Hallmarks of cancer.

Evading Growth Inhibition

Continuation to previous sheet→

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.

- **P53** is a classic tumor suppresser.

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

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

→ One mutant p53 allele high risk to get cancer .

→ Two mutant p53 allele you will get cancer .

- If you don't have p53 mutation wide variety of cancer will be avoided if you have p53 mutation you will have either senescence or apoptosis if DNA repair fails , to induce DNA repair you stop cell cycle and you inducing DNA repair genes.

Two status of P53 activity depending on DNA damage:

MDM-2 (MOUSE DOUBLE MINUTE_2) MDM-2 which is a ubiquitin ligase.

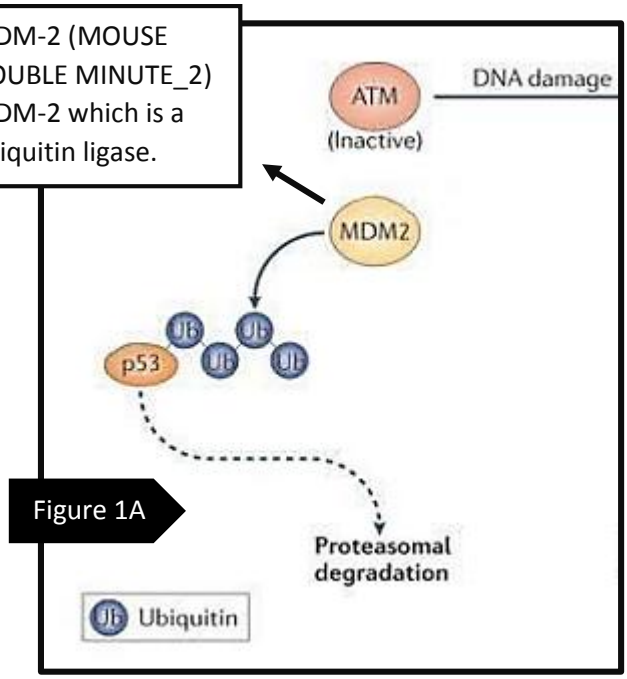
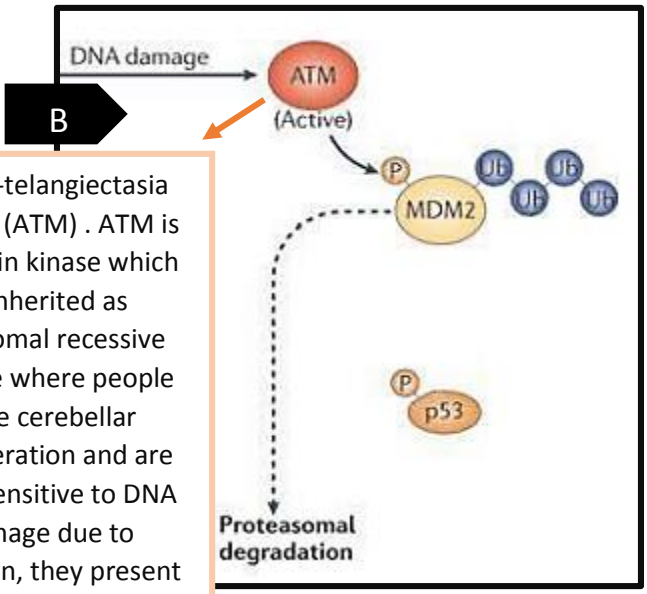


Figure 1A

Without DNA damage→

P53 is normally targeted by MDM-2→it add ubiquitin to P53→when p53 is ubiquitylated it is sent off for proteasomal degradation using the proteasomal-ubiquitin pathway. T1/2 for ubiquitylated p53 is about 20 min in normal circumstances (without DNA damage, anoxia, and abnormal oncogenes activity).



B

Ataxia-telangiectasia mutate (ATM) . ATM is a protein kinase which is inherited as 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.

With DNA damage→

When there is DNA damage , anoxia , abnormal oncogenes activity p53 escapes ubinqulation , but how does p53 sense dna damage ??

By Ataxia-telangiectasia mutate (ATM) . When active ATM →phosphorylates p53 and MDM2. When MDM2 is phosphorylated that serve as a signal for MDM-2 to be ubiquitylated and sent off to degradation (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

P53 role

- P53 can activate various transcription factors and it will induce cell cycle arrest . through activating these transcription factors it will induce apoptosis .
- P53 not only senses DNA damage it also sense anoxia and most important it sense abnormal oncogenes activity e.g. abnormal MYC/RAS activity which are related to growth proliferation, if we have over expression in MYC p53 going to stop it's activity .
- That's why in majority of cancers up to 37% you will found abnormality in p53 protein .
- Very rare syndrome : 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.
- 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.

P53 and cell cycle arrest

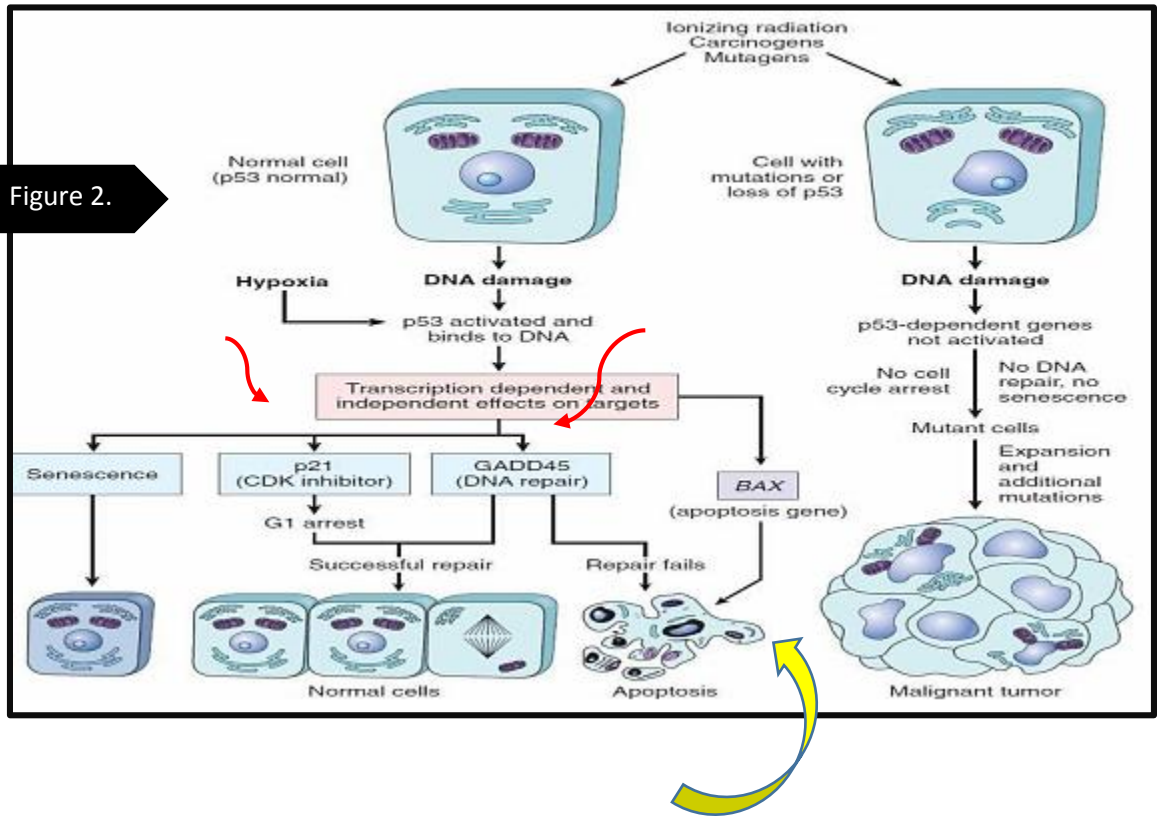
By affecting several transcriptional genes, p53 can →
[1] - Activate <u>temporary</u> cell cycle arrest (known as - quiescence), can re-enter the cell cycle.
[2]- Induce <u>permanent</u> cell cycle arrest (known as senescence), there is a chromatin modification where you close of the areas which we make them in sense with the transcription factors which are required for proliferation.
[3]- Trigger <u>apoptosis</u>.

*We don't know why p53 some time induce senescence over apoptosis.

How can p53 induce cell cycle arrest?

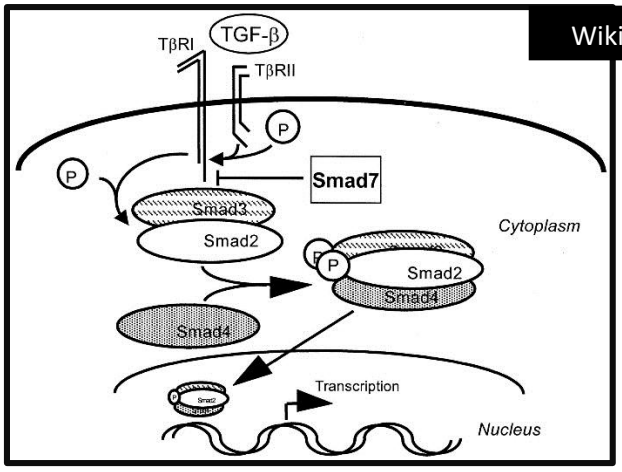
By regulating transcription of several downstream genes:
1. CDKN1A, (p21) is a CDK inhibitor; by inducing its transcription you are inducing the inhibition of the cell cycle.
2. miRNAs, some are under transcriptional control of p53, so by degrading them or prevent them to get into ribosomes we stop translation . Some miRNAs also target BCL-2 mRNA, this action downregulates production of BCL-2 which is an inhibitor of apoptosis.
3. GADD45A; Growth Arrest and DNA repair protein 45, it repairs DNA.

Figure 2.



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 mitochondria from the newly activated channels so we are inducing transcription of PUMA, it is a BH3 sensor, BCL-2 antagonist also induce miRNA which ALSO ANTAGONISE BCL-2 SO we reduce the level of bcl2 to induce APOPTOSIS. Now should DNA repair be successful then we will induce MDM-2 which send p53 to degrading so it produce it's own destroyers then p53 levels return to normal states .

TGF –β pathway



TGF-B pathway is a growth suppressor pathway.

- Consists of a family of 20 ligands that bind to serinethreonine kinase receptors.
- Their kinases have ligands, receptors and signal transducing proteins known as SMADS "سماد" :p.
- These SMADs enter the nucleus and form transcription complex where they induce production of CDK INHIBITORS or press production of MYC so we completely shut down the cell cycle . so SMADS are a very potent proliferator inhibitor.

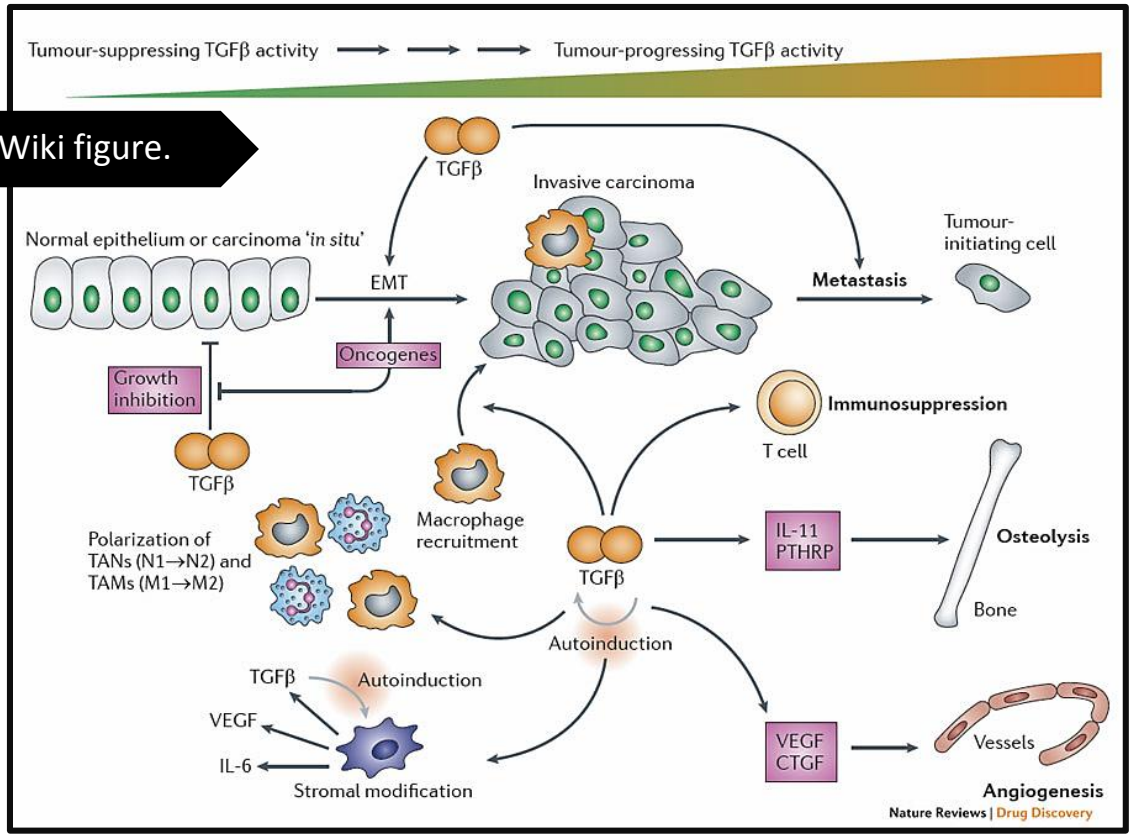
- So we want to stimulate or inhibit the TGF- β ?

We need both, TGF-B induce both immunoinvasion , Angiogenesis and epithelial to mesenchymal transition.

ϖ Let's discuss epithelial to mesenchymal transition:

→Epithelial cells have specific shapes and defined borders and stuck to the surrounding cells and you can say which way up and which down UNLIKE mesenchymal cells they have variety of shapes , they don't stuck to each other's and you can't know if the way is up or down .

Wiki figure.



So if an epithelial cell become more mesenchymal like it's that good or bad to the cancer?

• It's good for the cancer because it make invasion and metastasis.

• Now these cells which become more mesesnchymal won't stuck to it's neighbours it's free to move and doesn't have particular location so it can make metastasis (good for cancer bad for you) .

So if you stimulate TGF-B pathway you inhibiting proliferation but you increasing epithelial to mesenchymal transition so inducing immune_invasion and metastasis .

TGF-B pathway are mutated in various cancers.

→ 100% of all pancreatic cancers have a mutation in TGF-B.

→ MORE THAN 80% OF COLON CANCERS ALSO HAVE a MUTATED TGF-B PATHWAYS.

Another inhibitory pathway→

Cell-cell contact.

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).

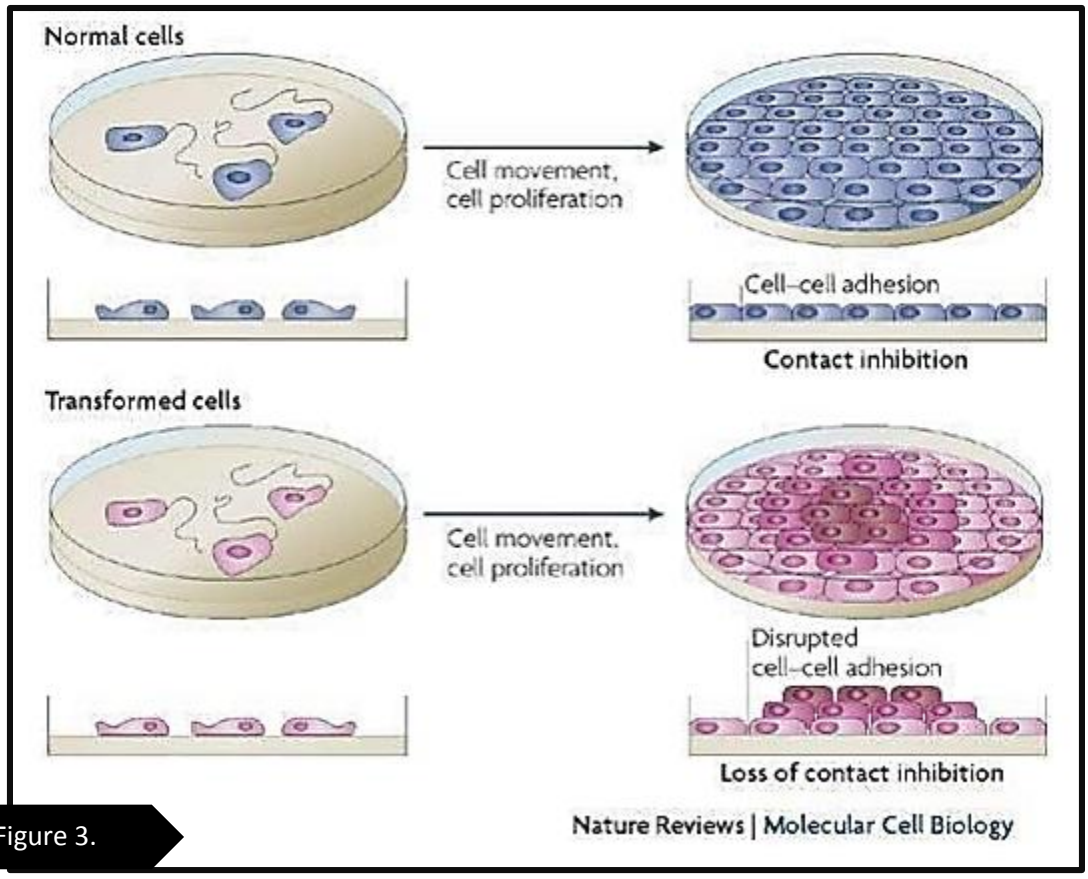


Figure 3.

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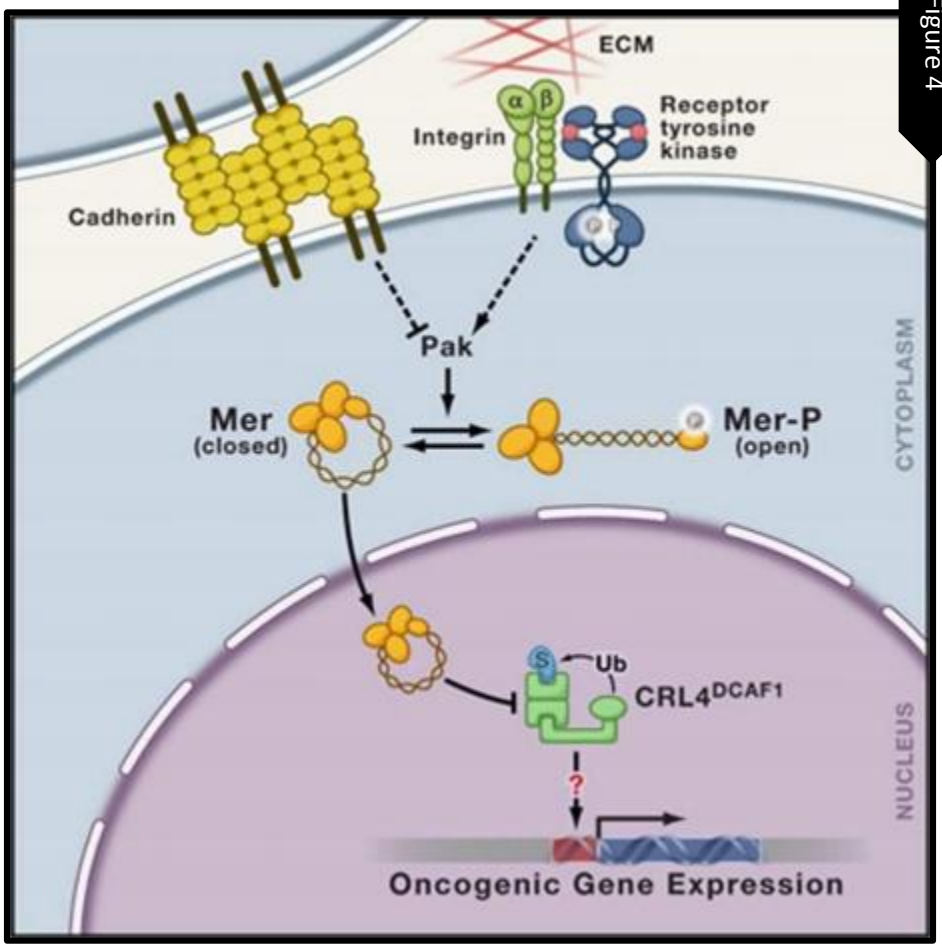
2 known pathways are involved in this cell-cell inhibition:

- NF2 pathway
- APC pathway

NF2 pathway

Figure 4



→ NF2 (also known as merlin) inhibits the transcription of oncogenes.
→(present in neurofibromin2).
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. Cancers known as the wound that never heals .

APC pathway

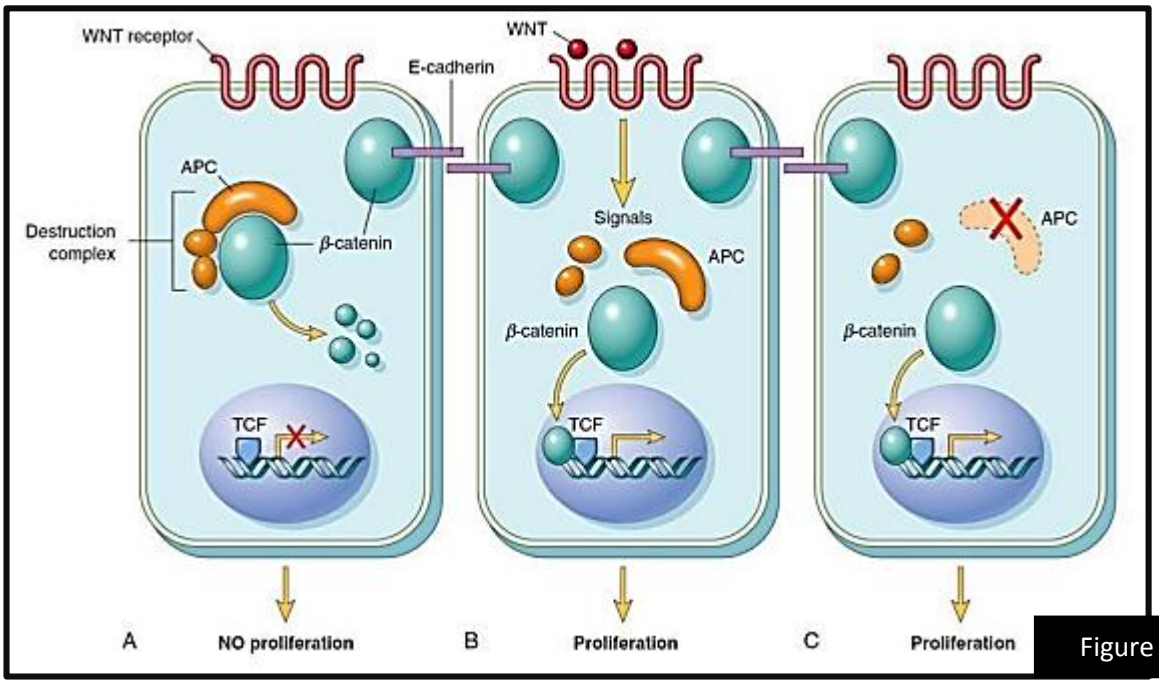


Figure 5

It's a tumor suppressor.

→ If you inherited single defective copy you will have a high risk for cancers, you need to lose both copies to get the tumor. Patients with an abnormal copy of the APC gene have familial adenomatous polyposis.

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.

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Those are negative regulators of genes that inhibit e-cadherin expression, which results in no contact inhibition.
ϖ APC has a role in epithelial to mesenchymal transition (EMT).
ϖ 70-80% of all colorectal cancer patients will have an APC mutation.
ϖ The rest will have a b-catenin mutation or anything in this complex.

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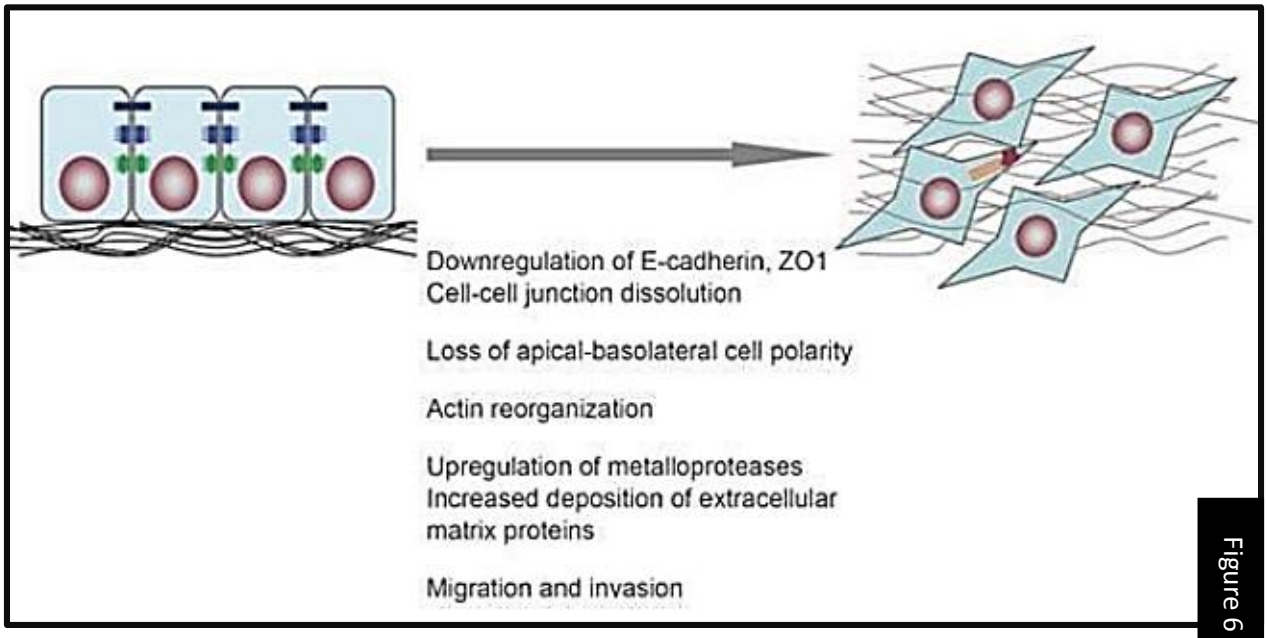


Figure 6

<u>Short introduction to EMT</u>
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.

END of the second hallmark ☺ ☺ ☺

Evasion of Cell Death

Review of apoptosis:

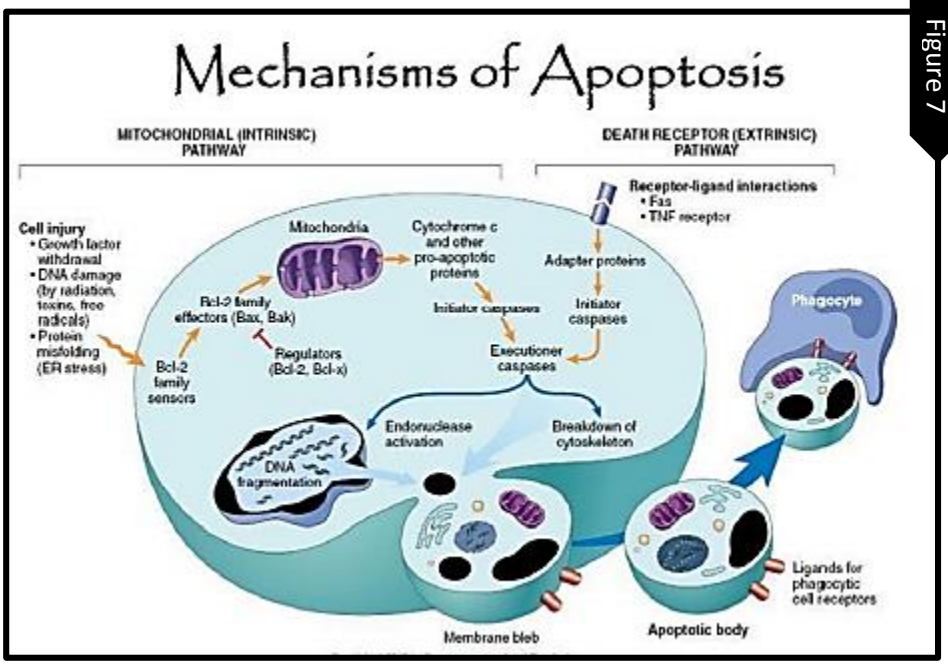
We have two pathways, the mitochondrial (intrinsic) pathway and the death receptor (extrinsic) pathway. Both pathways converge on initiator caspases (8 in extrinsic and 9 in intrinsic) which activate executioner caspases 3 and 6, following those cascade of events there is endonuclease activation and 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.

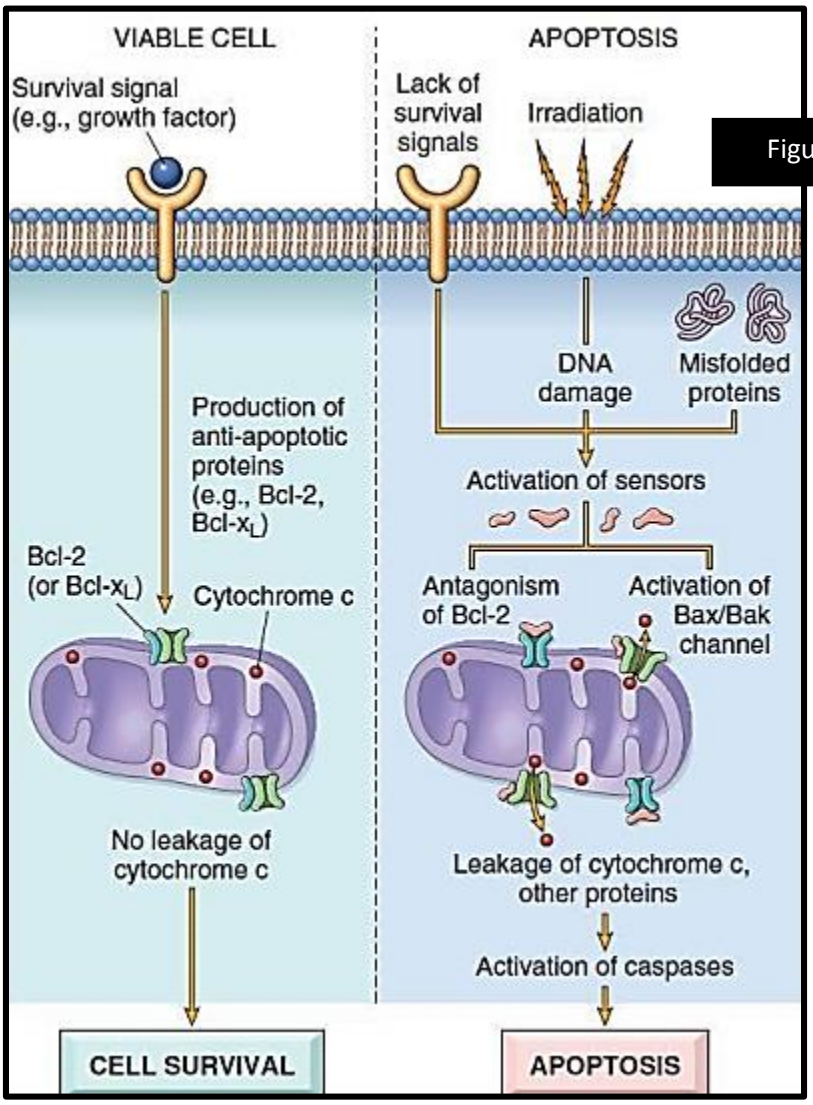
Figure 7



Intrinsic pathway

When the cell is receiving a survival signal (growth factor), it will produce antiapoptotic 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.

Figure 8



If the cell is not receiving growth factor signals, or there is DNA amage/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.

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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.

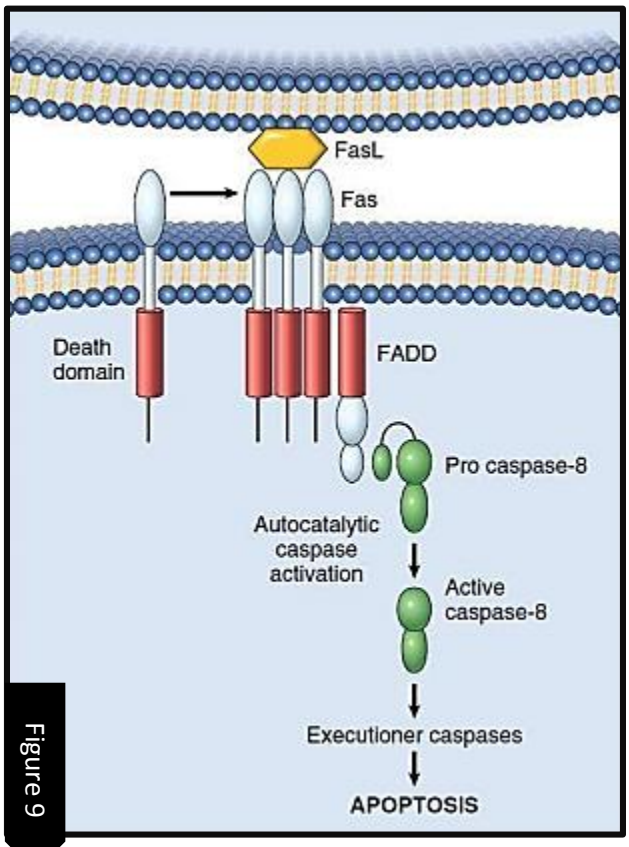


Figure 9

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).

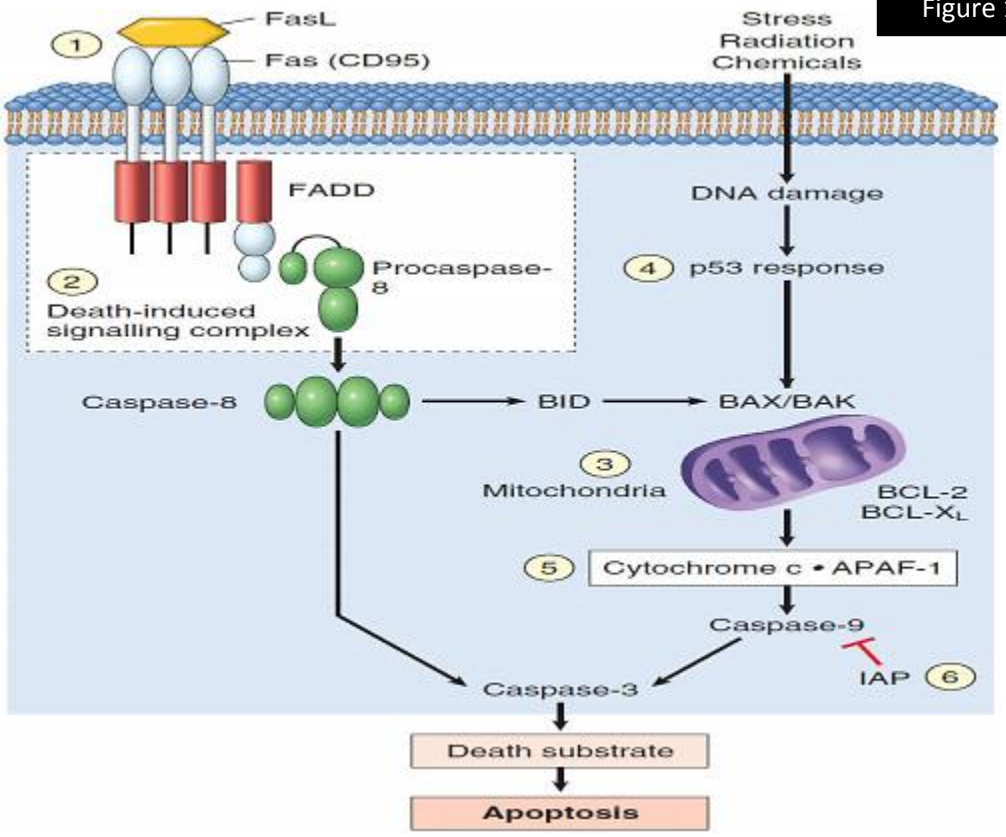
2) over-expressing FLIP inhibits apoptosis as FLIP can inhibit pro-caspase 8.

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.

Figure 10



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).

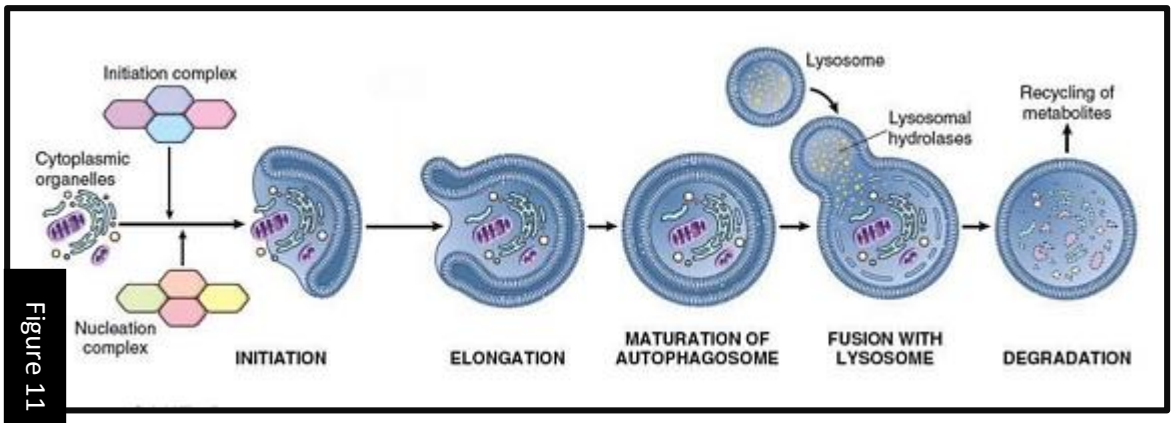


Figure 11