suppressor genes must be damaged for transformation to occur, referred to as recessive oncogenes.**

- **Genes that regulate apoptosis may be dominant, as are proto-oncogenes, or they may behave as tumor suppressor genes (recessive).**

- **Tumor suppressor genes are of 2 types :**
- **1- promoters genes**
- **2- caretakers genes**

- **Promoters are the traditional tumor suppressor genes, such as *RB* or *p53,***
- **mutation of these genes leads to cell transformation by releasing the control on cellular proliferation.**

- **Caretaker genes are responsible for processes that ensure the integrity of the genome, such as DNA repair.**
- **Mutation of caretaker genes does not directly transform cells by affecting proliferation or apoptosis.**
- **DNA repair genes affect cell proliferation or survival indirectly by influencing the ability to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and genes that regulate apoptosis.**

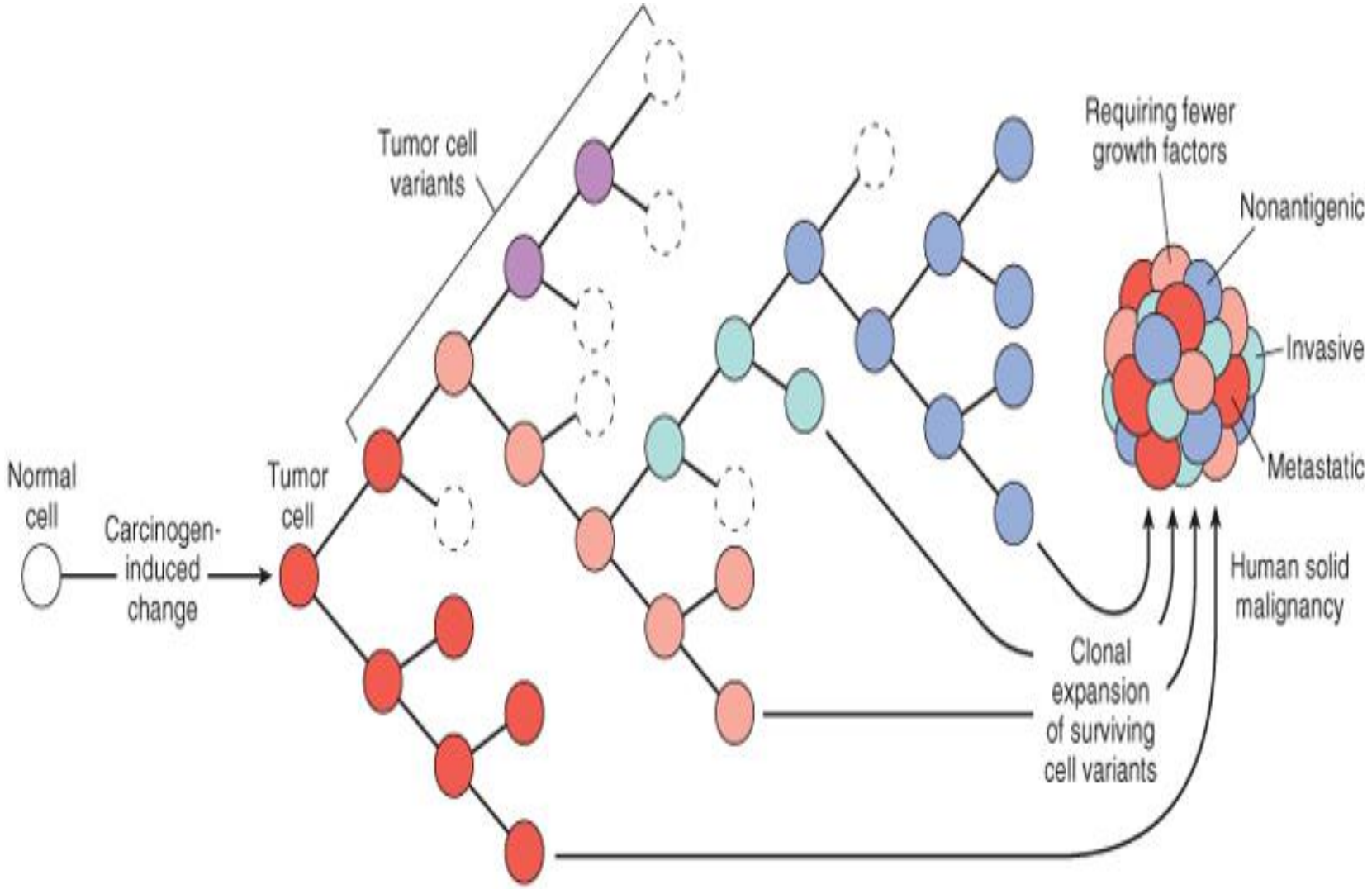
- ***Carcinogenesis is a multistep process* at both the phenotypic and the genetic levels, resulting from the accumulation of multiple mutations.**
- **Malignant neoplasms have several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases.**

- **Tumor progression**

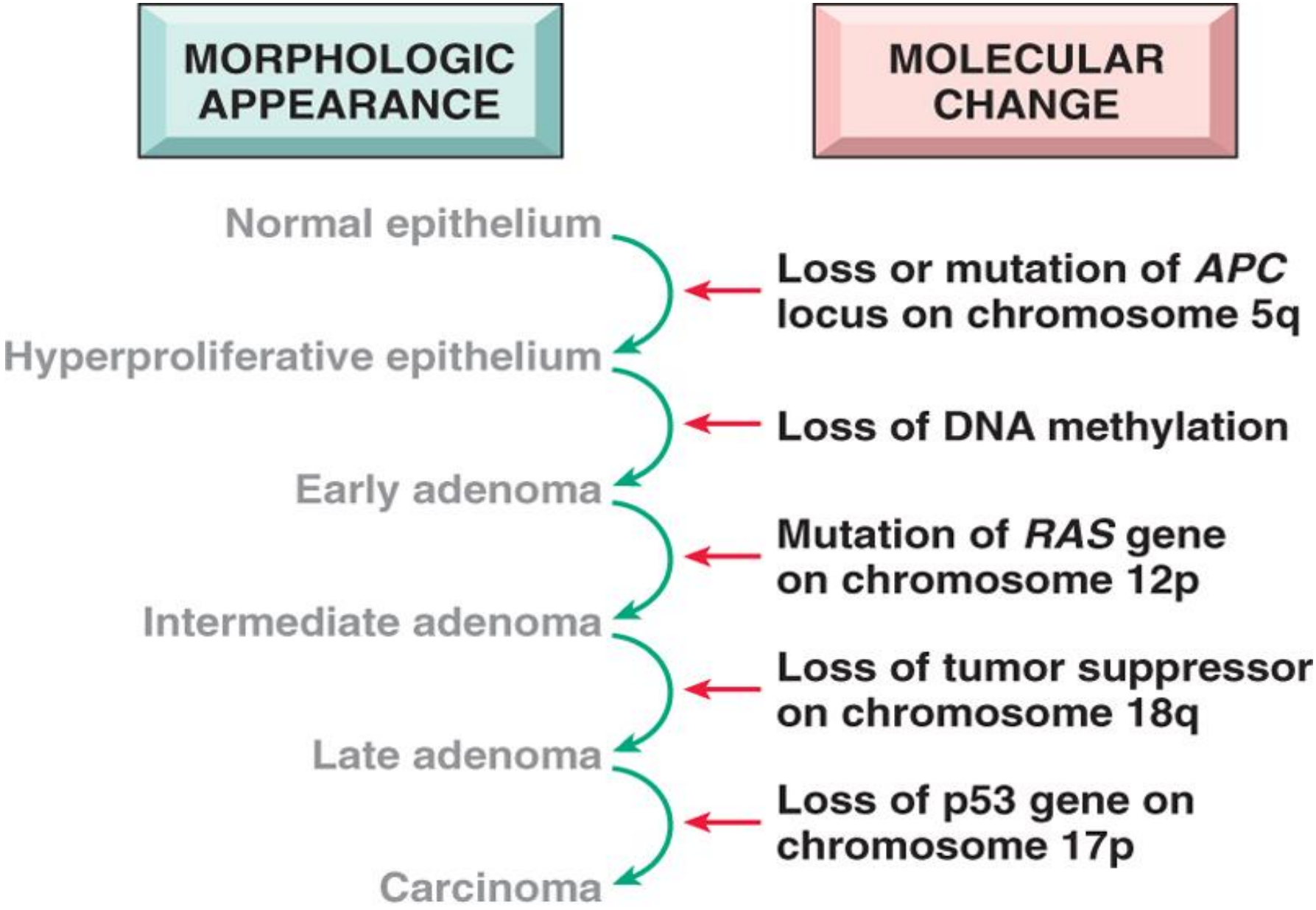
  over a period of time, many tumors become more aggressive and acquire greater malignant potential which is not simply represented by an increase in tumor size.

- **Tumor progression and associated heterogeneity results from multiple mutations that accumulate independently in different tumor cells, generating subclones with different characteristics**

- ***Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident, their constituent cells are extremely heterogeneous.***
- **During progression, tumor cells are subjected to immune and nonimmune selection pressures.**
- **E.g cells that are highly antigenic are destroyed by host defenses, whereas those with reduced growth factor requirements are positively selected.**
- **A growing tumor tends to be enriched for subclones that are capable of survival, growth, invasion, and metastasis**.



TRANSFORMATION	PROGRESSION	PROLIFERATION OF GENETICALLY UNSTABLE CELLS → TUMOR CELL VARIANTS HETEROGENEITY



Features of malignent cells

- 1-Self-sufficiency in growth signals
- 2-Insensitivity to growth-inhibitory signals
- 3-Evasion of apoptosis
- 4-Limitless replicative potential (i.e., overcoming cellular senescence and avoiding mitotic catastrophe)
- 5-Development of sustained angiogenesis
- 6-Ability to invade and metastasize
- 7-Genomic instability resulting from defects in DNA repair

Self-Sufficiency in Growth Signals

- **Genes that promote autonomous cell growth in cancer cells are called *oncogenes.***
- **They are derived by mutations in proto-oncogenes and are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals.**
- **Their products, called *oncoproteins*, resemble the normal products of proto-oncogenes except that oncoproteins are devoid of important regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals.**

- **The binding of a growth factor to its specific receptor on the cell membrane causes transient and limited activation of the growth factor receptor.**
- **→ activates several signal-transducing proteins on the inner leaflet of the plasma membrane**
- **→transmission of the transduced signal across the cytosol to the nucleus via second messengers or a cascade of signal transduction molecules**
- **→induction and activation of nuclear regulatory factors that initiate DNA transcription**
- **→progression of the cell into the cell cycle, resulting ultimately in cell division**

Growth Factors

- **All normal cells require stimulation by growth factors to undergo proliferation.**
- **Types :**
- **1- paracrine action.**

growth factors are made by one cell type and act on a neighboring cell to stimulate proliferation

2-autocrine action

Many cancer cells acquire growth self-sufficiency by acquiring the ability to synthesize the same growth factors to which they are responsive.

- Glioblastomas secrete platelet-derived growth factor (PDGF) and express the PDGF receptor,
- Many sarcomas make both transforming growth factor-α (TGF-α) and its receptor.
- Genes that encode homologues of fibroblast growth factors (e.g., *hst-1* and *FGF3*) have been detected in several gastrointestinal and breast tumors;
- FGF-2 is expressed in human melanomas but not normal melanocytes.

- Hepatocyte growth factor (HGF) and its receptor c-Met are both overexpressed in follicular carcinomas of the thyroid.
- In many instances the growth factor gene itself is not altered or mutated, but the products of other oncogenes (e.g., *RAS*) stimulate overexpression of growth factor genes and the subsequent development of an autocrine loop.

Growth Factor Receptors

- **Mutant receptor proteins deliver continuous mitogenic signals to cells, even in the absence of the growth factor in the environment.**
- **overexpression of growth factor receptors can render cancer cells hyper-responsive to levels of the growth factor that would not normally trigger proliferation.**

- E.g
- overexpression involve the epidermal growth factor (EGF) receptor family. *ERBB1,*
- the EGF receptor, is overexpressed in 80% of squamous cell carcinomas of the lung.
- In 50% or more of glioblastomas.
- In 80-100% of epithelial tumors of the head and neck.

- *HER2/NEU (ERBB2),* is amplified in 25-30% of breast cancers and adenocarcinomas of the lung, ovary, and salivary glands.
- These tumors are exquisitely sensitive to the mitogenic effects of small amounts of growth factors
- High level of HER2/NEU protein in breast cancer cells is a poor prognosis.

- The significance of *HER2/NEU* in the pathogenesis of breast cancers is illustrated by the clinical benefit derived from blocking the extracellular domain of this receptor with anti-*HER2/NEU* antibodies.
- Treatment of breast cancer with anti-HER2/NEU antibody (herciptin) proved to be clinically effective .

Signal-Transducing Proteins

- These signaling molecules couple growth factor receptors to their nuclear targets.
- Many such signaling proteins are associated with the inner leaflet of the plasma membrane, where they receive signals from activated growth factor receptors and transmit them to the nucleus, either through **second messengers** or through a **cascade of phosphorylation and activation of signal transduction molecules.**
- Two important members in this category are
- *1-RAS* gene
- *2-ABL gene*

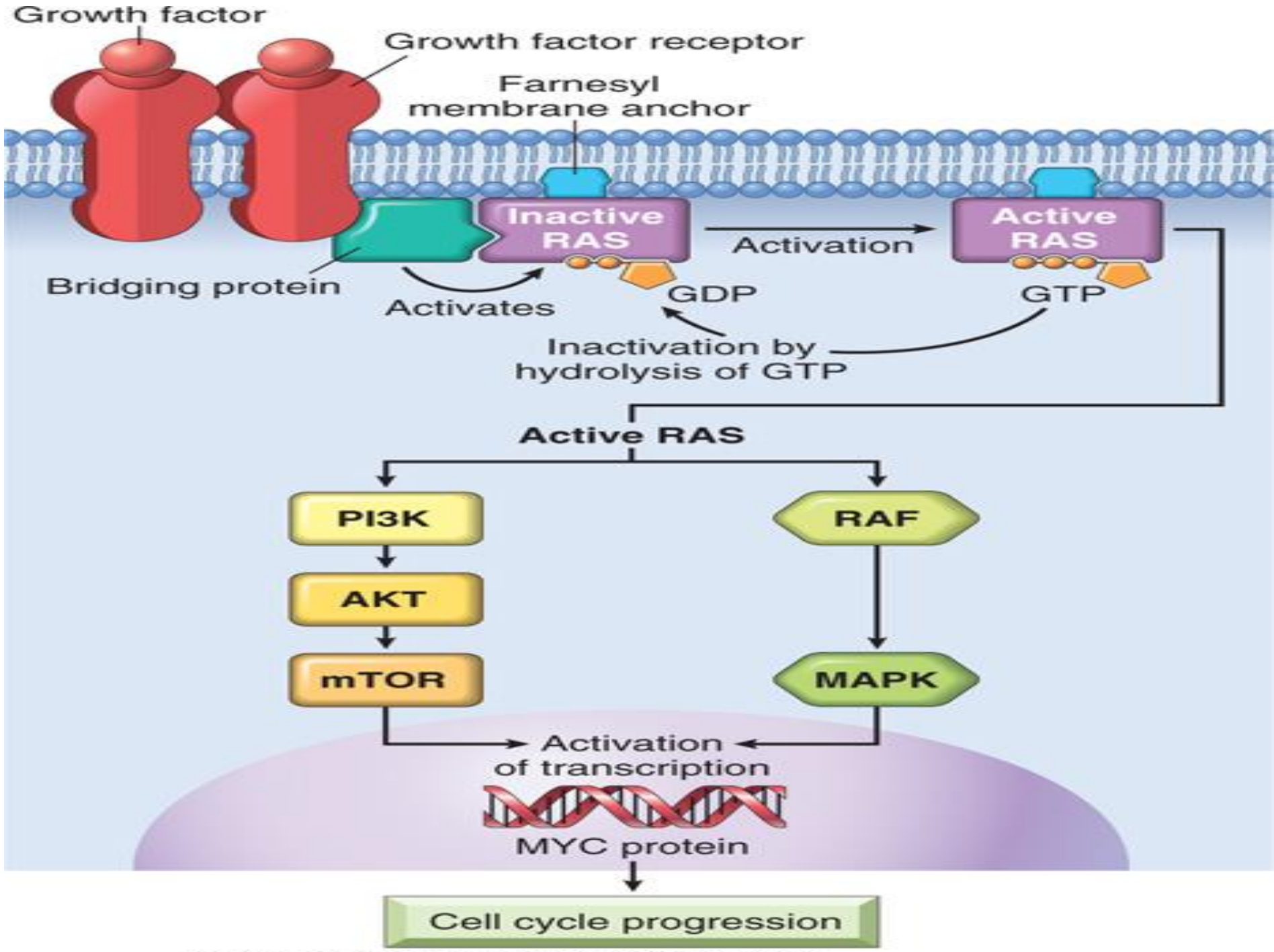
- *RAS* is the most commonly mutated proto-oncogene in human tumors.
- Approximately 30% of all human tumors contain mutated versions of the *RAS* gene
- The incidence is even higher in some specific cancers (e.g., colon and pancreatic adenocarcinomas).
- RAS is a member of a family of small G proteins that bind guanosine nucleotides (guanosine triphosphate [GTP] and guanosine diphosphate [GDP]).

- Normal RAS proteins flip back and forth between an excited signal-transmitting state and a quiescent state.
- RAS proteins are inactive when bound to GDP
- stimulation of cells by growth factors leads to exchange of GDP for GTP and subsequent activation of RAS.

- The activated RAS in turn stimulates down-stream regulators of proliferation, such as the *RAF-mitogen-activated protein (MAP) kinase mitogenic cascade,* which floods the nucleus with signals for cell proliferation.
- The excited signal-emitting stage of the normal RAS protein is short-lived
- Intrinsic guanosine triphosphatase (GTPase) activity hydrolyzes GTP to GDP, releasing a phosphate group and returning the protein to its quiescent inactive state.

- **The GTPase activity of activated RAS protein is magnified dramatically by a family of GTPase-activating proteins (GAPs), which act as molecular brakes that prevent uncontrolled RAS activation by favoring hydrolysis of GTP to GDP**.

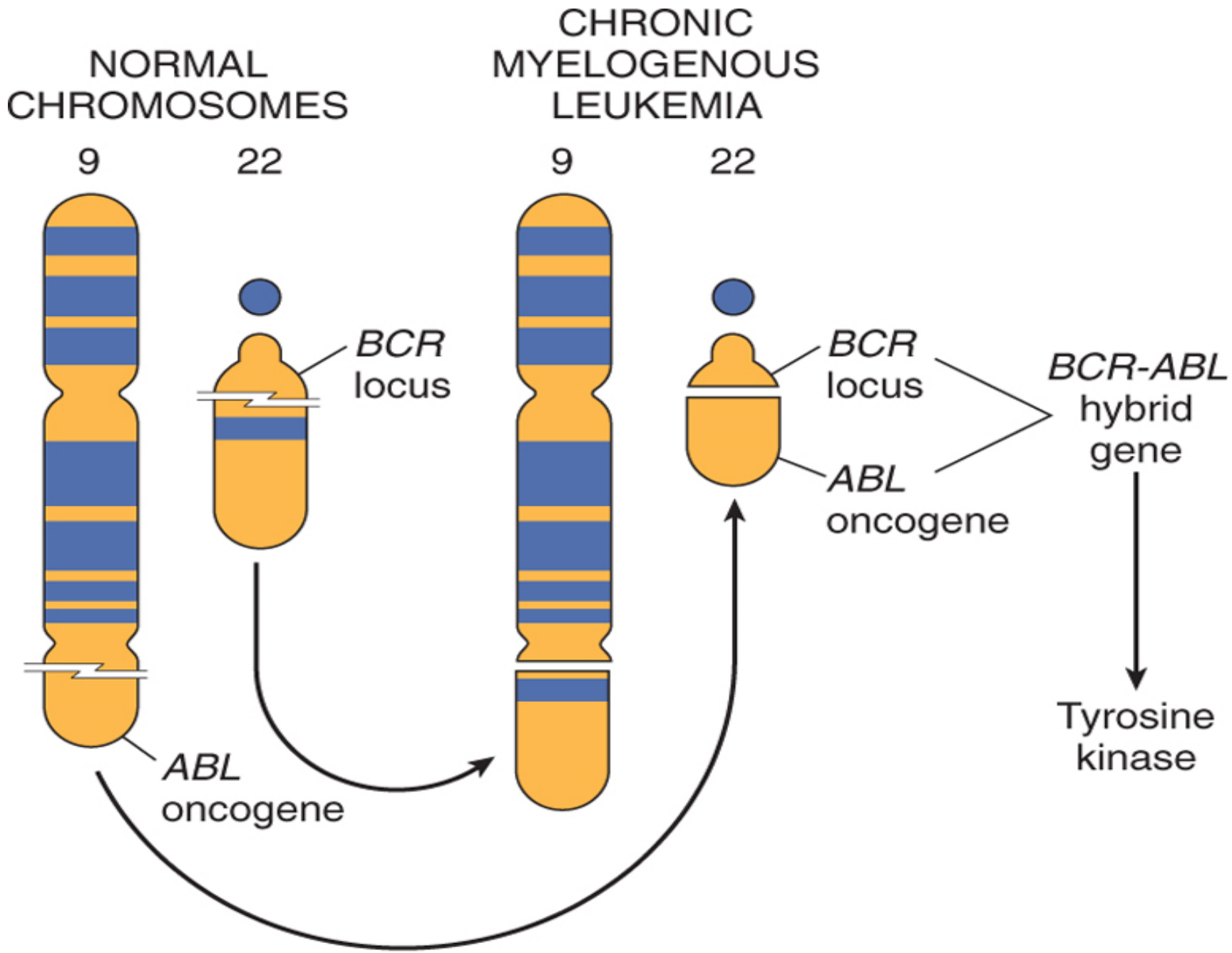
- **The *RAS* gene is most commonly activated by point mutations.**
- **Point mutations can affect :**
- **1-GTP-binding pocket**
- **2-the enzymatic region essential for GTP hydrolysis.**
- **Mutations at these locations interfere with GTP hydrolysis that is essential to convert RAS into an inactive form.**
- **RAS is thus trapped in its activated GTP-bound form, and the cell is forced into a continuously proliferating state**.



Kumar et al: Robbins Basic Pathology, 9e.

- **mutations in RAS protein would be mimicked by mutations in the GAPs that fail to restrain normal RAS proteins.**
- **E.g mutation of neurofibromin 1, a GAP, is associated with familial neurofibromatosis type 1**

- **The *ABL* proto-oncogene has tyrosine kinase activity that is dampened by internal negative regulatory domains.**
- **In chronic myeloid leukemia (CML) and acute lymphocytic leukemias,**
- ***When ABL* gene is translocated from its normal site on chromosome 9 to chromosome 22, where it fuses with part of the breakpoint cluster region *(BCR)* gene = Philadelphia (Ph) chromosome .**



- **The BCR-ABL hybrid protein has potent, unregulated tyrosine kinase activity, which activates several pathways, including the *RAS-RAF* cascade.**
- **Normal ABL protein localizes in the nucleus, where its role is to promote apoptosis of cells that suffer DNA damage.**
- **The *BCR-ABL* gene cannot perform this function, because it is retained in the cytoplasm as a result of abnormal tyrosine kinase activity.**

- **A cell with *BCR-ABL* fusion gene is dysregulated in two ways:**
- **1-inappropriate tyrosine kinase activity leads to growth autonomy.**
- **2- impairment of apoptosis.**

- **The crucial role of *BCR-ABL* in transformation has been confirmed by the dramatic clinical response of patients with chronic myeloid leukemia after therapy with an inhibitor of the BCR-ABL fusion kinase called imatinib mesylate (Gleevec).**

Nuclear Transcription Factors

- ***Growth autonomy may occur as a consequence of mutations affecting genes that regulate transcription of DNA.***
- ***MYC, MYB, JUN, FOS, and REL oncogenes, function as transcription factors that regulate the expression of growth-promoting genes, such as cyclins***.

- **the *MYC* gene is involved most commonly in human tumors.**
- **The *MYC* proto-oncogene is expressed in virtually all cells**
- **the MYC protein is induced rapidly when quiescent cells receive a signal to divide.**

- **In normal cells, MYC levels decline to near basal level when the cell cycle begins.**
- **In contrast, oncogenic versions of the *MYC* gene are associated with persistent expression or overexpression, contributing to sustained proliferation.**

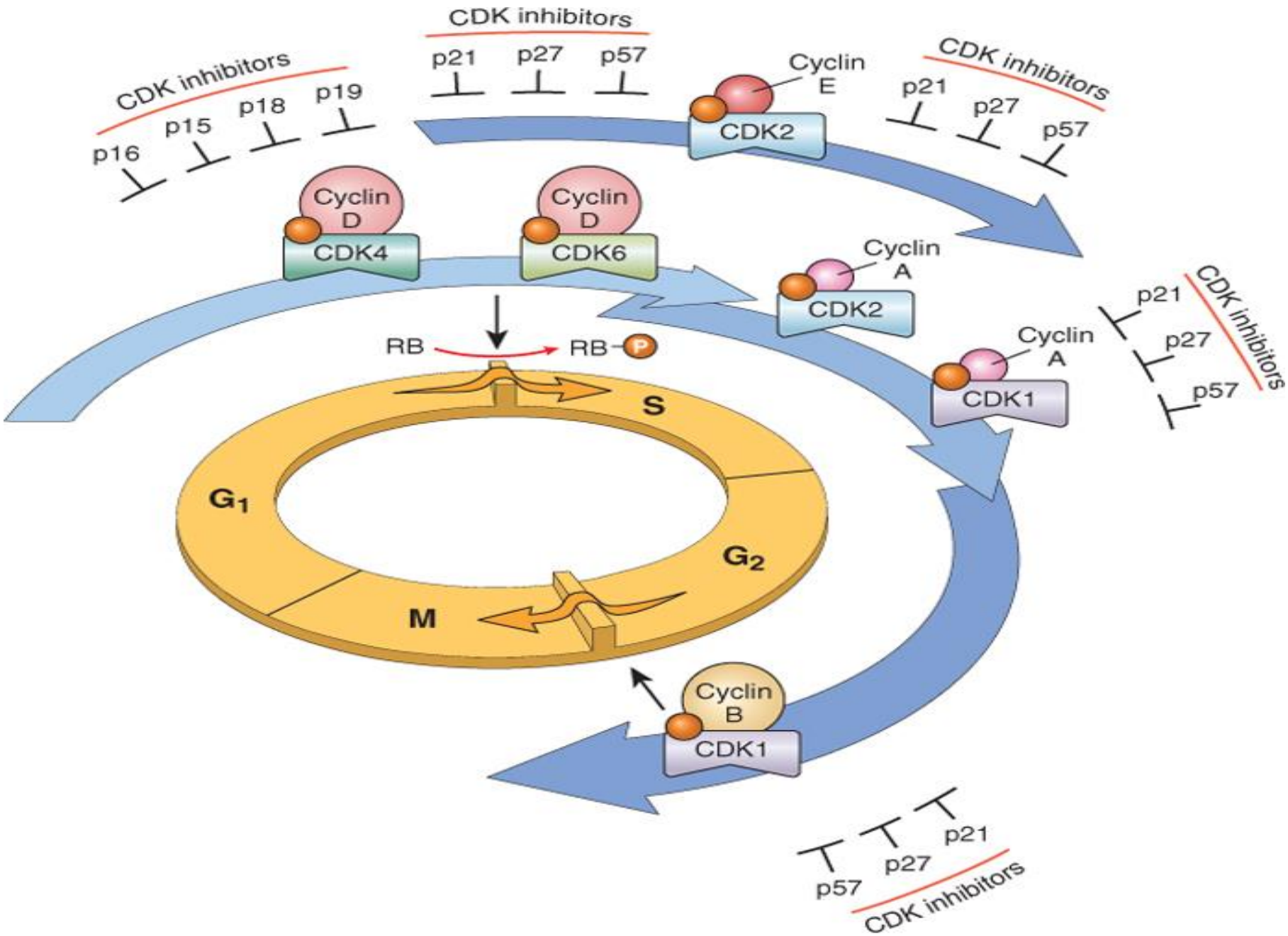
- **The MYC protein can either activate or repress the transcription of other genes.**
- **Those activated by MYC include several growth-promoting genes, including cyclin-dependent kinases (CDKs), whose products drive cells into the cell cycle.**
- **Genes repressed by MYC include the CDK inhibitors (CDKIs).**

- **MYC promotes tumorigenesis by increasing expression of genes that promote progression through the cell cycle and repressing genes that slow or prevent progression through the cell cycle.**

- **Dysregulation of the *MYC* gene resulting from a t(8;14) translocation occurs in Burkitt lymphoma, a B-cell tumor.**
- ***MYC* is also amplified in breast, colon, lung, and many other cancers;**
- ***N-MYC* and *L-MYC* genes are amplified in neuroblastomas and small-cell cancers of lung.**

Cyclins and Cyclin-Dependent Kinases (CDKs)

- **Cancers may become autonomous if the genes that drive the cell cycle become dysregulated by mutations or amplification.**
- **Progression of cells through the various phases of the cell cycle is controlled by CDKs.**
- **CDKs are activated by binding to *cyclins,* so called because of the cyclic nature of their production and degradation.**



- **The CDK-cyclin complexes phosphorylate crucial target proteins that drive the cell through the cell cycle.**
- **On completion of this task, cyclin levels decline rapidly.**
- **More than 15 cyclins have been identified; cyclins D, E, A, and B appear sequentially during the cell cycle and bind to one or more CDK**.

- **Mishaps affecting the expression of cyclin D or CDK4 seem to be a common event in neoplastic transformation.**
- **The cyclin D genes are overexpressed in many cancers, including those affecting the breast, esophagus, liver, and a subset of lymphomas.**

- **Amplification of the *CDK4* gene occurs in melanomas, sarcomas, and glioblastomas.**

- **Mutations affecting cyclin B and cyclin E and other CDKs also occur, but they are much less frequent than those affecting cyclin D/CDK4.**

- **Cyclins arouse the CDKs .**
- **CDK inhibitors (CDKIs) silence the CDKs and exert negative control over the cell cycle.**
- **One family of CDKIs, composed of three proteins :**
- **1- p21 [CDKN1A],**
- **2-p27 [CDKN1B],**
- **3-p57 [CDKN1C],**

inhibits the CDKs broadly

- **The other family of CDKIs has selective effects on cyclin D/CDK4 and cyclin D/CDK6.**
- **The four members of this family :**
- **1-p15 [CDKN2B]**
- **2-p16 [CDKN2A]**
- **3-p18 [CDKN2C]**
- **4-p19 [CDKN2D]**

are sometimes called INK4 (A-D) proteins.

- **Expression of these inhibitors is down-regulated by mitogenic signaling pathways, thus promoting the progression of the cell cycle.**
- **E.g**
- **p27 [CDKN1B], a CDKI that inhibits cyclin E, is expressed throughout G_1.**
- **Mitogenic signals inhibit p27 relieving inhibition of cyclin E-CDK2 and thus allowing the cell cycle to proceed.**

- **Interestingly, the p16(*CDKN2A*)gene locus, also called *INK4a/ARF,* encodes two protein products: the p16 INK4A and p14ARF**
- **Both block cell cycle progression but have different targets :**
- **1-p16 [CDKN2A] inhibits RB phosphorylation by blocking cyclin D-CDK4 complex**
- **2-p14ARF activates the p53 pathway by inhibiting MDM2**

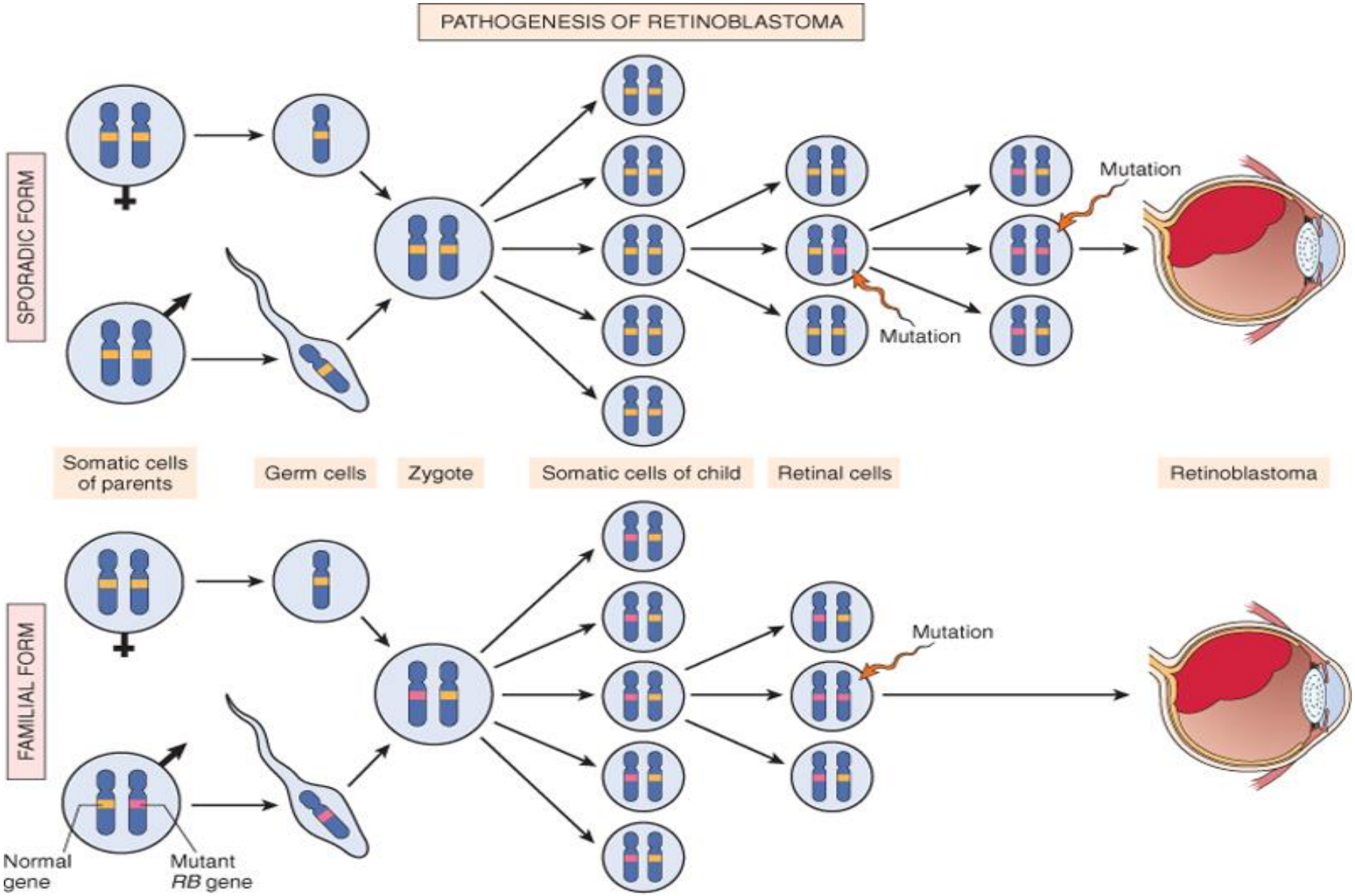
- **Both proteins function as tumor suppressors, and deletion of this locus, frequent in many tumors, impacts both the RB and p53 pathways.**
- **The CDKIs are frequently mutated or otherwise silenced in many human malignancies.**
- **Germ-line mutations of p16(*CDKN2A)* are associated with 25% of melanoma.**

- **Somatically acquired deletion or inactivation of p16(*CDKN2A)* is seen in :**
- **75% of pancreatic carcinomas**
- **40% to 70% of glioblastomas**
- **50% of esophageal cancers**
- **20% of non-small-cell lung carcinomas, soft tissue sarcomas, and bladder cancers.**

Insensitivity to Growth-Inhibitory Signals

- **Retinoblastoma *(RB)* gene, the first and prototypic cancer suppressor gene to be discovered.**
- **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; the other copy is normal.**
- **Retinoblastoma develops when the normal *RB* gene is lost in retinoblasts as a result of somatic**

- **Because 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.**

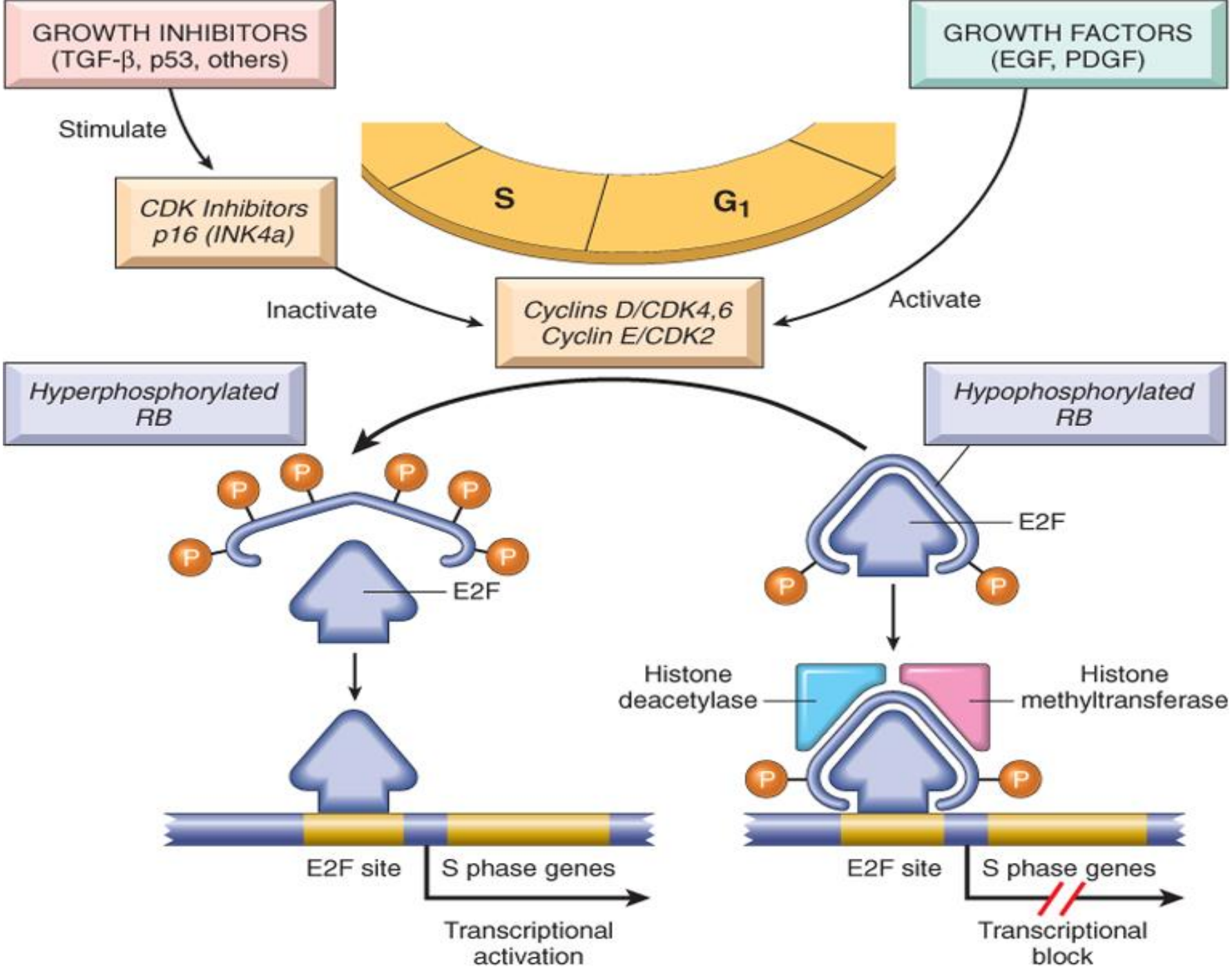
RB Gene and Cell Cycle

- **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.

- **The 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**
- **such 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.**

Transforming Growth Factor-β Pathway

- **TGF-β is a potent inhibitor of proliferation in most normal epithelial, endothelial, and hematopoietic cells.**
- **It regulates cellular processes by binding to a complex composed of TGF-β receptors I and II.**
- **Dimerization of the receptor upon ligand binding leads to a cascade of events that result in:**
- **1-transcriptional activation of CDKIs.**
- **2-suppression of growth-promoting genes such as *MYC, CDK2, CDK4,* and those encoding cyclins A and E.**

- **Mutations affecting the type II receptor are seen in cancers of the colon, stomach, and endometrium.**
- **Mutational inactivation of SMAD4, 1 of the 10 proteins known to be involved in TGF-β signaling, is common in pancreatic cancers.**
- ***In 100% of pancreatic cancers and 83% of colon cancers, at least one component of the TGF-β pathway is mutated.***
- **TGF-β can function to prevent or promote tumor growth, depending on the state of other genes in the cell.**
- **In many late-stage tumors, TGF-β signaling activates epithelial-to-mesenchymal transition (EMT), a process that promotes migration, invasion, and metastasis.**

Contact Inhibition
NF2 and APC

- **Contact inhibition" is abolished in cancer cells allowing them to pile on top of one another.**
- **Cell-cell contacts in many tissues are mediated by homodimeric interactions between transmembrane proteins called cadherins.**
- **E-cadherin mediates cell-cell contact in epithelial layers by mechanism not fully understood.**
- **One mechanism that sustains contact inhibition is mediated by the tumor suppressor gene *NF2*.**

- **NF2 product, neurofibromin-2, more commonly called merlin, facilitates E-cadherin mediated contact inhibition.**
- **Homozygous loss of *NF2* is known to cause a form of neural tumors associated with the condition called neurofibromatosis.**

Adenomatous Polyposis Coli-β-Catenin Pathway

- **The *APC* gene exerts antiproliferative effects in an unusual manner.**
- **It is a cytoplasmic protein whose dominant function is to regulate the intracellular levels of β-catenin.**
- **<u>β-catenin is a protein with many functions :</u>**

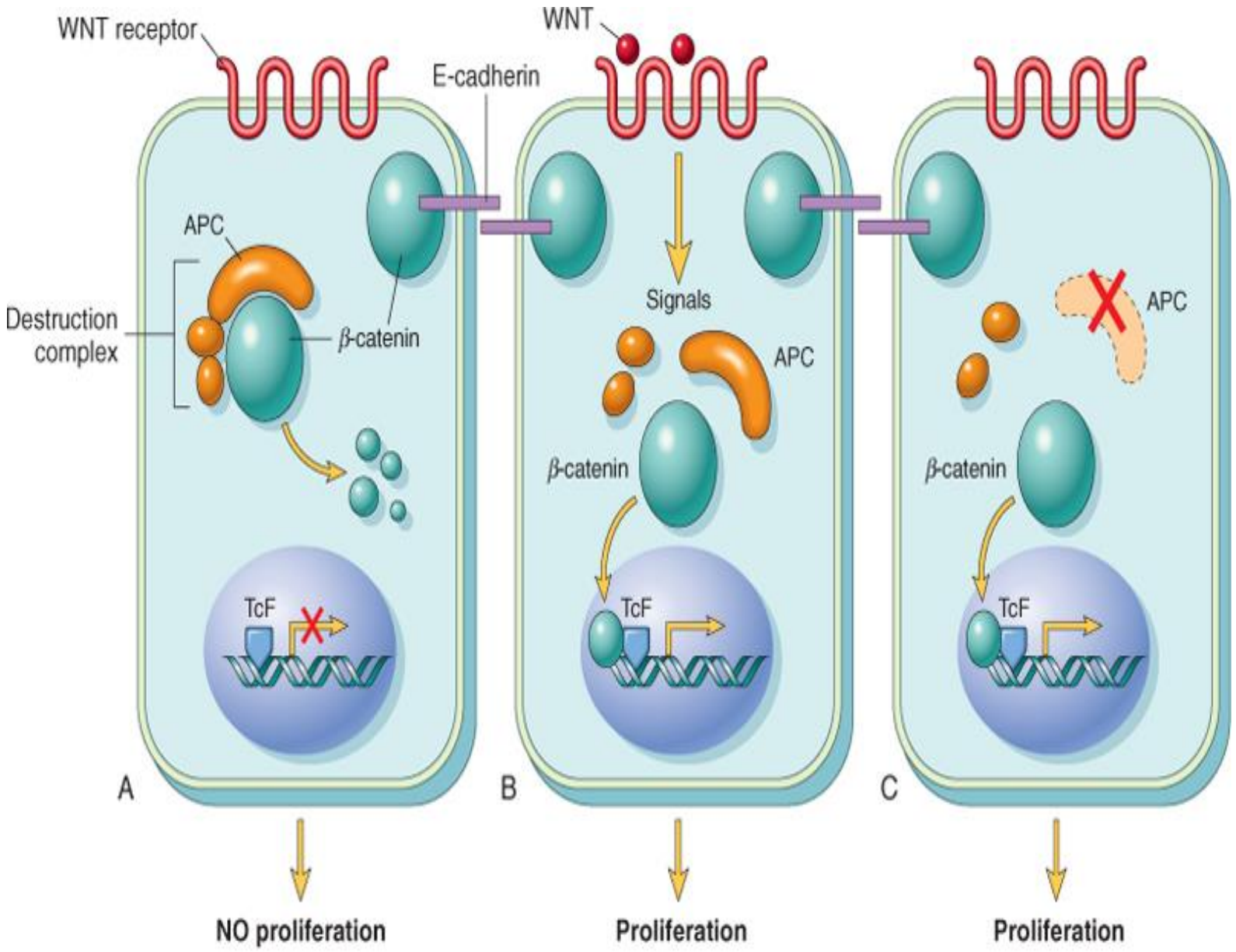
1- β-catenin binds to the cytoplasmic portion of E-cadherin, a cell surface protein that mediates intercellular interactions.

2- It can translocate to the nucleus and activate cell proliferation.

- **β-catenin is an important component of the so-called WNT signaling pathway that regulates cell proliferation.**
- **WNT is a soluble factor that can induce cellular proliferation.**
- **It does so by binding to its receptor and transmitting signals that prevent the degradation of β-catenin, allowing it to translocate to the nucleus where it acts as a transcriptional activator in conjunction with another molecule, called TcF .**

- **In quiescent cells which are not exposed to WNT, cytoplasmic β-catenin is degraded by a *destruction complex,* of which APC is an integral part .**
- **In malignant cells with loss of APC β-catenin degradation is prevented and the WNT signaling response is inappropriately activated in the absence of WNT .**

- **This leads to transcription of growth-promoting genes, such as cyclin D1 and *MYC and transcriptional regulators, such as TWIST and SLUG, that repress E-cadherin expression and thus reduce contact inhibition. .***



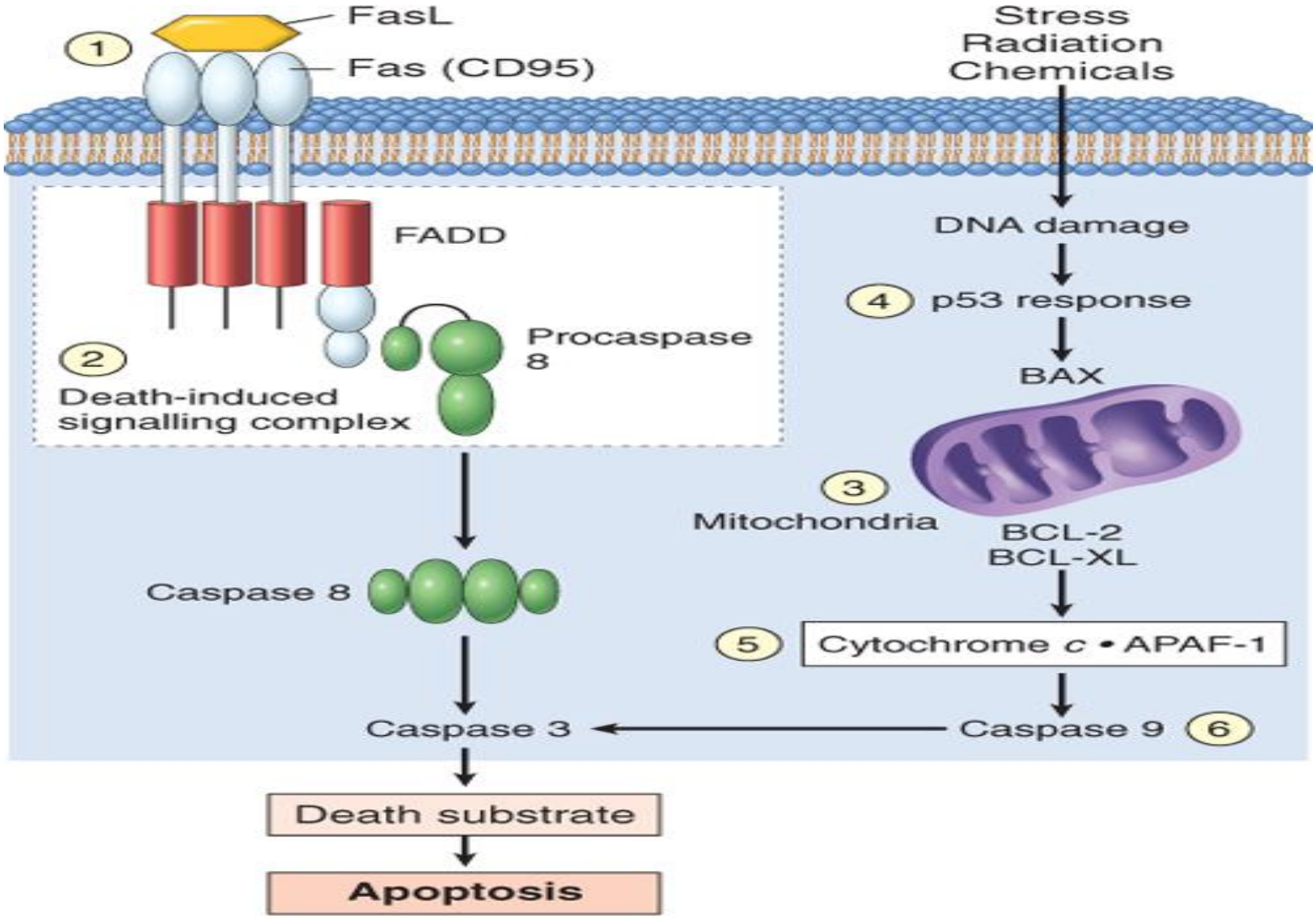
- ***APC*** **behaves as a typical tumor suppressor gene.**
- **Individuals born with one mutant allele develop hundreds to thousands of adenomatous polyps in the colon during their teens or 20s, which show loss of the other *APC* allele.**
- **Almost invariably one or more polyps undergo malignant transformation upon accumulation of other mutations in the cells within the polyp.**

- ***APC*** **mutations are seen in 70-80% of sporadic colon cancers.**
- **Colonic cancers that have normal *APC* genes show activating mutations of β-catenin that render them refractory to the degrading action of APC.**

Evasion of Apoptosis

- There are 2 distinct programs that activate apoptosis:
- 1- the extrinsic pathway (death receptor CD95/Fas).
- 2- the intrinsic pathway (DNA damage).

- Stimulation of either pathway results in activation of a normally inactive protease (caspase-8 or caspase-9), which initiates a proteolytic cascade involving "executioner" caspases that disassemble the cell in orderly fashion.
- The cellular remains are then efficiently consumed by the cellular neighbors and professional phagocytes without stimulating inflammation.



Extrinsic pathway of apoptosis

- **TNF receptor (CD95 ,Fas)is bound to its ligand CD95L → trimerization of the receptor and thus its cytoplasmic *death domains α* → attract the intracellular adaptor protein FADD → recruits procaspase 8 to form the death-inducing signaling complex.**

- **Procaspase 8 is activated by cleavage into smaller subunits, generating caspase 8.**
- **Caspase 8 then activates down-stream caspases such as caspase 3 (*executioner caspase)* that cleaves DNA and other substrates to cause cell death.**

Intrinsic pathway of apoptosis

- **It is triggered by a variety of stimuli, including :**
- **1- withdrawal of survival factors.**
- **2-stress.**
- **3-injury.**
- **Activation of this pathway leads to permeabilization of mitochondrial outer membrane, with resultant release of molecules, such as cytochrome *c,* that initiate apoptosis.**

- **Cytochrome *c* leaks into the cytosol where it binds to APAF-1 activating caspase 9.**
- **caspase-9 can cleave and activate the executioner caspases.**

- **The integrity of the mitochondrial outer membrane is regulated by pro-apoptotic and anti-apoptotic members of the BCL2 family of proteins.**
- **The pro-apoptotic proteins, BAX and BAK, are required for apoptosis and directly promote mitochondrial permeabilization.**

- **Their action is inhibited by the anti-apoptotic members of this family exemplified by BCL2 and BCL-XL.**
- **A third set of proteins (so-called BH3-only proteins) including BAD, BID, and PUMA, regulate the balance between the pro- and anti-apoptotic members of the apoptic genes.**

- **The BH3-only proteins promote apoptosis by neutralizing the actions of anti-apoptotic proteins like BCL2 and BCL-XL.**
- **When the sum total of all BH3 proteins expressed "overwhelms" the anti-apoptotic BCL_2/BCL_{XL} protein barrier, BAX and BAK are activated and form pores in the mitochondrial membrane.**

- **Because of the pro-apoptotic effect of BH3 only proteins, efforts are underway to develop of BH3 mimetic drugs.**

- **Malignent cells can escape apoptosis through different ways :**
- **1-Reduced levels of CD95 may render the tumor cells less susceptible to apoptosis by Fas ligand (FasL).**
- **2-Some tumors have high levels of FLIP, a protein that can bind death-inducing signaling complex and prevent activation of caspase 8.**

- **3-Reduced egress of cytochrome *c* from mitochondrion as a result of up-regulation of BCL2.**
- **4- Reduced levels of pro-apoptotic BAX resulting from loss of p53.**
- **5-Loss of APAF-1.**
- **6-Up-regulation of inhibitors of apoptosis.**

- ***The best established gene in this group is BCL_2 gene in protecting tumor cells from apoptosis.***
- **85% of B-cell lymphomas of the follicular type carry a characteristic t(14;18) (q32;q21) translocation.**
- **14q32, the site where immunoglobulin heavy-chain genes are found.**
- **Juxtaposition of this transcriptionally active locus with *BCL_2* (located at 18q21) causes overexpression of the BCL_2 protein.**

- **This in turn increases the BCL2/BCL-XL buffer, protecting lymphocytes from apoptosis and allowing them to survive for long periods.**
- **A steady accumulation of B lymphocytes will follow resulting in lymphadenopathy and marrow infiltration.**

- **Because BCL2-overexpressing lymphomas arise in large part from reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow growing) compared with many other lymphomas.**

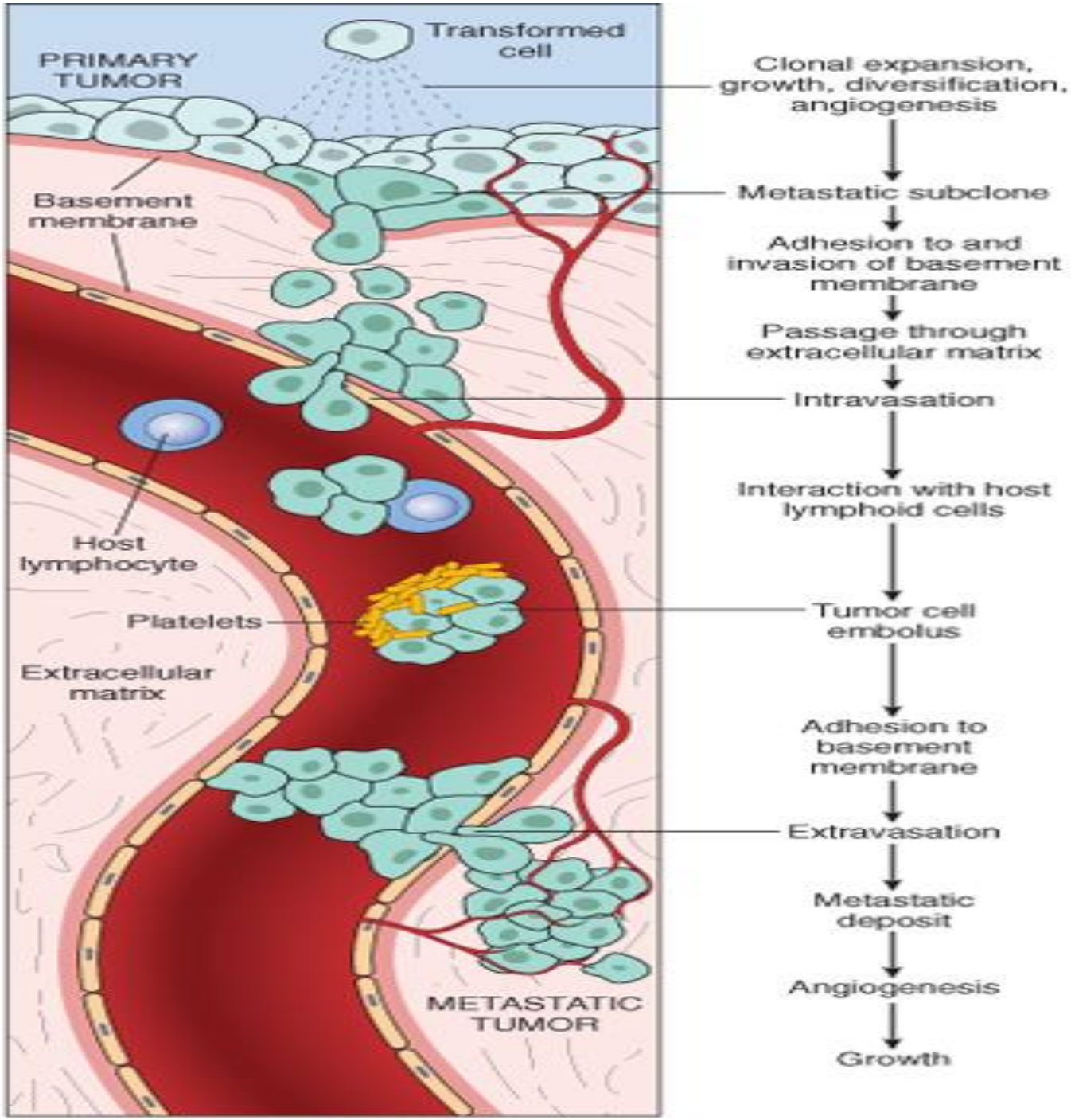
Ability to Invade and Metastasize

- **The metastatic cascade can be subdivided into two phases:**
- **1-invasion of ECM and vascular dissemination.**
- **2-homing of tumor cells.**

Invasion of Extracellular Matrix (ECM

- **human tissues are organized into a series of compartments separated from each other by two types of ECM:**
- **1-basement membranes .**
- **2-interstitial connective tissue.**

- **each of these components of ECM is composed of :**
- **1-collagens.**
- **2-glycoproteins.**
- **3-proteoglycans.**



- **Invasion of the ECM is an active process that requires four steps :**
- **1-Detachment of tumor cells from each other.**
- **2-Degradation of ECM .**
- **3-Attachment to novel ECM components .**
- **4-Migration of tumor cells .**

- ***loosening*** **of tumor cells needs to loss of E-cadherins that act as intercellular glues that keep the cells together.**
- **Their cytoplasmic portions bind to β-catenin .**
- **E-cadherin can transmit antigrowth signals by sequestering β-catenin.**

- ***<u>E-cadherin function is lost in almost all epithelial cancers by :</u>***
- ***1- mutational inactivation of E-cadherin genes.***
- ***2- by activation of β-catenin genes.***
- ***3-by inappropriate expression of the SNAIL and TWIST transcription factors, which suppress E-cadherin expression .***

- **oncogenes are SNAIL and TWIST, which encode transcription factors whose primary function is to promote a process called epithelial-to-mesenchymal transition (EMT).**
- **In EMT, carcinoma cells down-regulate certain epithelial markers (e.g., E-cadherin) and up-regulate certain mesenchymal markers (e.g., vimentin and smooth muscle actin).**
- **These changes are believed to favor the development of a promigratory phenotype that is essential for metastasis.**
- **Loss of E-cadherin expression seems to be a key event in EMT, and SNAIL and TWIST are transcriptional repressors that promote EMT by down-regulating E-cadherin expression.**
- **EMT has been documented mainly in breast cancers.**

- **The second step in invasion is local *degradation of the basement membrane and interstitial connective tissue*.**
- **Tumor cells may either secrete proteolytic enzymes themselves or induce stromal cells (e.g., fibroblasts and inflammatory cells) to elaborate proteases.**

- **Multiple different families of proteases are present :**
- **1-matrix metalloproteinases (MMPs).**
- **2-cathepsin D.**
- **3-urokinase plasminogen activator.**

- **MMPs regulate tumor invasion not only by remodeling insoluble components of the basement membrane and interstitial matrix but also by releasing ECM-sequestered growth factors.**
- **Cleavage products of collagen and proteoglycans also have chemotactic, angiogenic, and growth-promoting effects.**

- **MMP-9 is a gelatinase that cleaves type IV collagen of the epithelial and vascular basement membrane and also stimulates release of VEGF from ECM-sequestered pools.**

- **Benign tumors of the breast, colon, and stomach show little type IV collagenase activity**
- **Malignant tumors overexpress this enzyme.**
- **The levels of metalloproteinase inhibitors are reduced so that the balance is tilted greatly toward tissue degradation.**

- **overexpression of MMPs and other proteases have been reported for many tumors. Because of these observations, attempts are being made to use protease inhibitors as therapeutic agents.**

- **The third step in invasion involves *changes in attachment of tumor cells to ECM proteins*.**
- **Normal epithelial cells have receptors, such as integrins, for basement membrane laminin and collagens that are polarized at their basal surface.**
- **These receptors help to maintain the cells in a resting, differentiated state.**
- **Loss of adhesion in normal cells leads to induction of apoptosis.**

- **Cleavage of the basement membrane proteins ,collagen IV and laminin by MMP-2 or MMP-9 generates novel sites that bind to receptors on tumor cells and stimulate migration.**

- ***Locomotion*** **is the final step of invasion.**
- **Migration is a complex, multistep process that involves many families of receptors and signaling proteins that eventually impinge on the actin cytoskeleton.**
- **Such movement seems to be potentiated and directed by tumor cell-derived cytokines, such as autocrine motility factors.**

- **In addition, cleavage products of matrix components (e.g., collagen, laminin) and some growth factors (e.g., insulin-like growth factors I and II) have chemotactic activity for tumor cells.**
- **Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor/scatter factor (HGF/SCF), which bind to receptors on tumor cells.**
- **Concentrations of HGF/SCF are elevated at the advancing edges of the highly invasive brain tumor glioblastoma multiforme, supporting their role in motility.**

Vascular Dissemination and Homing of Tumor Cells

- In the bloodstream, some tumor cells form emboli by aggregating and adhering to circulating leukocytes, particularly platelets.
- Aggregated tumor cells are thus afforded some protection from the antitumor host effector cells.
- Most tumor cells however circulate as single cells.

- **Extravasation of free tumor cells or tumor emboli involves adhesion to the vascular endothelium followed by egress through the basement membrane into the organ parenchyma by mechanisms similar to those involved in invasion.**

- **The site of extravasation and the organ distribution of metastases generally can be predicted by the location of the primary tumor and its vascular or lymphatic drainage.**
- **Many tumors metastasize to the organ that represents the first capillary bed they encounter after entering the circulation.**
- **In many cases the natural pathways of drainage do not readily explain the distribution of metastases.**

- **e.g.lung cancers tend to involve the adrenals with some regularity but almost never spread to skeletal muscle.**
- **<u>The mechanisms of site-specific homing involves :</u>**
- **1-the expression of adhesion molecules by tumor cells whose ligands are expressed preferentially on the endothelium of target organs.**
- **2-chemokines and their receptors.**
- **chemokines participate in directed movement (chemotaxis) of leukocytes.**

- **Human breast cancer cells express high levels of the chemokine receptors *CXCR4* and *CCR7.***
- **The ligands for these receptors (i.e., chemokines CXCL12 and CCL21) are highly expressed only in those organs where breast cancer cells metastasize.**
- **It is speculated that blockade of chemokine receptors may limit metastases.**

- **After extravasation, tumor cells are dependent on a receptive stroma for growth.**
- **Tumors may fail to metastasize to certain target tissues because they present a nonpermissive growth environment.**
- **The precise localization of metastases cannot be predicted with any form of cancer.**

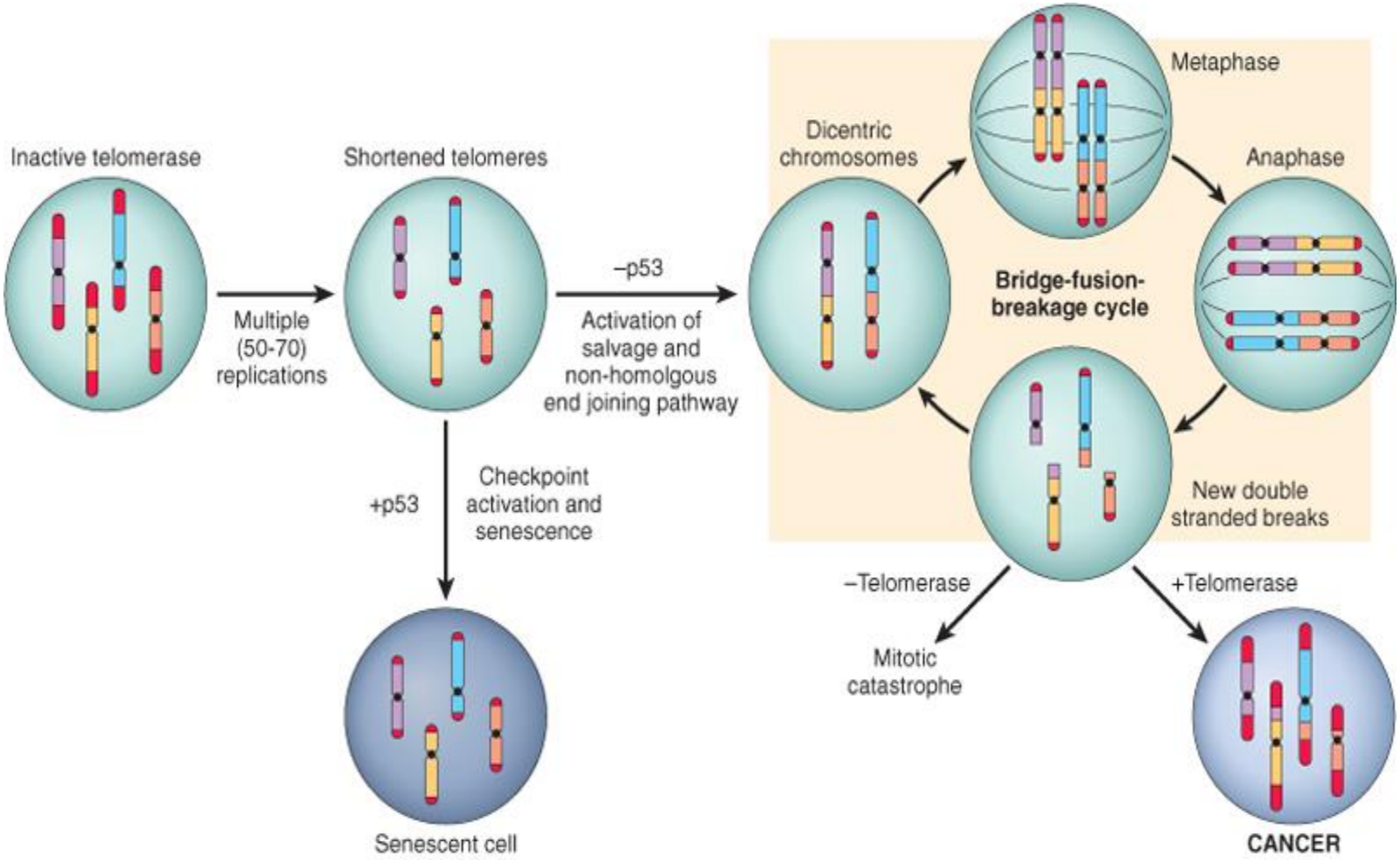
- **Tumor cells can be detected in the bloodstream and in small foci in the bone marrow even in patients in whom gross metastatic lesions never develop.**
- **The prolonged survival of micrometastases without progression, is well described in melanoma and in breast and prostate cancer.**

Limitless Replicative Potential

- **Most normal human cells have a capacity of 60 to 70 doublings.**
- **After this the cells lose the capacity to divide and enter senescence.**
- **This phenomenon is due to progressive shortening of *telomeres* at the ends of chromosomes.**

- **Short telomeres are recognized by the DNA repair machinery leading to cell cycle arrest mediated by *p53* and *RB.***
- **Cells in which the checkpoints are disabled by *p53* or *RB* mutations the nonhomologous end-joining pathway is activated as a last-ditch effort to save the cell joining the shortened ends of two chromosomes.**

- **This inappropriately activated repair system results in dicentric chromosomes that are pulled apart at anaphase resulting in new double-stranded DNA breaks.**
- **The resulting genomic instability from the repeated bridge-fusion-breakage cycles eventually produces mitotic catastrophe characterized by massive cell death.**



- ***It follows that for tumors to grow indefinitely loss of growth restraints is not enough.***
- ***Tumor cells must also develop ways to avoid both cellular senescence and mitotic catastrophe .***

- **If during crisis a cell manages to reactivate telomerase, the bridge-fusion-breakage cycles cease and the cell is able to avoid death.**
- **During this period of genomic instability that precedes telomerase activation, numerous mutations could accumulate.**
- **Passage through a period of genomic instability probably explains the complex karyotypes frequently seen in human carcinomas.**

- **Telomerase, active in normal stem cells, is normally absent from, or at very low levels in most somatic cells.**
- **Telomere maintenance is seen in virtually all types of cancers.**
- **In 85-95% of cancers, this is due to up-regulation of the enzyme telomerase.**

- **In the progression from colonic adenoma to colonic adenocarcinoma, early lesions had a high degree of genomic instability with low telomerase expression, whereas malignant lesions had complex karyotypes with high levels of telomerase activity, consistent with a model of telomere-driven tumorigenesis in human cancer.**

- **Unregulated proliferation in incipient tumors leads to telomere shortening, followed by chromosomal instability and mutation accumulation.**
- **Reactivation of telomerase in these cells causes extension of telomeres and mutations become fixed contributing to tumor growth.**

Development of Sustained Angiogenesis

- **Tumors cannot enlarge beyond 1-2 mm in diameter unless they are vascularized.**
- **Cancer cells can stimulate neo-angiogenesis during which new vessels sprout from previously existing capillaries or in some cases vasculogenesis in which endothelial cells are recruited from the bone marrow .**

- **Tumor vasculature is abnormal , leaky, dilated, and have a haphazard pattern of connection.**
- **<u>Neovascularization has a dual effect on tumor growth:</u>**
- **1-Perfusion supplies needed nutrients and oxygen**
- **2-Newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, such as insulin-like growth factors, PDGF, and granulocyte-macrophage colony-stimulating factor.**

- **Angiogenesis is required not only for continued tumor growth but also for access to the vasculature and hence for metastasis.**
- ***Angiogenesis is thus a necessary biologic correlate of neoplasia, both benign and malignant.***

- Tumor angiogenesis is controlled by a balance between pro-angiogenic and inhibitory factors.
- The angiogenesis inducer is vascular endothelial growth factor (VEGF).
- The angiogenesis inhibitor is thrombospondin-1 (TSP-1).

- **Early in their growth most human tumors do not induce angiogenesis. They remain small or in situ for years until the angiogenic switch terminates this stage of vascular quiescence.**
- **Normal p53 induces synthesis of TSP-1.**

- **The molecular basis of the angiogenic switch involves increased production of angiogenic factors and/or loss of angiogenesis inhibitors.**
- **These factors may be produced :**
- **1-directly by the tumor cells themselves .**
- **2-by inflammatory cells (e.g., macrophages) .**
- **3-by stromal cells associated with the tumors.**

- **Proteases, elaborated either by the tumor cells directly or from stromal cells in response to the tumor, also are involved in regulating the balance between angiogenic and anti-angiogenic factors.**
- **Many proteases can release the angiogenic basic FGF stored in the extracellular matrix (ECM).**

- **There are potent angiogenesis inhibitors:**

1- angiostatin (plasminogen)

2- endostatin (collagen)

3- vasculostatin (transthyretin)

4- TSP-1is produced by stromal fibroblasts themselves in response to signals from the tumor cells.

- **The angiogenic switch is controlled by several physiologic stimuli, such as hypoxia.**
- **Relative lack of oxygen → activation of hypoxia-induced factor-1α (HIF1α), an oxygen-sensitive transcription factor → stimulates production of pro-angiogenic cytokines as VEGF.**

- **HIF1α is continuously produced, but in normal conditions the von Hippel-Lindau protein (VHL) binds to HIF1α, leading to ubiquitination and destruction of HIF1α.**

- **In hypoxic conditions, such as a tumor that has reached a critical size**
- **The lack of oxygen → prevents HIF1α recognition by VHL protein →no destruction of HIF1α → HIF1α translocates to the nucleus and activates transcription of its target genes, such as VEGF.**

- **VHL acts as a tumor suppressor gene, and germ-line mutations of the *VHL* gene are associated with hereditary *VHL syndrome :***

1- renal cell cancers

2- pheochromocytomas

3- hemangiomas of the CNS

4- retinal angiomas

5- renal cysts

- **VEGF also increases the expression of ligands that activate the Notch signaling pathway, which regulates the branching and density of the new vessels.**
- **Anti-VEGF antibody is now approved for the treatment of several types of cancers.**

Reprogramming Energy Metabolism

- **Reprogramming of energy metabolism is so common to tumors that it is now considered a hallmark of cancer.**
- **Even in the presence of ample oxygen cancer cells shift their glucose metabolism away from efficient mitochondrial oxidative phosphorylation to glycolysis.**
- **This phenomenon, called the Warburg effect and also known as aerobic glycolysis.**

- Aerobic glycolysis is less efficient than mitochondrial oxidative phosphorylation, producing 2 molecules of ATP per molecule of glucose, versus 36.
- Yet tumors that adopt aerobic glycolysis, such as Burkitt lymphoma, are the most rapidly growing of human cancers.
- Indeed, in clinical practice, the "glucose hunger" of such tumors is used to visualize tumors by positron emission tomography (PET) scanning, in which the patient is injected with 18F-fluorodeoxyglucose, a nonmetabolizable derivative of glucose.
- Most tumors are PET-positive, and rapidly growing ones are markedly so.

- **Rapidly dividing normal cells, such as those in the embryo, also adopt Warburg metabolism, indicating that this mode of metabolism is favored when rapid growth is required.**
- **Tumor cells before division must also double all of its other components, including membranes, proteins, and organelles which requires increased uptake of nutrients, particularly glucose and amino acids.**

- **In rapidly growing cells glucose is the primary source of the carbons that are used for synthesis of lipids (needed for membrane assembly) as well as other metabolites needed for nucleic acid synthesis.**
- **This pattern of glucose carbon use is achieved by shunting pyruvate toward biosynthetic pathways at the expense of the oxidative phosphorylation pathway and ATP generation.**
- **tumor cells that adapt this altered metabolism are able to divide more rapidly and outpace competing tumor cells that do not**

Genomic Instability-Enabler of Malignancy

- **The importance of DNA repair in maintaining the integrity of the genome is highlighted by several inherited disorders in which genes that encode proteins involved in DNA repair are defective.**
- **Individuals born with such inherited defects in DNA repair proteins are at a greatly increased risk of developing cancer.**

- **Typically, genomic instability occurs when both copies of the gene are lost.**
- **Defects can involve 3 types of DNA repair systems :-**

1-mismatch repair.

2-nucleotide excision repair.

3-recombination repair.

Hereditary Nonpolyposis Colon Cancer Syndrome(HNPCC)

- **HNPCC syndrome is characterized by familial carcinomas of the colon affecting predominantly the cecum and proximal colon**
- **It results from defects in genes involved in DNA mismatch repair.**

- **When a strand of DNA is being repaired, these genes act as "spell checkers."**
- **E.g if there is an erroneous pairing of G with T rather than the normal A with T, the mismatch repair genes correct the defect.**
- **Without these genes errors gradually accumulate in several genes, including proto-oncogenes and cancer suppressor genes.**

- **Mutations in at least 4 mismatch repair genes have been found to underlie HNPCC .**
- **Each affected individual inherits one defective copy of one of several DNA mismatch repair genes and acquires the second hit in colonic epithelial cells.**
- **DNA repair genes behave like tumor suppressor genes in their mode of inheritance, but in contrast to tumor suppressor genes (and oncogenes), they affect cell growth only indirectly-by allowing mutations in other genes during the process of normal cell division.**

- **One of the hallmarks of patients with mismatch repair defects is microsatellite instability (MSI).**
- **Microsatellites are tandem repeats of 1-6 nucleotides found throughout the genome.**

- **In normal people the length of these microsatellites remains constant.**
- **In patients with HNPCC, these satellites are unstable and increase or decrease in length.**
- **HNPCC accounts only for 2-4% of all colonic cancers.**
- **MSI can be detected in about 15% of sporadic cancers.**

- **The growth-regulating genes that are mutated in HNPCC include those encoding TGF-β receptor type II, BAX, and other oncogenes and tumor suppressor genes.**

Xeroderma Pigmentosum

- **Patients with xeroderma pigmentosum are at increased risk for the development of cancers of the skin exposed to the ultraviolet (UV) light contained in sun rays.**
- **The basis of this disorder is defective DNA repair.**
- **UV light causes cross-linking of pyrimidine residues, preventing normal DNA replication.**
- **Such DNA damage is repaired by the nucleotide excision repair system.**
- **Several proteins are involved in nucleotide excision repair and an inherited loss of any one can give rise to xeroderma pigmentosum.**

Diseases with Defects in DNA Repair by Homologous Recombination

- **A group of autosomal recessive disorders comprising :**
- **1-Bloom syndrome**
- **2-Ataxia-telangiectasia**
- **3-Fanconi anemia**
- **characterized by hypersensitivity to :**
- **1- DNA-damaging agents, such as ionizing radiation (Bloom syndrome and ataxia-telangiectasia),**
- **2-DNA cross-linking agents, such as nitrogen mustard (Fanconi anemia).**

- **Their phenotype is complex and includes, in addition to predisposition to cancer, features such as :**
- **1-neural symptoms (ataxia-telangiectasia & Fanconi anemia)**
- **2-developmental defects (Bloom syndrome).**

- **The gene mutated in ataxia-telangiectasia is *ATM,* which seems to be important in recognizing and responding to DNA damage caused by ionizing radiation.**

- **Mutations in two genes, *BRCA1* and *BRCA2,* account for 80% of cases of familial breast cancer.**
- **In addition to breast cancer, *BRCA1* mutations substantially increase risk of :**
- **1-epithelial ovarian cancers in women.**
- **2-prostate cancer in men.**

- **mutations in the *BRCA2* gene increase the risk of breast cancer in both men and women as well as cancer of the :**
- **1-ovary.**
- **2-prostate.**
- **3-pancreas.**
- **4-bile ducts.**
- **5-stomach.**
- **6-melanocytes.**
- **7-B-lymphocytes**

- **Both copies of *BRCA1* and *BRCA2* must be inactivated for cancer to develop.**
- **Although linkage of *BRCA1* and *BRCA2* to familial breast cancers is established these genes are rarely inactivated in sporadic cases of breast cancer.**
- ***BRCA1* and *BRCA2* are different from other tumor suppressor genes, such as *APC* and *p53,* which are inactivated in both familial and sporadic cancers.**

TUMOR IMMUNITY

- ***immune surveillance*** **to refer to recognition and destruction of newly appearing tumor cells, which are seen as foreign by the host immune system**

Tumor Antigens

- **2 categories based on their patterns of expression:**
- ***1-tumor-specific antigens.***

  which are present only on tumor cells and not on any normal cells.
- **2-*tumor-associated antigens.***

  present on tumor cells and also on some normal cells.

- **This classification is imperfect because many antigens thought to be tumor specific turned out to be expressed by some normal cells as well.**
- **The modern classification of tumor antigens is based on their molecular structure and source.**

1-Products of Mutated Oncogenes and Tumor Suppressor Genes

- **Antigens in this category are derived from mutant oncoproteins and cancer suppressor proteins.**
- **Unique tumor antigens arise from products of β-*catenin, RAS, p53,* and *CDK4* genes.**
- **The mutant proteins are present only in tumors, their peptides are expressed only in tumor cells.**
- **These antigens are shared by different tumors.**

2-Products of Other Mutated Genes

- **Because of the genetic instability of tumor cells many genes are mutated in these cells including genes whose products are not related to the transformed phenotype and have no known function.**
- **Products of these mutated genes are potential tumor antigens.**
- **These antigens are extremely diverse because the carcinogens that induce the tumors may randomly mutagenize virtually any host gene.**

- **Mutated cellular proteins are found more frequently in chemical carcinogen- or radiation-induced animal tumors than in spontaneous human cancers.**
- **They can be targeted by the immune system, since there is no self-tolerance against them.**

3-Overexpressed or Aberrantly Expressed Cellular Proteins

- **Tumor antigens may be normal cellular proteins that are abnormally expressed in tumor cells and elicit immune responses.**
- **Human melanomas tumor antigens are structurally normal proteins that are produced at low levels in normal cells and overexpressed in tumor cells.**
- **E.g Tyrosinase, an enzyme involved in melanin biosynthesis is expressed only in normal melanocytes and melanomas.**

- **T-cells from melanoma patients recognize peptides derived from tyrosinase raising the possibility that tyrosinase vaccines may stimulate such responses to melanomas.**
- **It may be surprising that these patients are able to respond to a normal self-antigen.**
- **The probable explanation is that tyrosinase is normally produced in such small amounts and in so few cells that it is not recognized by the immune system and fails to induce tolerance.**

- **"cancer-testis" antigens, are encoded by genes that are silent in all adult tissues except the testis .**
- **These antigens are tumor specific.**
- **Prototypic of this group is the MAGE family of genes.**
- **MAGE antigens are tumor specific but not unique for individual tumors.**

- **MAGE-1 is expressed on :**
- **1-37% of melanomas**
- **2-lung, liver, stomach, and esophageal carcinomas.**
- **Similar antigens called GAGE, BAGE, and RAGE have been detected in other tumors.**

4-Tumor Antigens Produced by Oncogenic Viruses

- **The most potent of these antigens are proteins produced by latent DNA viruses.**
- **E.g HPV and EBV.**
- **Vaccines against HPV antigens have been found effective in prevention of cervical cancers in young females.**

5-Oncofetal Antigens

- **Oncofetal antigens or embryonic antigens such as carcinoembryonic antigen (CEA) and α-fetoprotein (αFP).**
- **Both are expressed during embryogenesis but not in normal adult tissues.**
- **Derepression of the genes that encode these antigens causes their reexpression in colon and liver cancers.**
- **Used as serum markers for cancer.**

6-Altered Cell Surface Glycolipids and Glycoproteins

- **These altered molecules include :**
- **1-gangliosides.**
- **2-blood group antigens.**
- **3-mucins.**
- **such antigens are not specifically expressed on tumors.**
- **they are present at higher levels on cancer cells than on normal cells.**
- **This class of antigens is a target for cancer therapy with specific antibodies.**

- **These include :**
- **1-CA-125 , expressed on ovarian carcinomas.**
- **2-CA-19-9, expressed on ovarian carcinomas.**
- **3-MUC-1, expressed on breast carcinomas.**

- **Unlike many other types of mucins, MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium.**
- **In ductal carcinomas of the breast, the molecule is expressed in an unpolarized fashion and contains new tumor-specific carbohydrate and peptide epitopes.**
- **These epitopes induce both antibody and T-cell responses in cancer patients and are therefore being considered as candidates for tumor vaccines.**

7-Cell Type-Specific Differentiation Antigens

- **Tumors express molecules that are normally present on the cells of origin.**
- **These antigens are called *differentiation antigens,* because they are specific for particular lineages or differentiation stages of various cell types.**
- **E.g lymphomas may be diagnosed as B-cell-derived tumors by the detection of surface markers characteristic of this lineage, such as CD10 and CD20.**
- **These differentiation antigens are typically normal self-antigens and therefore they do not induce immune responses in tumor-bearing hosts.**

CLINICAL ASPECTS OF NEOPLASIA

- any tumor benign & malignent may cause morbidity and mortality.
- Both malignant and benign tumors may cause problems because of :
- (1) location and impingement on adjacent structures.
- (2) functional activity such as hormone synthesis or the development of paraneoplastic syndromes.
- (3) bleeding and infections when the tumor ulcerates through adjacent surfaces.
- (4) rupture or infarction.
- (5) cachexia or wasting.

Effects of Tumor on Host

- **Location is crucial in both benign and malignant tumors.**
- **A small (1-cm) pituitary adenoma can compress and destroy the surrounding normal gland and give rise to hypopituitarism.**
- **A 0.5-cm leiomyoma in the wall of the renal artery may lead to renal ischemia and serious hypertension.**
- **A small carcinoma within the common bile duct may induce fatal biliary tract obstruction.**

- **Hormone production is seen with benign and malignant neoplasms arising in endocrine glands.**
- **Adenomas and carcinomas arising in the β-cells of the islets of the pancreas can produce hyperinsulinism, sometimes fatal.**
- **some adenomas and carcinomas of the adrenal cortex elaborate corticosteroids that affect the patient (e.g., aldosterone, which induces sodium retention, hypertension, and hypokalemia).**
- **Such hormonal activity is more likely with benign tumors rather than with a corresponding carcinoma.**

- **A tumor may ulcerate through a surface, with consequent bleeding or secondary infection.**
- **Benign or malignant neoplasms that protrude into the gut lumen may become caught in the peristaltic pull of the gut causing intussusception and intestinal obstruction or infarction.**

Cancer Cachexia

- **Progressive loss of body fat and lean body mass accompanied by profound weakness, anorexia, and anemia.**
- **There is some correlation between the size and extent of spread of the cancer and the severity of the cachexia.**
- **Cachexia is not caused by the nutritional demands of the tumor.**

- **Although patients with cancer are often anorexic, current evidence indicates that cachexia results from the action of soluble factors such as cytokines produced by the tumor and the host rather than reduced food intake.**
- **In patients with cancer, calorie expenditure remains high, and basal metabolic rate is increased, despite reduced food intake.**

- This is in contrast to the lower metabolic rate that occurs as an adaptational response in starvation.
- The basis of these metabolic abnormalities is not fully understood.
- TNF produced by macrophages in response to tumor cells or by the tumor cells themselves mediates cachexia.
- TNF suppresses appetite and inhibits the action of lipoprotein lipase inhibiting the release of free fatty acids from lipoproteins.
- A protein-mobilizing factor called proteolysis-inducing factor, which causes breakdown of skeletal muscle proteins by the ubiquitin-proteosome pathway has been detected in the serum of cancer patients.
- Other molecules with lipolytic action also have been found.

Paraneoplastic Syndromes

- **Symptom complexes that occur in patients with cancer and that cannot be readily explained by local or distant spread of the tumor or by the elaboration of hormones indigenous to the tissue of origin of the tumor.**
- **They appear in 10-15% of patients with cancer.**

- **<u>It is important to recognize them for several reasons:</u>**
- **1-They may represent the earliest manifestation of an occult neoplasm.**
- **2-They may represent significant clinical problems and may even be lethal.**
- **3-They may mimic metastatic disease and confound treatment.**

- ***The most common syndromes are :***
- ***1-Hypercalcemia.***
- ***2-Cushing syndrome.***
- ***3-Nonbacterial thrombotic endocarditis.***
- ***4-Others as*** **clubbing of the fingers and hypertrophic osteoarthropathy in patients with lung carcinomas.**

- **The neoplasms most often associated with these and other syndromes are :**
- **1-lung & breast cancers.**
- **2-hematologic malignancies.**

Hypercalcemia

- **Hypercalcemia in cancer patients is multifactorial, but the most important mechanism is :**
- **1-the synthesis of a parathyroid hormone-related protein (PTHrP) by tumor cells.**
- **2-TGF-α, a polypeptide factor derived from malignent cells that activates osteoclasts and the active form of vitamin D.**

- **widespread osteolytic metastatic disease of bone can cause *hypercalcemia resulting from bone destruction but it is not a paraneoplastic syndrome*.**

Cushing syndrome

- **Cushing syndrome as a paraneoplastic phenomenon is usually related to ectopic production of ACTH or ACTH-like polypeptides by cancer cells.**
- **Small-cell carcinoma of the lung.**
- **Sometimes one tumor induces several syndromes concurrently.**
- **E.g bronchogenic carcinomas may elaborate products identical to or having the effects of ACTH, antidiuretic hormone, parathyroid hormone, serotonin, human chorionic gonadotropin, and other bioactive substances.**

Hypercoagulability

- leading to venous thrombosis & nonbacterial thrombotic endocarditis .

Grading of Cancer

- **The *grading* of a cancer attempts to establish some estimate of its aggressiveness or level of malignancy.**
- **It is based on :**
- **1-the cytologic differentiation of tumor cells.**
- **2-the number of mitoses within the tumor.**

- **The cancer may be classified as grade I, II, III, or IV, in order of increasing anaplasia.**

- **Criteria for the individual grades vary with each form of neoplasia .**
- **Difficulties in establishing clear-cut criteria have led in some instances to descriptive characterizations as :**
- **Well-differentiated**
- **Moderately-differentiated.**
- **Poorly-differentiated.**
- **Anaplastic tumors .**

Staging of cancer

- ***Staging*** **of cancers is based on :**
- **1-the size of the primary lesion.**
- **2-its extent of spread to regional lymph nodes.**
- **3-the presence or absence of metastases.**

- **This assessment is usually based on clinical and radiographic examination (CT scan & MRI) and in some cases surgical exploration.**

- **<u>Two methods of staging are currently in use:</u>**
- **1-the TNM system (*T,* primary tumor; *N,* regional lymph node involvement; *M,* metastases)**
- **2-the AJC (American Joint Committee) system..**

- **In the TNM system, T1, T2, T3, and T4 describe the increasing size of the primary lesion;**
- **N0, N1, N2, and N3 indicate progressively advancing node involvement;**
- **M0 and M1 reflect the absence or presence of distant metastases.**

- **In the AJC method, the cancers are divided into stages 0 to IV, incorporating the size of primary lesions and the presence of nodal spread and of distant metastases.**
- ***staging has proved to be of greater clinical value than grading.***

Staging of breast carcinoma

stage	Tumor	Lymph Nodes	Metastasis	Prognosis 5-yr survival
0	DCIS or LCIS	0	M 0	92%
I	Invasive carcinoma 2 cm or less in diameter	0	M 0	87%
II	< or = 5cm > 5cm	< or 3 0	M 0 M 0	75%
III	< or = 5 cm >5 cm Any size	4 or more Any number 10 or more	Skin or chest wall involvement	46%
IV	Any size	Any number	M 1	13%

TNM Staging of Colon Cancers

- **Tumor**
- T0 none evident
- Tis = in situ (limited to mucosa)
- T1 = invasion of lamina propria or submucosa
- T2 = invasion of muscularis propria
- T3 = invasion through muscularis propria into subserosa or nonperitonealized perimuscular tissue
- T4 = invasion of other organs or structures
- **Lymph Nodes (N)**
- 0 = none evident
- 1 = 1 to 3 positive pericolic nodes
- 2 = 4 or more positive pericolic nodes
- 3 = any positive node along a named blood vessel

- **Distant Metastases (M)**
- 0 = none evident
- 1 = any distant metastasis
- **5-Year Survival Rates**
- T1 = 97%
- T2 = 90%
- T3 = 78%
- T4 = 63%
- Any T; N1; M0 = 66%
- Any T; N2; M0 = 37%
- Any T; N3; M0 = data not available
- Any M1 = 4%

Laboratory Diagnosis of Cancer

- **1-Morphologic Methods :**
- **(H&E stain)**
- A-excision or biopsy.
- B-fine-needle aspiration.
- C-cytologic smears (Papanicolaou) .
- D-Frozen sections.

- ***Immunocytochemistry*** **:**
- **Cytokeratin**
- **prostate-specific antigen (PSA) =prostate carcinoma.**
- **estrogen receptors =breast cancer.**

- ***Flow cytometry***
- **is used routinely in the classification of leukemias and lymphomas.**
- **Fluorescent antibodies against cell surface molecules and differentiation antigens are used to obtain the phenotype of malignant cells.**

- **2-Tumor Markers** :
- **A-PSA**
- **Prostatic carcinoma can be suspected when elevated levels of PSA are found in the blood.**
- **PSA levels are often elevated in cancer.**
- **PSA levels also may be elevated in benign prostatic hyperplasia**
- ***PSA test suffers from both low sensitivity and low specificity*.**

- **B-carcinoembryonic antigen (CEA).**
- **carcinomas of the colon, pancreas, stomach, and breast.**
- **C-α-fetoprotein.**
- **produced by :**
- **1- hepatocellular carcinomas.**
- **2-yolk sac remnants in the gonads.**
- **3-teratocarcinomas.**
- **4-embryonal cell carcinomas.**
- **5-neural tube defect of the fetus.**
- **CEA and α-fetoprotein assays lack both specificity and sensitivity**

- **3-Molecular Diagnosis**