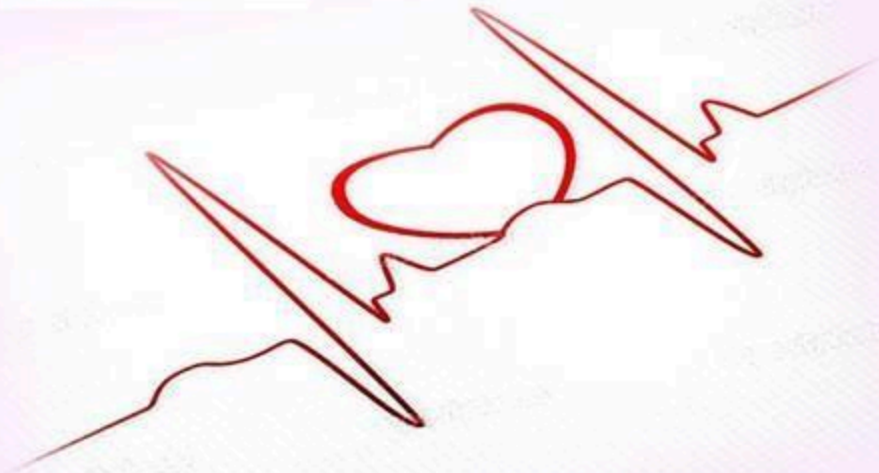
PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE



Lecture Number: 20

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Designed By: Majida Al-foqaraa'

Hallmarks of Cancer

The following sheet is made from 2 records (section 2 & 3)

Before starting, the doctor mentioned a mistake in sheet 18 page 7 (8 with cover)

"Amplification of NMYC gene (present in neurons specifically) which is a transcription factor is associated with 25-30 % of the cases of neuroblastomas and it's associated with poor prognosis (because we don't have the proper molecular techniques to diagnose it, but in the next 5-10 years this can happen)..."

The underlined sentence is incorrect...

*"It is associated with poor prognosis because we do not have the proper molecular techniques to **TREAT it NOT diagnose it**!"*

Topics discussed:

- ✓ Continuation of the second hallmark; evading growth inhibition
  - -TP53
  - -TGF-B Pathway
  - Contact inhibition
- ✓ EMT
- ✓ Evasion of cell death
  - -Apoptosis
  - -Autophagy

Evading Growth Inhibition

TP53; Guardian of the Genome

NOTE: When we are discussing *dysregulation of growth and increased proliferation*, we would be talking about *oncogenes*, while when we are discussing *dysregulation of growth inhibition*, we would be talking about *tumor suppressor genes* in particular.

The first pathway regulating growth inhibition is retinoblastoma, we discussed it in the previous lecture, we went over cell proliferation, cell cycle, how RB governs the cycle by governing the G_1-S transition. When looking at the majority of cancers,

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you will find an abnormal G_1-S transition by dysregulating one of four parts of the pathway;

1) inactivating a cyclin-dependent kinase inhibitor like p16

2+3) over-expressing or amplifying cyclin-D or cyclin dependent kinase 4 (CDK4)

4) mutating RB itself

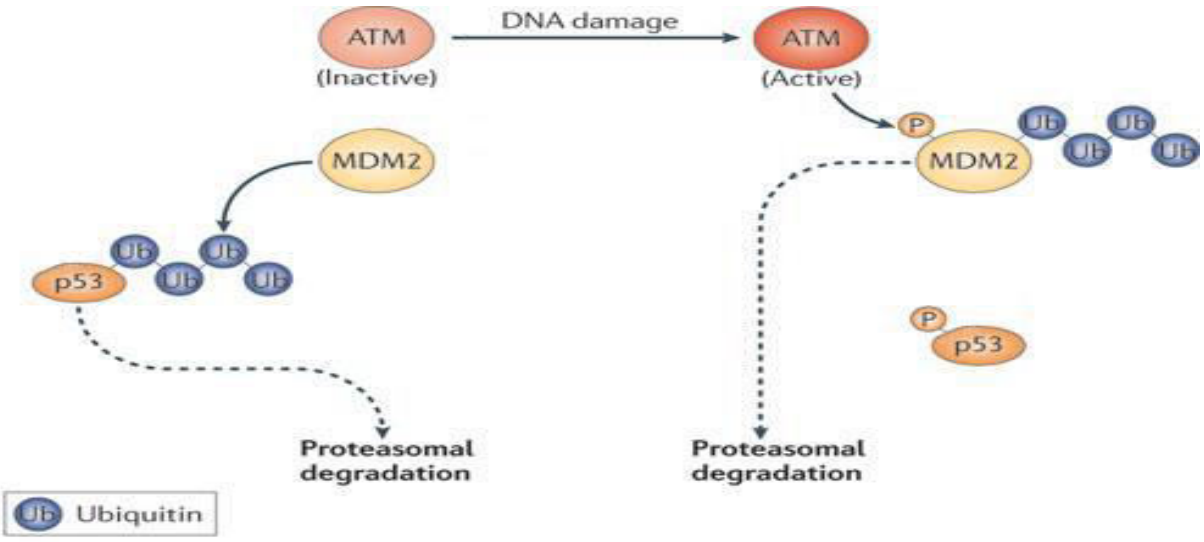
RB is the prototypical tumor suppressor, another tumor suppressor also related to growth inhibition is p53, the gene is called **TP53.**

P53 was previously discussed in apoptosis, when you have DNA damage, p53 can induce apoptosis if the DNA damage is un-repairable or severe.

P53 is a classic tumor suppressor, when it is inactive, i.e not sensing DNA damage or anoxia or abnormal oncoprotein activity, p53 is ubiquitylated by MDM2 (mouse double minute 2) which is a ubiquitin ligase; it adds ubiquitins to a protein. When p53 is ubiquitylated it is sent off for proteasomal degradation using the proteasomal-ubiquitin pathway.

Recall from previous lecture: mdm2 can be inhibited by P14R (one of the proteins produced at the CDK locus which can be potentially inhibited by hypermethylation in cancer)

If p53 is subjected to DNA, anoxia or abnormal oncogene activity, p53 escapes ubiquitylation.



How does it sense DNA damage?

- ✓ By Ataxia-telangiectasia mutate (ATM)

ATM is a serine-threonine protein kinase, it is inherited as an autosomal recessive disease where people have cerebellar degeneration and are extra sensitive to DNA damage due to radiation, they present with an increased risk of cancer.

When this protein is active, it phosphorylates p53 and MDM2. When MDM2 is phosphorylated this targets it to be ubiquitylated, (here we are inhibiting the inhibitor of p53 hence we are activating p53), this protein can also affect transcription of multiple-downstream genes.

When this protein is mutated (inactive) it can no longer phosphorylate p53, hence p53 is ubiquitylated and sent to the proteasomal degradation pathway.

Normally in a cell without DNA damage, p53 is ubiquitylated by MDM2, this gives it a half life of 20minutes.

In Li-Fraumeni syndrome, there is one mutant allele inherited, people with this syndrome have a 25-fold increased risk of cancer compared to the general population.

Recall RB counts as an autosomal dominant disease as it increases the general risk of cancer, however on the cellular level, it counts as autosomal recessive for a phenotype of a specific cancer.

P53 is a **tumor suppressor** and you need to lose (2) p53 genes on the cellular level in order to get cancer, however it is inherited as autosomal dominant because the phenotype is increased risk of cancer. (JUST LIKE RB)

Compared to RB, people with a mutated allele can get a wider range of cancer! Why?

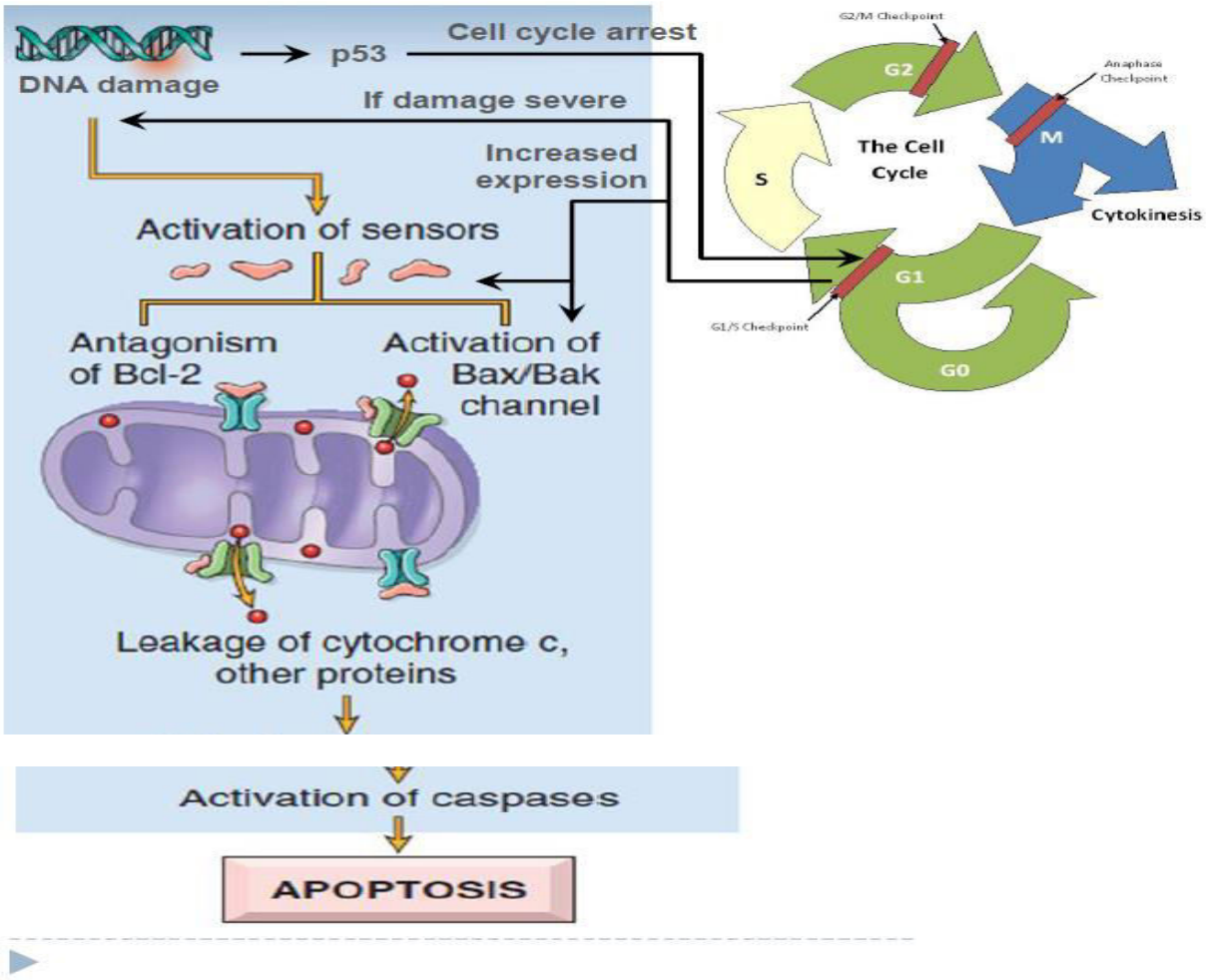
Since it is a *mutated phenotype*, it doesn't only affect the cell cycle, but also it accumulates mutations which could be wide-ranging.

By affecting several transcriptional genes, p53 can:

1. activate temporary cell cycle arrest (known as -quiescence)

2. induce permanent cell cycle arrest (known as senescence) , due to severe DNA damage

3. trigger apoptosis



Not only does p53 sense DNA damage, but also it <u>senses anoxia and oncogene expression</u> e.g. abnormal MYC/RAS activity which are related to growth proliferation, p53 can sense this abnormal activity and induce the cells to quiesce, senesce or die. So how does it do this?

By regulating transcription of several downstream genes:

1. CDKN1A, (p21) is a CDK inhibitor; by inducing its transcription you are inducing the inhibition of the cell cycle.

2. miRNAs, some are under transcriptional control of p53, they target cyclins, when you downregulate the production of cyclins you can no longer go through the cell cycle. (this provides "breathing time" for the cell to repair any DNA damage).

Some miRNAs also target BCL-2 mRNA, this action downregulates production of BCL-2 which is an inhibitor of apoptosis.

REMEMBER: Inhibiting an inhibitor = Activation

3. GADD45A ; Growth Arrest and DNA repair protein 45, it repairs DNA.

If the DNA repair is taking too long or not effective, p53 induces the intrinsic pathway of apoptosis using BAX which is activated by sensors allowing cytochrome C to leak out from the newly activated channels.

4. BAX, it is a proapoptotic protein, its sensors antagonize BCL-2

5. PUMA, it is a BH3 sensor, BCL-2 antagonist, when its transcription is increased, apoptosis is induced.

6. MDM2, a protein that destroys p53, it happens to be a transcriptional target of p53, when DNA repair is effective and we no longer need p53, transient signaling pathway takes place where p53 induces the transcription of its destructor. (negative feedback inhibition)

Summary : if you have a normal cell with a functioning p53, and this cell is subjected to DNA damage or hypoxia, the following take place:

P53 activated → targets transcribed → CDK-inhibitor targeted (to arrest the cells)

targets transcribed → miRNA (inhibits cyclins)

targets transcribed → GADD45 (repairs DNA)

If we have successful repair, MDM2 is transcribed and the cell goes back to normal continuing its cycle, however, if we do not have effective repair, p53 induces:

1) apoptosis by increasing BAX/BH3 (PUMA) and decreasing BCL-2 & BCL-XL

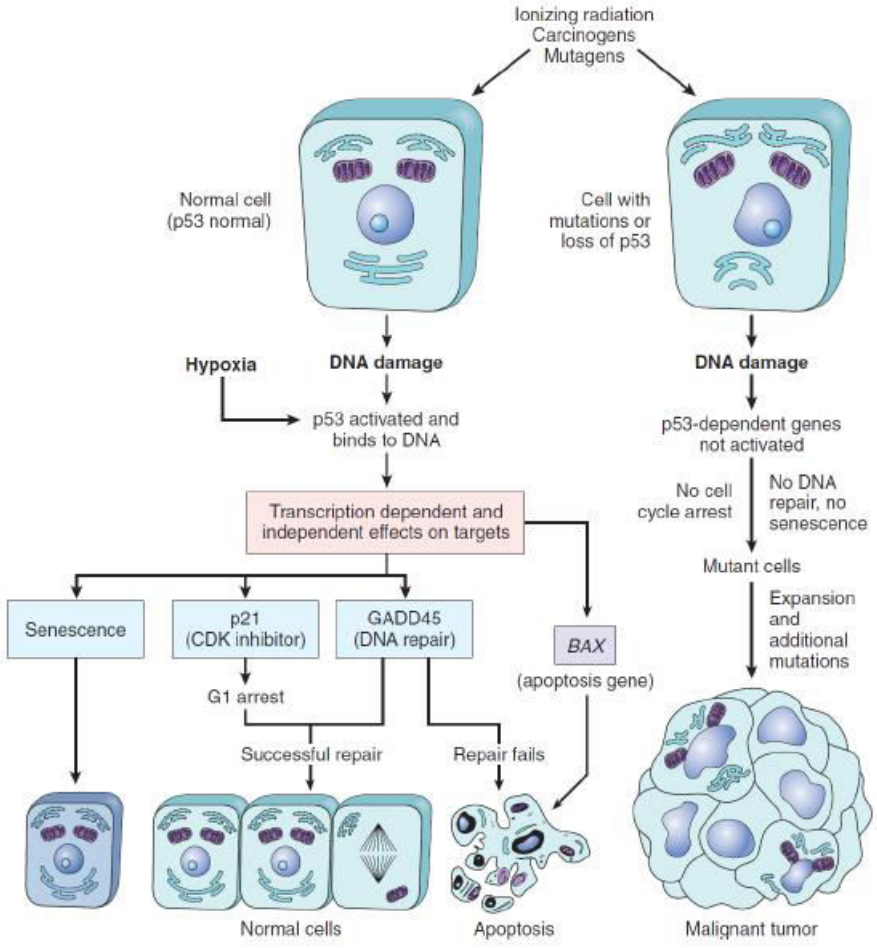
or

2) senescence; permanent exit from the cell cycle in response to DNA damage, where P53 and/or RB pathways are activated.

Note: we do not fully understood why one would occur in preference to the other. (apoptosis and senescence)

During Senescence, a massive epigenetic change occurs to what kind of genes are transcribed. If you do not have normal p53, DNA damage ⟶ P53 not activated, no cell cycle inhibition ⟶ no repair or death, and mutant cells will expand if this mutation is beneficial.

NOTE: not all mutations are beneficial to the cell, some kill it, others make it more favourable for its growth. Natural selection takes place; one cell gains an advantage over the surrounding cells, this cell will be selected and will continue to grow to the next generation.



TP53 : Guardian of the genome

Summary

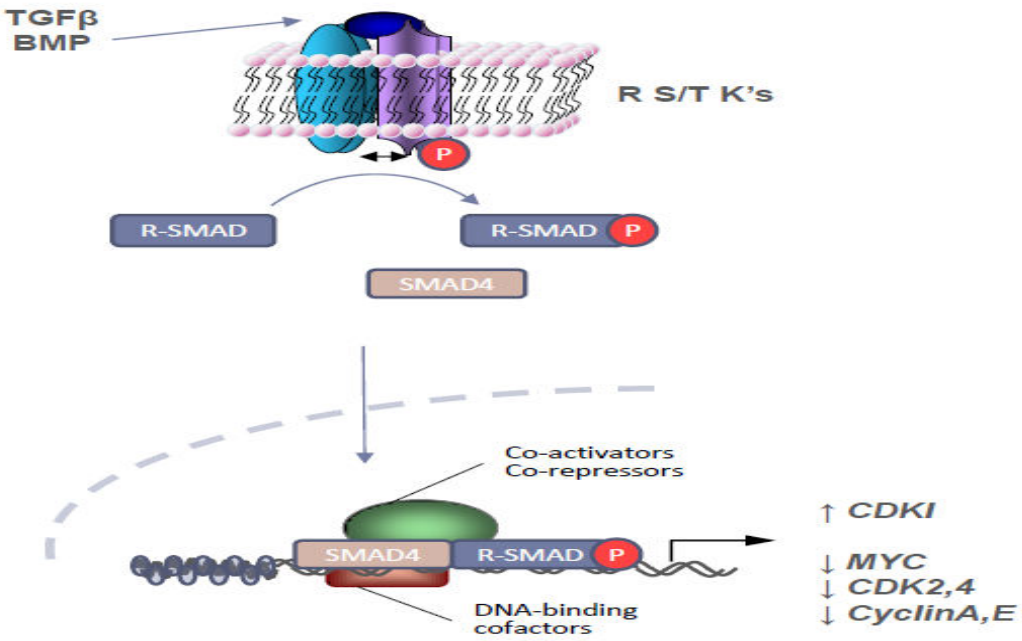
❖ P53 is a target of oncogenic viruses: HPV, HBV and EBV

- ❖ HPV can inactivate RB and affect p53.
- ❖ Viruses can inactivate 2 major parts of G1-S transition
- ❖ They are oncogenic as they allow inappropriate progression through G1-S checkpoint.

TGF-B pathway

- Consists of a family of 23 ligands that bind to serine-threonine kinase receptors.
- Their kinases have ligands, receptors and signal-transducing proteins known as SMADS
- They translocate to the nucleus and increase or decrease transcription of certain proteins e.g. in the growth inhibitory pathway, they increase production of cyclin-dependent kinase inhibitors as well as inhibit MYC (transcription protein that increases other proteins responsible for cell proliferation) consequently inhibiting cell proliferation.
- This pathway is frequently mutated in cancer

  - ✓ Type 2 receptors mutations are found in colon, stomach and endometrial cancer.
  - ✓ SMAD4 mutations are present in pancreatic cancer



100% of pancreatic cancers surveyed to-date, have a TGF-B pathway mutation which turns off anti-proliferation ability of TGF-B .

REMEBER: in epigenetic context for notch signaling, depending on the cell you could either have pro or anti-carcinogenic effect.

Here, this is the first example of **time related duality of function** of the pathway,

i.e it is time dependent, carcinogenesis is a multistep process, if a cancer activates an oncogene, it will accumulate certain mutations that are downstream of TGF-B .

e.g. CDKN2A hypermethylation ⟶ here we are inhibiting the production of the CDK inhibitor.

Another example is when you have increased MYC production or over-expression of cyclin-dependent kinase 4 (CDK-4).

When you signal the TGF-B pathway, you will find these proteins are no longer under the control of the TGF-B pathway, hence it is no longer an effective growth inhibitor.

Keep in mind *Angiogenesis* is also induced by TGF-B.

Furthermore, TGF-B is an immuno-suppressor; it can help in immuno-invasion of harmful cells.

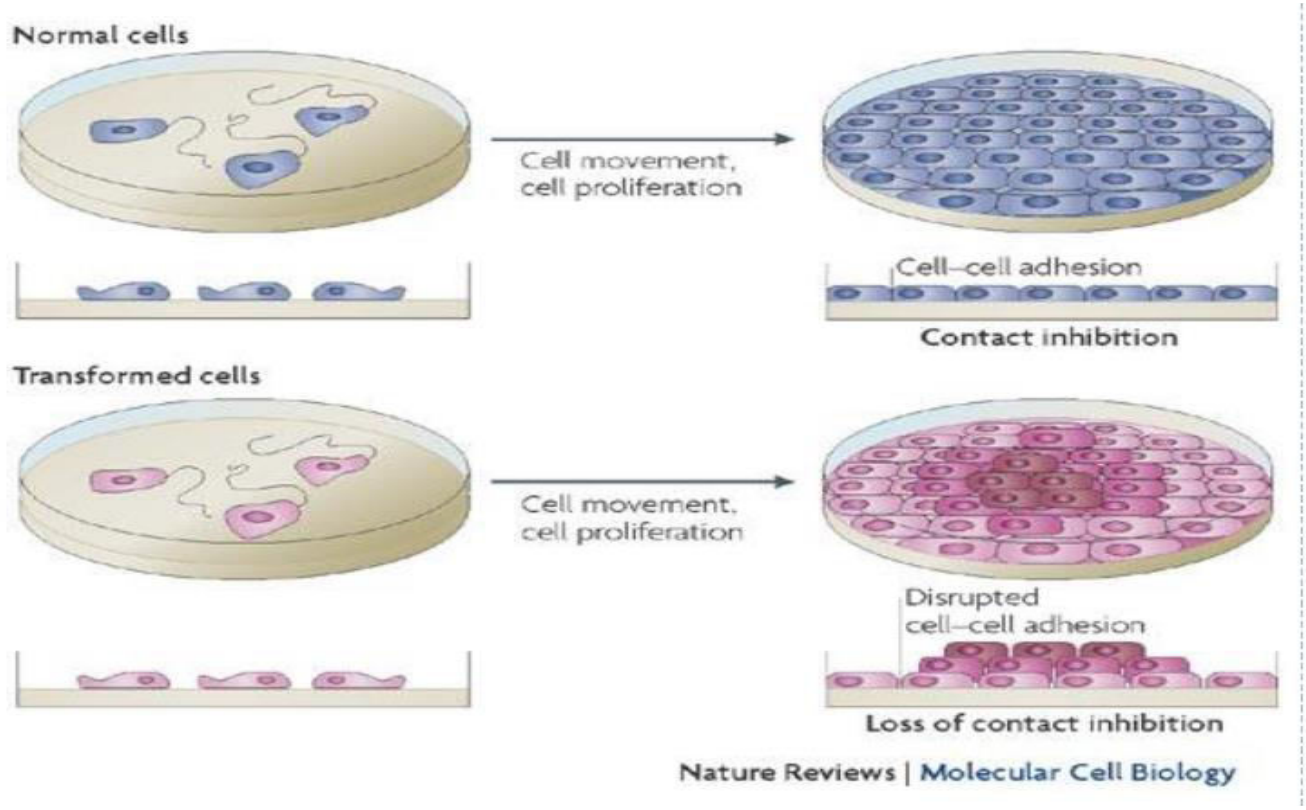
TGF-B can also induce *epithelial to mesenchymal transition*.

Early in cancer, if you activate TFG-B, it acts as an **anticarcinogen.**

However, Late in cancer, it acts a **procarcinogen** as you are allowing cells to evade immune system, invade basement membrane and produce angiogenic factors which favor the cells' growth.

One last thing that inhibits cell growth is *cell-cell contact*, once the cells have created an epithelial barrier they do not need proliferate unless there's a gap (i.e they lost a cell). Normally if you plant epithelial cells on a dish, cells will move, grow and proliferate until a monolayer is seen, then the cells will stop growing due to cell-cell contact inhibition.

Nonetheless, if the cells are transformed (mutated cancer cells) they aren't inhibited by contact inhibition and will continue growing layers and cells over each other. (this mechanism is not fully understood yet)

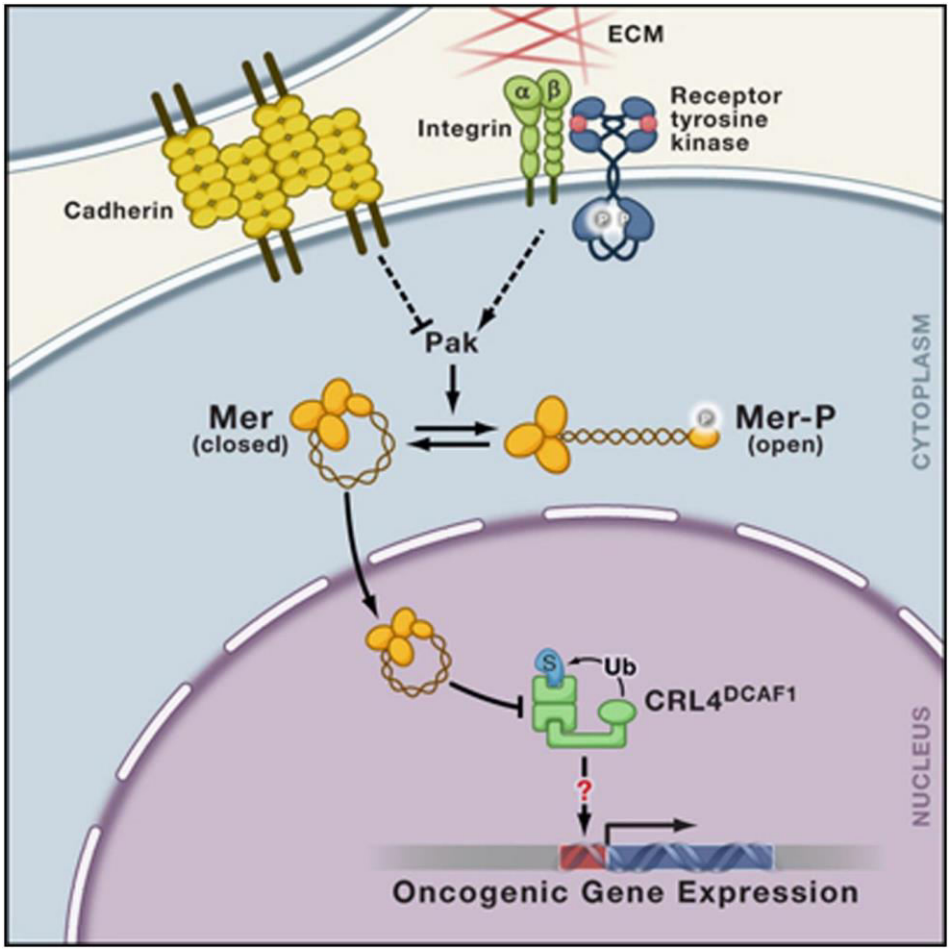


2 known pathways are involved in this cell-cell inhibition

1) NF2 pathway (present in neurofibromin2)

NF2 (also known as merlin) inhibits the transcription of oncogenes.

When there is cell to cell adhesion, cadherins signal from one cell to the next resulting in the formation of merlin which can enter the nucleus and inhibit oncogene production. If there is no cell to cell signaling (no cadherins signaling) the cell are receiving signals mostly from the ECM instead of neighboring cells, merlin will not go to nucleus and won't do its function, therefore *transcription of oncogenes inducing proliferation* won't be inhibited.



2) APC pathway (present in colon cancer)

APC is responsible for destroying a transcription factor when there is no signaling, this transcription factor is b-catenin, it is bound in an inactive pool with e-cadhirin forming what is called a destruction complex.

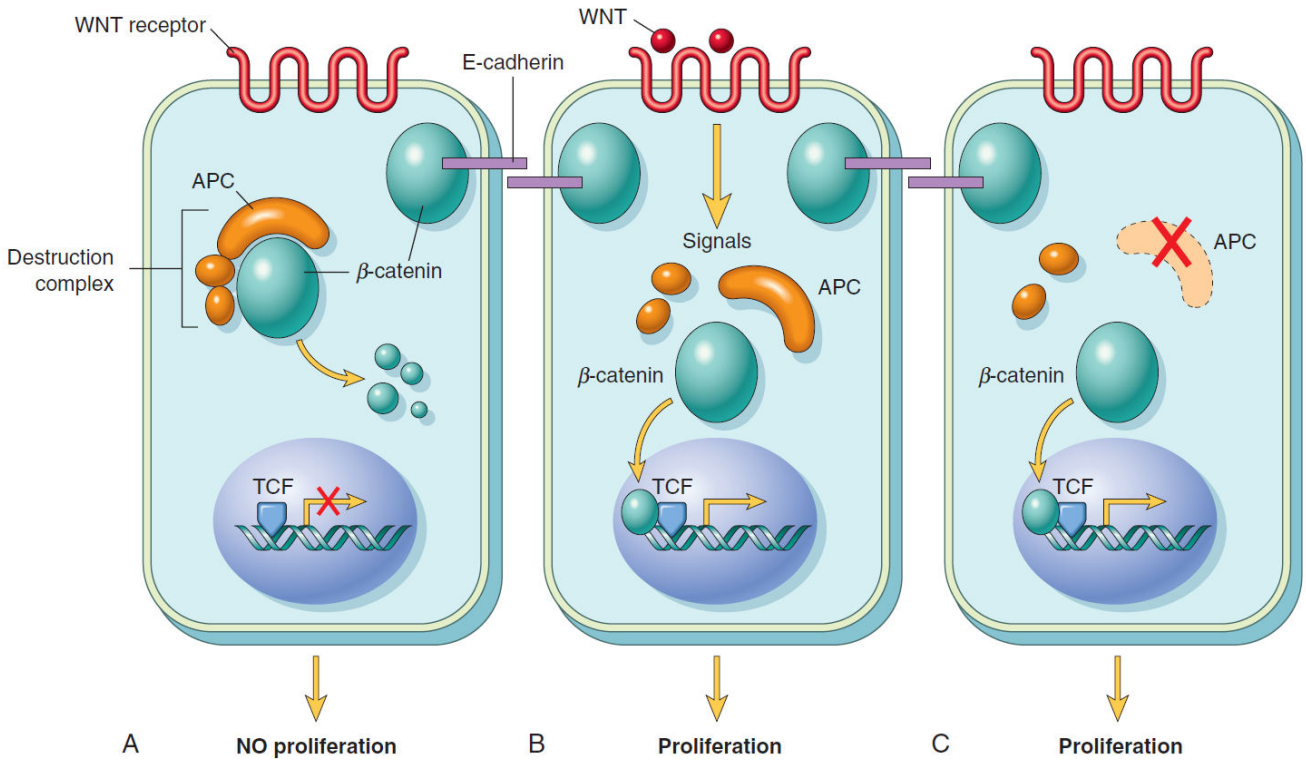
If E-cadhirin is lost, potentially, b-catenins would accumulate.

Normally if APC is there, b-catenin is destroyed and hence not able to enter the nucleus.

However, when there's a signal from a receptor called frizzled, WNT (a ligand which is also as a soluble growth factor) enables the destruction complex to dissociate; allowing b-catenin to escape and go to the nucleus along with other factors like TCF. This induces transcription of certain genes which cause proliferation.

If APC is not functional, even without the signal, there will be proliferation.

Patients with an abnormal copy of the APC gene have familial adenomatous polyposis.



b-catenin targets:

1) growth-promoting genes (positive regulation)

- Cyclin D1
- MYC

2) Transcriptional regulators (negative regulation)

- TWIST
- SLUG/SNAIL

Those are negative regulators of genes that inhibit e-cadherin expression, which results in no contact inhibition.

- ❖ APC has a role in epithelial to mesenchymal transition (EMT)
- ❖ 70-80% of all colorectal cancer patients will have an APC mutation
- ❖ The rest will have a b-catenin mutation or anything in this complex

Short introduction to EMT

Epithelial cells are usually:

- Mono-layered (3D), two dimensionally they would look like a line.
- Attached to cells surrounding them
- Sit on a basement membrane
- Know which way is up and which way is down (apical and basal)

In Cancer transformation:

Downregulation of e-cadehrins and zonula occludens (junctions) causes cells to separate, and cell-cell junctions to dissolve.

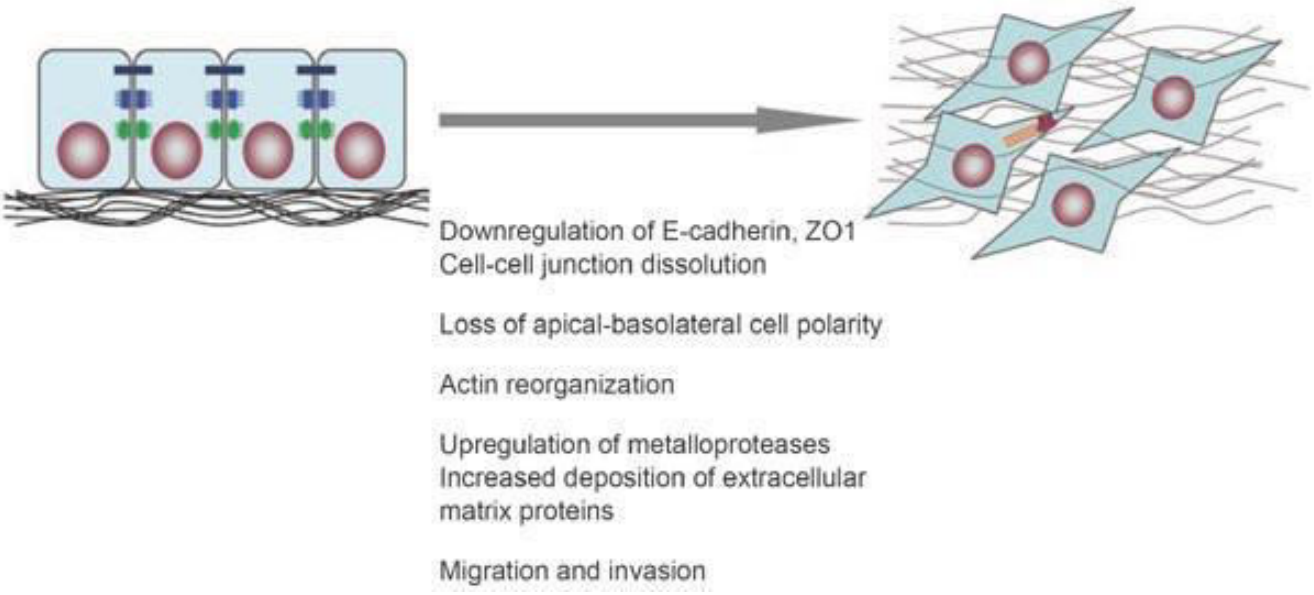
Polarity is lost, the cells will change shape and look like mesenchymal cells.

Actin is reorganized (due to new shape) , this is also important for cell movement.

Upregulate metabolic proteases to degrade ECM.

Laying new ECM (remodeling) which makes it easier for cells to move.

All these cause migration and invasion.



*Both TGF-B and b-catetnin can induce ETM

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Evasion of Cell Death

Review of apoptosis:

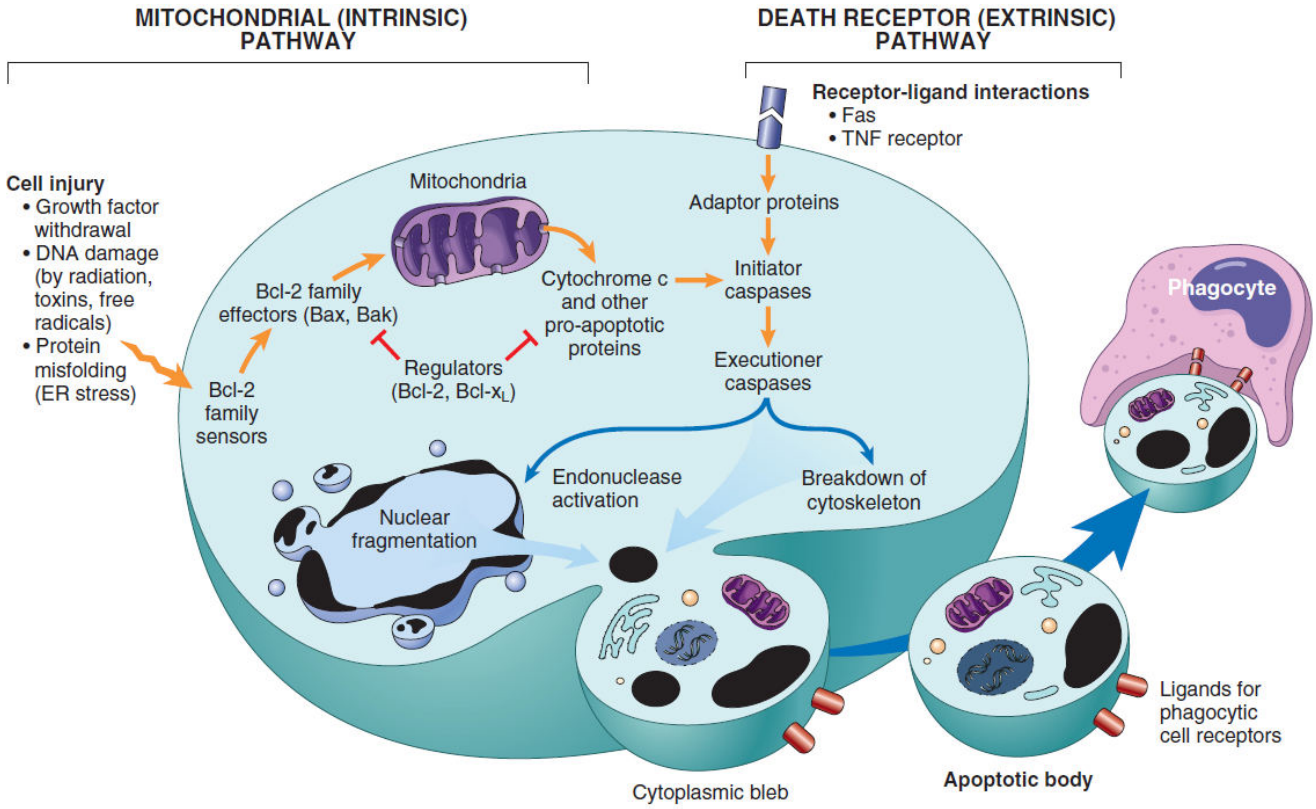
We have two pathways, the mitochondrial (intrinsic) pathway and the death receptor (extrinsic) pathway. Both pathways converge on initiator caspases (8 in extrinsic and 9 in intrinsic) which activate executioner caspases 3 and 6, following those cascade of events there is endonuclease activation and breakdown of cytoskeleton forming apoptotic bodies which are targeted for Phagocytosis. The cells die off by apoptosis and no inflammation is induced.

How do the 2 pathways differ?

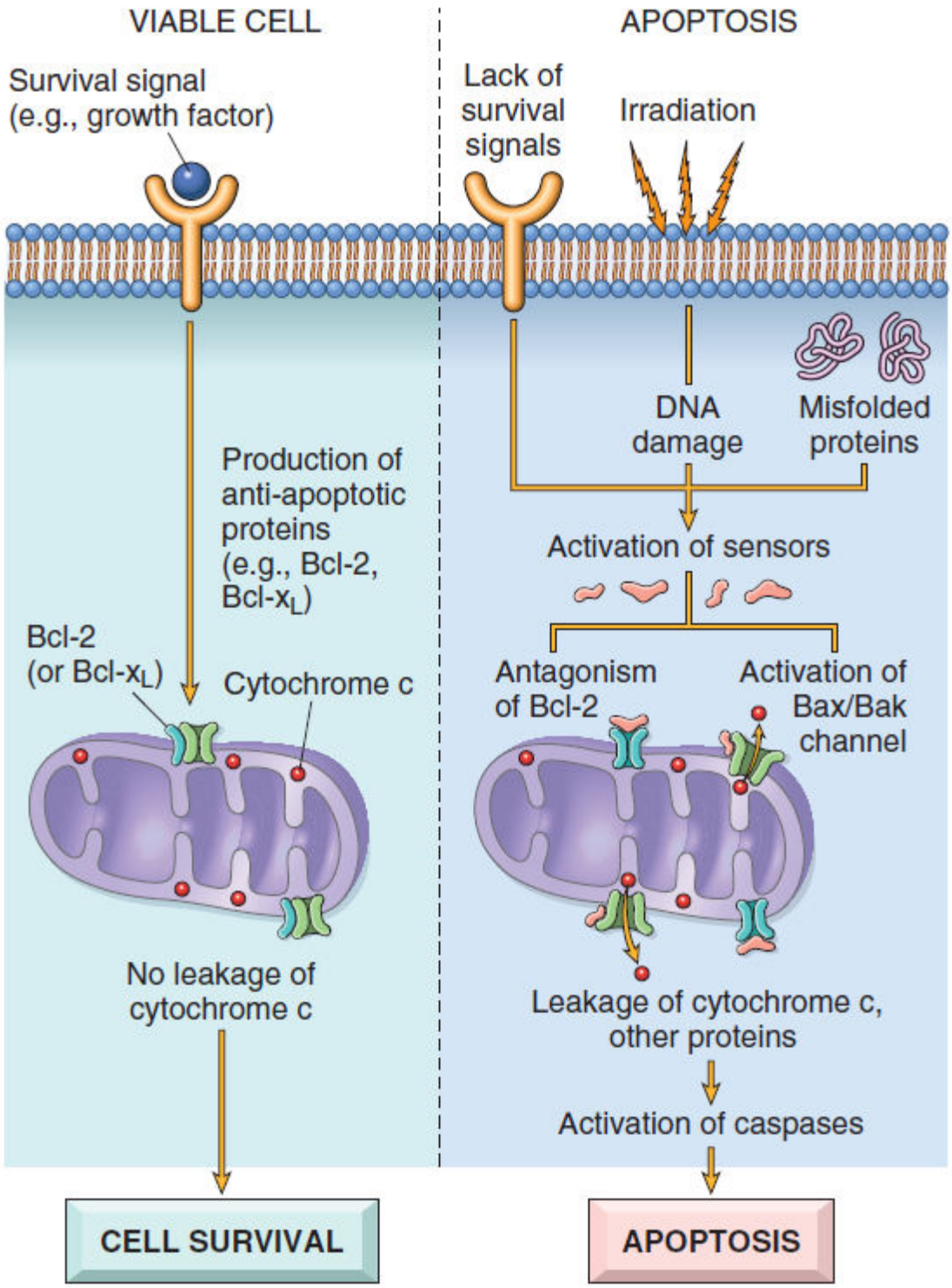
In the intrinsic pathway, we are signaling through the mitochondria, with the leakage of certain proteins (cytochrome c) that signal the initiator caspases etc…

In the extrinsic pathway there is a ligand which attaches to a surface receptor that transduces the signal which goes to the caspase proteins.

NOTE: not all cell-signaling ends up in the nucleus and not all cell-signaling results in protein transcription.



When the cell is receiving a survival signal (growth factor), it will produce anti-apoptotic proteins (BCL-XL & BCL-2) which insert into the mitochondrial membrane inhibiting pro-apoptotic protein (BAX & BAK) channels. So typically, the mitochondria isn't leaky to cytochrome C.



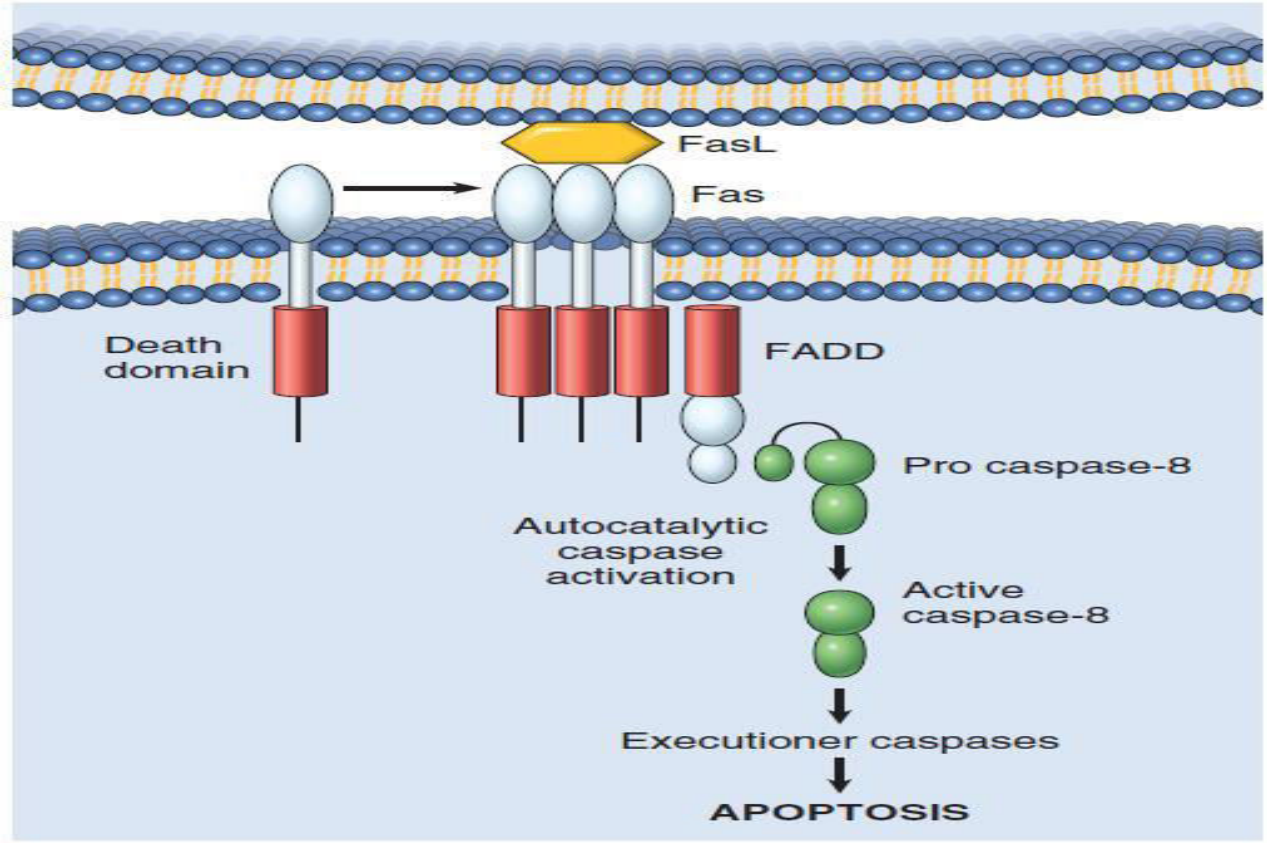
If the cell is not receiving growth factor signals, or there is DNA damage/Misfolding of proteins, sensors of the BH3 family will antagonize the activity of BCL-2 and BCL-XL, activating BAX and BAK, subsequently making the mitochondria leaky to cytochome C. Cytochrome C activates the initiator caspase for induction of apoptosis.

REMINDER: P53 senses DNA damage through ATM, P53 induces cell cycle arrest through increased transcription of p21 (cyclin dependent kinase inhibitor) and induces the transcription of miRNA which inhibits production of cyclins. If the DNA repair is unsuccessful, apoptosis takes over.

Extrinsic pathway

Ligands bind to receptors (TNF or FAS), formation of the death domain occurs, death domain recruits FADD protein which then recruits pro-caspase 8, caspase 8 is then activated resulting in the activation of executioner caspases which induce apotosis.

Caspase 8 can activate BH3 receptors for activation of the intrinisic pathway too.



How does Cancer affect the apoptotic pathway in order to evade cell death?

1) reducing CD95 (surface receptors) due to epigenetic change (cell can't sense stress hence no apoptosis)

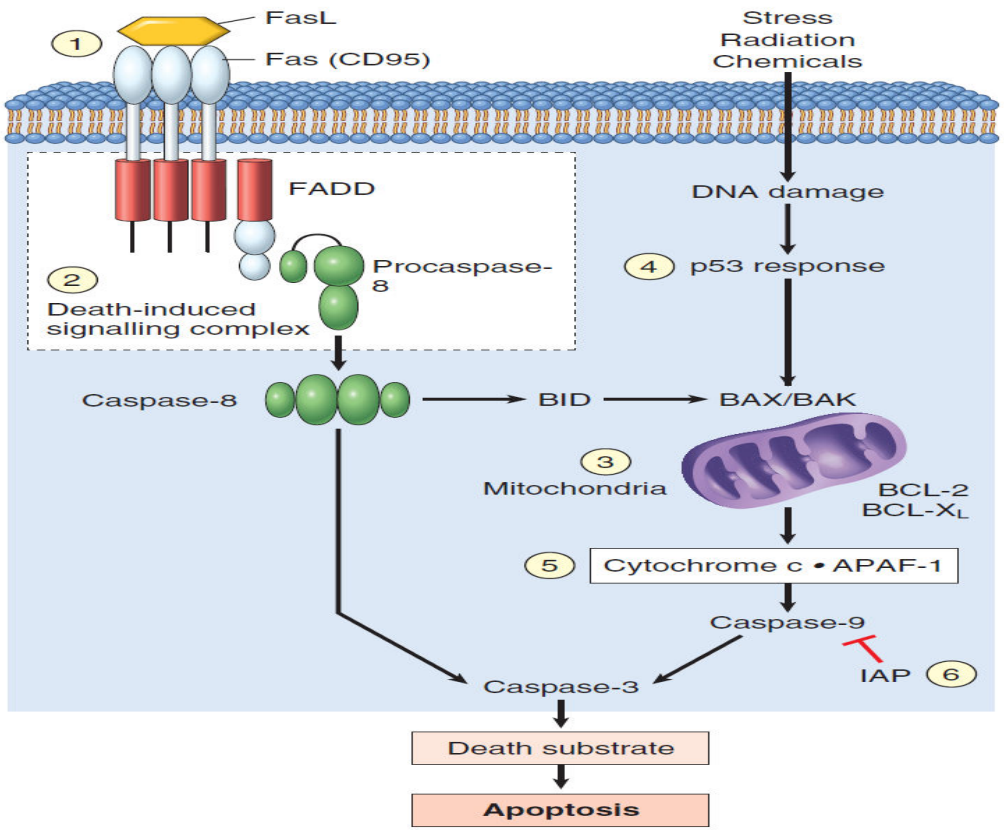
2) over-expressing FLIP inhibits apoptosis as FLIP can inhibit pro-caspase 8

3) By the over-expression of BCL-2, this can be found in 85% of follicular B cell Lymphoma, there is a translocation t(14:18) , chromosome p14 contains the immunoglobulin heavy light chain which is controlled by a very active promoter, so this translocation results in the over-expression of BCL-2 gene, which is a major anti-apoptotic protein.

4) mutation of ATM, less functional p53 proteins, no sensor of DNA damage NO attempted repair/death.

NOTE: cytochrome C + APAF1 are required to activate caspase 9 when it comes to the intrinsic pathway.

5) over-expression of IAPs which are inhibitors of caspase 9, survivin is a prime example.



AUTOPHAGY

Beclin-1 is a BH3 sensor, it can either induce apoptosis or autophagy.

Reminder: Autophagy is a survival mechanism when there is nutrient deprivation, it is used for organelle turn-over.

Early on in a cancer, if we induce autophagy through the same pathways which induce apoptosis, we may be able to kill these cancer cells (here it is anticarcinogenic). Later on if this cancer has grown beyond its blood supply and is now nutrient deficient, the cancer can inappropriately activate autophagy and destroy some non-essential organelles for the cancer cells to survive (here it is procarcinogenic).

Summary (from Robbin's Basic Pathology)

- P53 is the central monitor of stress in the cell and be activated by anoxia, inappropriate oncogene signaling or DNA damage. Activated p53 controls the expression and activity of genes involved in cell cycle arrest, DNA repair, cellular senescence and apoptosis.
- DNA damage leads to activation of p53 by phosphorylation. Activated p53 drives transcription of CDKN1A (p21), which prevents RB phosphorylation, thereby causing a G1-S block in the cell cycle. This pause allows the cells to repair DNA damage.
- If DNA damage cannot be repaired, p53 induces cellular senescence or apoptosis.
- Of human tumors, 70% demonstrate biallelic loss of TP53. Patients with the rare Li-Fraumeni syndrome inherit one defective copy in the germ line and lose the second one in somatic tissues; such persons develop a variety of tumors.
- As with Rb, p53 can be incapacitated by binding to proteins encoded by oncogenic DNA viruses such as HPV.
- TGF-β inhibits proliferation of many cell types by activation of growth-inhibiting genes such as CDKIs and suppression of growth-promoting genes such as MYC and those encoding cyclins.
- TGF-β function is compromised in many tumors by mutations in its receptors (colon, stomach, endometrium) or by mutational inactivation of SMAD genes that transduce TGF-β signaling (pancreas).

- E-cadherin maintains contact inhibition, which is lost in malignant cells.
- APC gene exerts antiproliferative actions by regulating the destruction of the cytoplasmic protein β-catenin. With a loss of APC, β-catenin is not destroyed, and it translocates to the nucleus, where it acts as a growth-promoting transcription factor.
- In familial adenomatous polyposis syndrome, inheritance of a germ line mutation in the APC gene and sporadic loss of the sole normal allele causes the development of hundreds of colonic polyps at a young age. Inevitably, one ormore of these polyps evolves into a colonic cancer. Somatic loss of both alleles of the APC gene is seen in approximately 70% of sporadic colon cancers
- Apoptosis can be initiated through extrinsic or intrinsic pathways.
- Both pathways result in the activation of a proteolytic cascade of caspases that destroys the cell.
- Mitochondrial outer membrane permeabilization is regulated by the balance between pro-apoptotic (e.g., BAX, BAK) and anti-apoptotic molecules (BCL2, BCL-XL). BH- 3–only molecules activate apoptosis by tilting the balance in favor of the pro-apoptotic molecules.
- In 85% of follicular B cell lymphomas, the anti-apoptotic gene BCL2 is activated by the t(14;18) translocation.
- Stress may also induce cells to consume their components in a process called autophagy. Cancer cells may accumulate mutations to avoid autophagy, or may corrupt the process to provide parts for continued growth.

POP QUIZ :P

1. A patient with chronic hepatitis B virus is at a greatly increased risk of developing hepatocellular carcinomas due to:

A) The consistent integration of the virus in the vicinity of protooncogenes

B) the ability of the virus to capture protooncogenes from the host DNA

C) Virus-induced injury to liver cells followed by extensive regeneration

D) the ability of viral genes to inactivate RB and p53 expression

E) the ability of the virus to cause immunosuppression of the host

2. Loss of both alleles results in a retinoblastoma.

A) Oncogenes on chromosome 13

B) C-neu

C) C-sis

D) RAS

E) N-MYC

3. a 30 year old male presents with multiple benign subcutaneous tumors that are attached to nerves. Further examination reveals numerous skin lesions. Opthalmoscopic examination shows hamartomatous nodules on the iris. Which of the following mechanisms of transformation is most likely related to the mutation of this patient inherited?

A) Persistent activation of RAS gene

B) increased production of epidermal growth factor

C) decreased susceptibility to apoptosis

D) Impaired functioning of mismatch repair genes

E) inactivation of the RB gene

4. A child is born with a single functional copy of a tumor suppressor gene. At the age of 5 , the remaining normal allele is lost through mutation, as a result the ability to control the transition from G1 to S phase of the cell cycle is lost. Which of the following neoplasms is most likely to arise by means of this mechanism?

A) Retinoblastoma

B) Breast carcinoma

C) Adenocarcinoma of colon

D) cerebral astrocytoma

E) Chronic myloid leukemia

5. which of the following principles of carcinogenesis is best illustrated by the study of molecular alterations that occur during the evolution of a spordiac colonic adenoma into an invasive carcinoma?

A) protooncogenes can be activated by chromosomal translocation

B) malignant transformation involves accumulation of mutations in protooncogenes and tumor suppressor genes in a step wise manner*

C) extensive regeneration of tissues increases the risk of cancer-causing mutations

D) inherited defects in DNA repair increase the susceptibility to the development of cancers

E) Overexpression of growth factor receptor genes is associated with poor prognosis.

6. A 40 year-old male develops generalized lymph node enlargement and hepatosplenomegaly. Lymph node biopsy reveals a malignant tumor of lymphoid cells. Immunoperoxidase staining of the tumor cells with antibody to BCL-2 is positive in the lymphocytic cell nuclei. By which of the following mechanisms has this lymphoma occurred?

A) increased tyrosine kinase activity

B) lack of apoptosis

C) gene amplifications

D) reduced DNA repair

E) loss of cell cycle inhibition

7. a 38-year-old female presents with abdominal distention, and a CT scan demonstrates bowel obstruction with a 6-cm mass in the jejenum. A burkitt lymphoma of the small bowel is resected, and a portion of the tumor sent for flow cytometry analysis shows a high S phase. Mutational activation of which of the following nucleus oncogenes is likely to present in the tumor?

A) c-erb B2 also known as HER2

B) p53

C) RAS

D) MYC

E) APC

Answers:

1C	2A	3A	4A
5B		6B	7D

Explanations:

1. Although HBV does not encode for any transforming proteins, the regnerating hepatocytes are more likely to develop mutations such as inactivation of p53. HBD does not have a consistent site of integration in the liver cell nuclei, nor does it contain viral oncogenes. However there is no convincing evidance that HBV can bind p53 or RB proteins.

2. _____

3. this patient has clinical features of neurofibromatosis type 1. The nf-1 gene encodes a GTP-ase activating protein that facilitates the conversion of active (GTP-bound) RAS to inactive (GDP-bound) RAS. Loss of NF-1 prevents such conversion and traps RAS in the active signal-transmitting stage. All the other genes are also involved in carciogensis although in different tumors.

4. the RB gene is the classic example of the two-hit mechanism for the loss of tumor suppression. About 60% of these tumors are sporadic. Others are familial and there is inheritance of a mutated copy of the RB gene. Loss of the second copy in retinoblasts leads to the occurrence of retinoblastoma in childhood. Why patients who inherit a mutant RB gene through germ line develop retinoblastoma and not most other tumors is unknown. The RB gene controls the G1 to S transition of the cell cycle; with the loss of both copies, this important checkpoint in the cell cycle is lost.

5. development of colonic adenocarcinoma typically takes years, during which time a number of mutations occur within the mucosa, including mutations involving such genes as APC, hMSH2, k-ras, DCC, and p53. The accumulation of mutations, rather than their occurrence in a specific order, is most important for the development of carcinoma. Activation of protooncogenes, extensive regeneration,

faulty DNA repair genes, and amplification of growth factor receptor genes all contribute to the development of malignancies but are not sufficient by themselves to produce a carcinoma from a colonic adenoma.

6. Overexpression of the bcl-2 gene prevents apoptosis, allowing the accumulation of cells in the lymphoid tissues, increased tyrosine kinase activity results from the mutations affecting abl oncogene. Gene amplifications are typically seen to affect the c-erb b2 and myc oncogenes. Reduced DNA repair occurs in the inherited disorder xeroderma pigmentosa. Loss of cell cycle inhibition results from loss of tumor suppressor genes such as p53.

7. the MYC oncogene is commonly activated in Burkitt lymphoma because of a t(8:14) translocation. The MYC gene binds DNA to cause transcriptional activation of growth-related genes such as that for cyclin D1, resulting in activation of the cell cycle. P53 and APC are tumor suppressor genes that are inactivated in many cancers, including APC colon cancer, RAS oncogene encodes a GTP-binding protein that is located under the cell membrane. HER2 encodes growth factor receptor located on the cell surface.

I'd like to dedicate this sheet to Dina Al-Majali, Aseel Saudi, Lujain Sarar and most importantly Dr.Mazen. ✌

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