NEOPLASIA SHEET

Lectures 5&6

DONE BY:
MAHMOUD ALJUNAIDI

Neoplasia sheet

(lectures 5&6)

بما يخص المحاضرة الأولى في الشيت الأول, تم اضفاء تعديل بسيط (زيادة معلومات) لم تكن موجودة في الملف الأول القديم, يرجى الاطّلاع عليهم في اخر الملف الأول.

❖ Neoplasia 5:

➢ Insensitivity to Growth-Inhibitory Signals:

- The signal of tumor suppressor genes have **anti-growth signals** that can prevent cell proliferation in different mechanisms:
    1. May cause dividing cells to **enter G0** (quiescence),
    2. May cause the **re-entery** of cells in a **postmitotic**, **differentiated pool** and **lose replicative potential.**

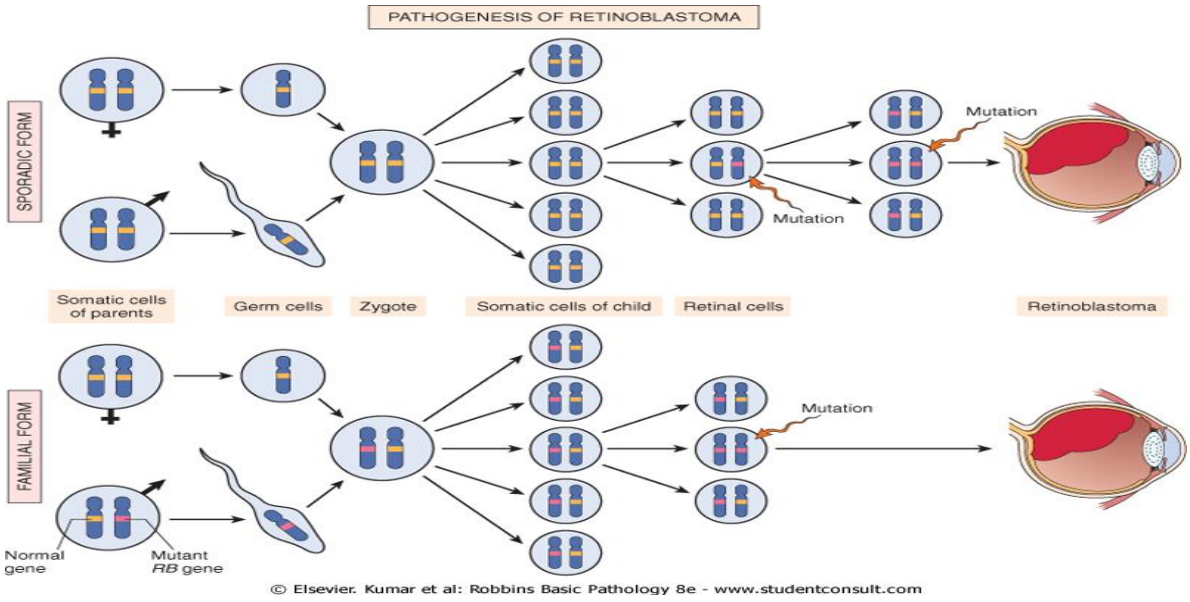
➢ Retinoblastoma gene (RB): Governor of the Cell Cycle:

- **RB** gene is a key **negative regulator** of the cell cycle, is **directly** or **indirectly inactivated** in most human cancers.
- 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.**
- The function of the RB protein is to **regulate the G1/S checkpoint**, the portal through which cells must pass before DNA replication commences.
- The transition from G1 to S is an **extremely important** checkpoint in the cell cycle. (refer to the cell cycle next page)

✓ 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** (tumor).
- These involve the ***RB*** gene, located on chromosome **13q14**.
- **Both of the normal** alleles of the *RB* locus must be **inactivated** (two hits) for the development of retinoblastoma.
- In familial cases, children **inherit one defective copy** of the *RB* gene in the germ line while the **other copy is normal**.
- Retinoblastoma develops when the normal ***RB* gene is lost in retinoblasts as a result of somatic mutation.**

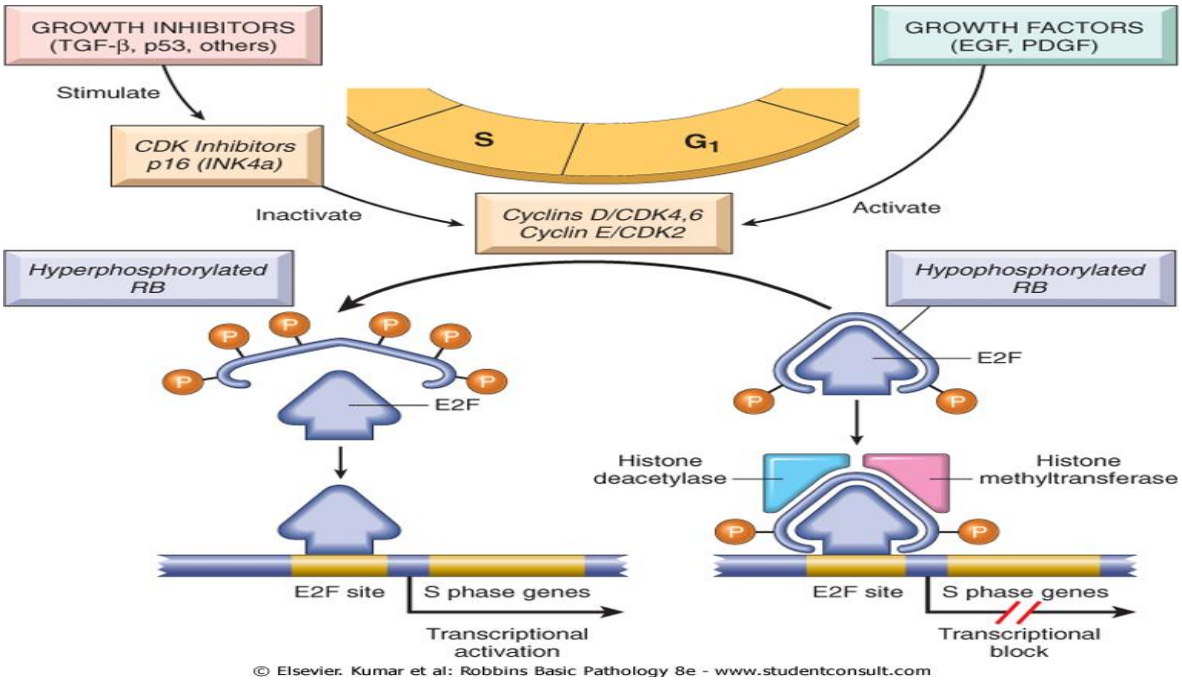
- In retinoblastoma families **only a single** somatic **mutation** is required for **expression** of the **disease.**
- The familial transmission follows an **autosomal <u>dominant</u>** inheritance pattern.
- In sporadic cases, **both** normal *RB* alleles are lost by somatic mutation in one of the 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 :
    1) Breast cancer
    2) Small-cell cancer of the lung
    3) 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 <u>hypo</u>phosphorylated*** and an **inactive <u>hyper</u>phosphorylated** 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 <u>immediately</u> **after mitosis** ends. (M Phase-S Phase-M Phase-S Phase ...)

- 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 (سكون).
    2) Permanently, called senescence (شيخوخة).
- 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.



❖ Cell cycle and mitogenic signaling:

- 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 [a]**histone deacetylases** and [b]**histone methyltransferases**, which bind to the promoters of E2F-responsive genes such as cyclin E.

- These enzymes (A & B) **modify chromatin** at the promoters to make **DNA insensitive to transcription factors,** so no transcription.
- 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, examples:
  - RB stimulates myocyte-, adipocyte-, melanocyte-, and macrophage-specific transcription factors.
- RB pathway is important to:
  1) Control of cell cycle progression at G_1
  2) Induce cell differentiation
  3) Induce senescence

➢ **Mutations** in other **genes that control RB phosphorylation** can mimic the effect of ***RB*** **loss.**

- Genes are mutated in many cancers that seem to have normal *RB* genes. Examples,

  - 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 (Mutations in ATM and ATR), 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)***- *recall inhibitors*.
- 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 **varies**.
- The most common types of tumors are:
  - sarcomas, breast cancer, leukemia, brain tumors, and carcinomas of the adrenal cortex.

Here's a quiz on this lecture.

❖ Neoplasia 6:

➢ Transforming Growth Factor-β Pathway:

- **TGF-β**, a member of a family of **dimeric** growth factors that includes bone morphogenetic proteins and activins.
- In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a **potent inhibitor** of proliferation.
- 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 the **transcriptional activation** of CDKIs with growth-suppressing activity as well as repression of growth-promoting genes such as ***MYC*** and ***CDK4***.
- In many forms of cancer the **growth-inhibiting effects** of the TGF-β pathways are **impaired by mutations** affecting TGF-β signaling.
- These mutations may **alter** the **type II TGF-β receptor** or **SMAD** molecules that serve to transduce anti-proliferative signals from the receptor to the nucleus.
- Mutations affecting the type II receptor are seen in:
    1. Colon cancer.
    2. Stomach cancer.
    3. Endometrium cancer.
    4. Pancreatic cancer (mutational inactivation of SMAD4).
- **Loss** of TGF-β–mediated growth **control** occurs at a level **downstream of the core TGF-β signaling pathway**; for example, there may be loss of p21 expression and/or overexpression of *MYC*.
- These tumor cells can then **use other elements of the TGF-β induced program**, including immune system suppression or promotion of angiogenesis to **facilitate tumor progression**.
- TGF-β can function to **prevent** or **promote** tumor growth depending on the state of other genes in the cell.

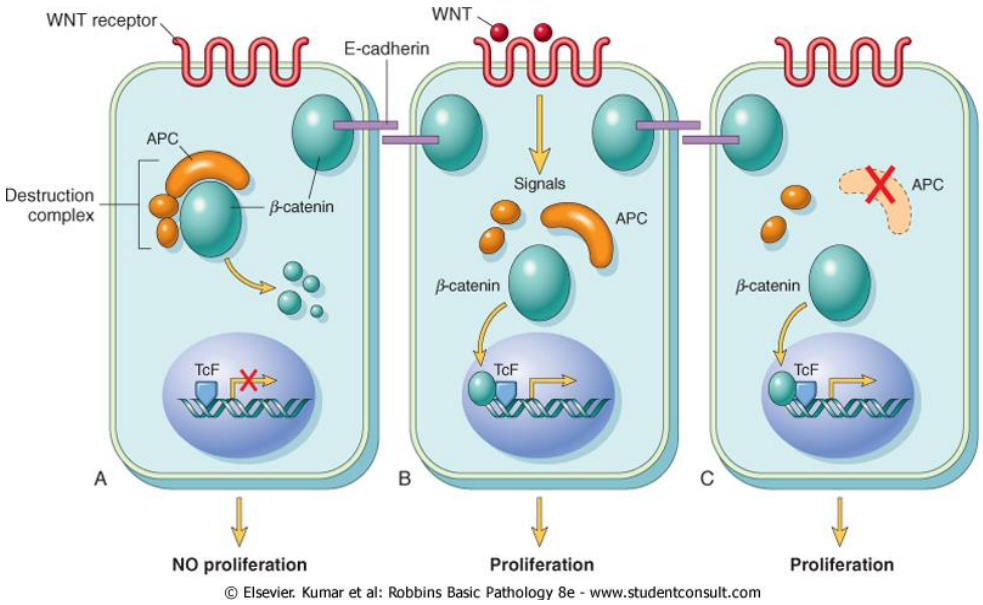
➢ Contact Inhibition, NF2, and APC:

- When cancer cells are grown in the laboratory their proliferation fails to be inhibited when they come in contact with each other. (definition)
- This is in sharp contrast to **nontransformed cells** which **stop** proliferating once they **form confluent monolayers**.
- **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.

- Two mechanisms have been proposed to explain how E-cadherin maintains contact inhibition:
    1. One mechanism is mediated by the tumor suppressor gene *NF2*.
        - **Neurofibromin-2** (called *merlin*) acts downstream of E-cadherin in a signaling pathway that helps for **maintain contact inhibition**.
        - **Homozygous loss of *NF2*** is known to cause certain neural tumors as **neurofibromatosis type 2**.
    2. E-cadherin may regulate contact inhibition through β-catenin signaling pathway.
        - APC gene mutation and adenomatous polyposis coli.

➢ 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**.
- β-catenin is a protein with many functions:
    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.
- With loss of APC (in malignant cells), **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***.
- ***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.



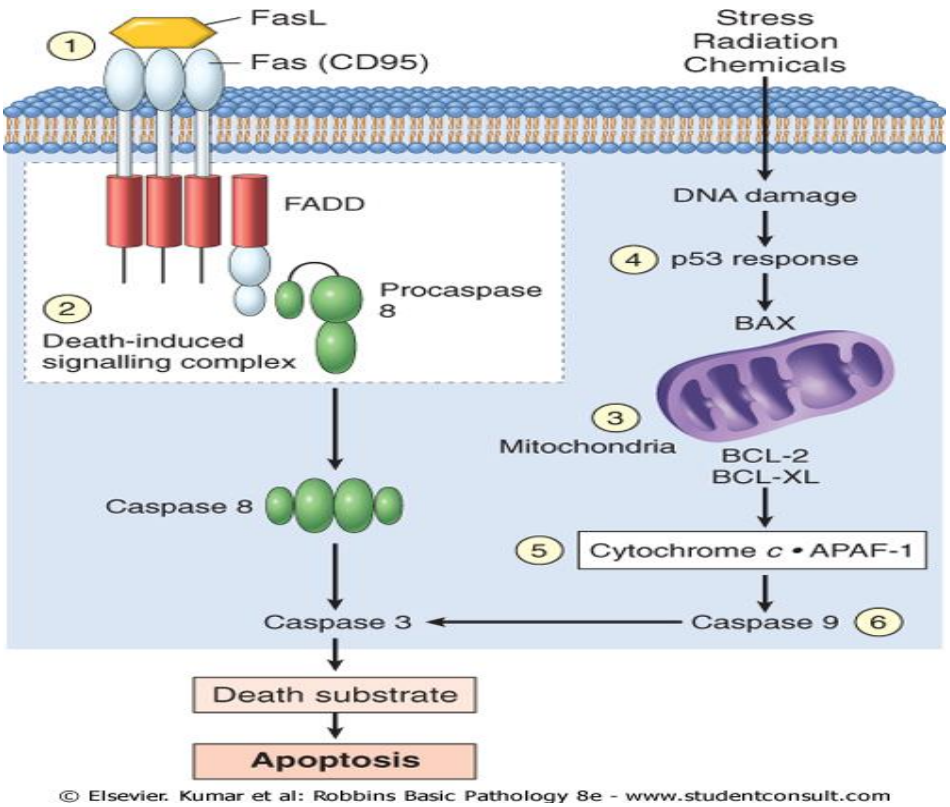
- ***APC* mutations** are seen in 70-80% of **sporadic colon cancers**.
- Colonic cancers **that have normal *APC* genes** show <u>activating mutations of β-catenin</u> that render them refractory to the degrading action of APC.

➢ <u>Evasion of Apoptosis:</u>

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

Overview of apoptosis →

To be explained, look at it then come back again.



1) Extrinsic pathway:

- The extrinsic pathway is initiated when **CD95 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** a typical **executioner caspase** that cleaves DNA and other substrates to cause cell death.

2) Intrinsic pathway:

- The intrinsic pathway of apoptosis 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.**

✓ **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 BCL2 family of proteins.
- 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 BCL2/BCLXl protein barrier, **BAX and BAK are activated and form pores in the mitochondrial membrane.**
- Cytochrome *c* leaks into the cytosol where it binds to **APAF-1**, **activating caspase 9.**
- Like caspase 8 of the extrinsic pathway, caspase 9 can **cleave** and **activate** the **executioner caspases**.

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

✓ So now, how do malignant cells evade apoptosis?

- **Malignant 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.
- Of all these genes the role of **BCL2** in protecting tumor cells from apoptosis is best established.
- 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, is also involved in the **pathogenesis of Burkitt lymphoma**.
- **Juxtaposition** of this transcriptionally active locus **with *BCL2*** (located at 18q21) causes **overexpression of the BCL2 protein**.
- This in turn increases the BCL2/BCL-XL buffer protecting lymphocytes from apoptosis and allowing them to survive for long periods.
- Steady accumulation of B lymphocytes resulting in lymphadenopathy and marrow infiltration.
- 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.

➢ **Limitless Replicative Potential:**

- Most normal human cells have a capacity of 60-70 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.**
- Tumor cells, unlike normal cells, are **capable of limitless replication**.
- Markedly shortened telomeres are **recognized** by the **DNA repair machinery** as double-stranded DNA breaks leading to **cell cycle arrest and senescence mediated by *TP53* and *RB*.**

- In cells in which ***TP53*** or ***RB*** mutations are **disabled** by mutations, the **nonhomologous end-joining pathway** is activated in a last effort to **save the cell joining the shortened ends of two 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 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 (apoptosis).**
- It follows that for tumors to grow **indefinitely**, as they often do, **loss of growth restraints is not enough.**
- Tumor cells must also develop ways to **avoid** both **cellular senescence** and **mitotic catastrophe**.

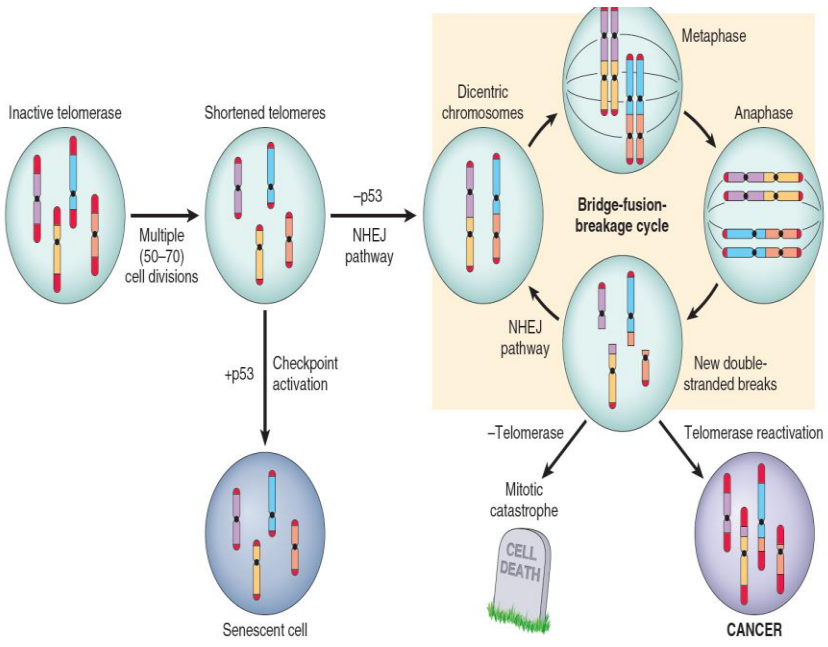


Fig. 6.26 Escape of cells from replicative senescence and mitotic catastrophe caused by telomere shortening.

- 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 cancer.
- In 85-95% of cancers due to up-regulation of the enzyme telomerase.

- A few tumors use other mechanisms, termed **alternative lengthening of telomeres**, which probably depend on **DNA recombination**.
- In the **progression** from colonic adenoma **to colonic adenocarcinoma**, early lesions had a **high degree of genomic instability with low telomerase expression.**
- **Malignant** lesions had **complex karyotypes** with high levels of telomerase activity consistent with a model of **telomere-driven tumorigenesis** in human cancer.

Here's a quiz on this lecture.

OVERALL QUIZ (LECTURES 5 AND 6)

FEEDBACK

* Resources used and for you to use:

1. lecture 5 (DR. Maha Shomaf)
2. lecture 5- pt.1 (2022)
3. lecture 5- pt.2 (2022)
4. lecture 6 – pt.1 (2022)
5. lecture 6 - pt. 2 (2022- skip start)

V1	Overall quiz link not working → link fixed.