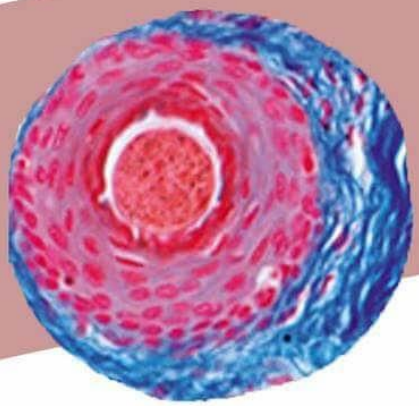
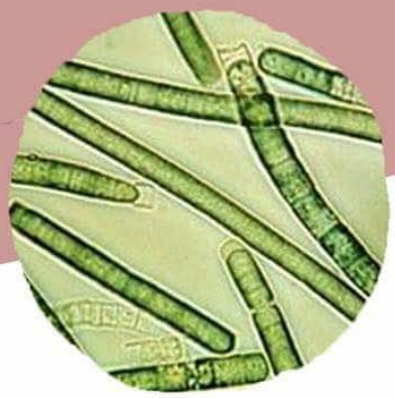
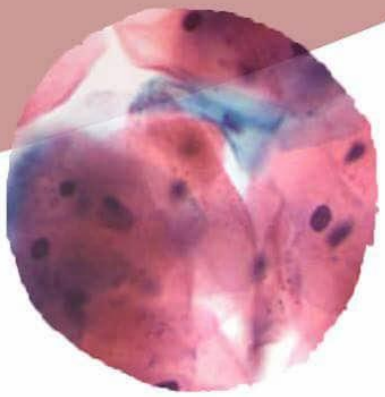
INTRODUCTION TO PATHOLOGY

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Sheet# 19

"You don't HAVE to do this you GET to do this"

(Page 185-190 in the book)

**** Last time we stopped at TP53--- called the *Guardian of the Genome* (the other major inhibitor of cell proliferation) ****

P53 causes cell cycle arrest to allow DNA repair and if the DNA is not repaired it will induce apoptosis

P53 encoding tumor suppressor gene (the same thing as TP53) is one of those genes when you lose one copy you get that autosomal dominant disease called *Li-Fraumeni syndrome*. These patients are rare and they do not get a tumor unless they use the second copy on a molecular level. Unlike Retinoblastoma, which has a wide but limited wide subset of tumors*, Li-Fraumeni syndrome patients* get an even wider subset of twos.

Q: Why would Li-Fraumeni syndrome patients, people that have mutations in p53 get an even wider subset of twos compared to Retinoblastoma?

Answer: *Student's answer*: because P53 is for mutations and cell cycle. Retinoblastoma → mainly affecting cell growth

p53→ not only are you affecting that G1-S check point but we also get a mutator phenotype, it will cause accumulation of mutations resulting in a much wider variety of tumors.

Accumulation of P53 activation prevents neoplastic transformation by three mechanisms:

- Inducing cell cycle arrest temporarily through the production of cyclin dependent kinase inhibitors → *Quiescence*
- Major chromatid changes inducing cell cycle arrest permanently→ *Senescence*
- *Apoptosis*

In the case of quiescence & senescence → they are typically are irreversible and we don't fully understand why P53 would induce one versus the other.

A variety of stresses may trigger P53:

1. Anoxia (we don't fully understand why)
2. Abnormal oncoprotein activity [example: MYC or RAS → may be overactive, therefore P53 senses this and stops their over activity by inducing one of the 3 major functions mentioned above (**apoptosis, senescence, or quiescence**)
3. DNA damage

Just so we don't get confused with the weird names, the 6 transcriptional targets for P53 are: 1. CDKN1A (p21), 2.GADD45 (DNA repair), 3.BAX (channel), 4.PUMA (Bcl-2 antagonist), 5.miRNA (microRNA , it decreases BCl2 and cyclins), and 6.MDM2

How is p53 sensing the DNA damage? It senses the damage by a protein called ATM (ataxia telangiectasia mutated). If a patient has two abnormal copies of ATM, they have cerebellar degeneration (patient has loss of balance), that's why they have ataxia, as well a variety of tumors, now what ATM does is that when it detects DNA damage, it is a kinase, which means it will phosphorylate something, in this case it phosphorylates another protein called MDM2. (Mouse double minute 2 which is a ubiquitin ligase)

Remember: we mentioned in autophagy that we could get rid of proteins by ubiquitylating. MDM2 is one of those proteins that can add ubiquitin to the proteins that need to be degraded. In this case, what MDM2 does t*hroughout the normal cell cycle, is add ubiquitin to P53 = consistent production of P53* and MDM2 always adds to ubiquitin, and therefore P53 is constantly degraded.

Result: (P53 has a short half life when MDM2 is not phosphorylated of about 20 minutes) → DNA damage occurs → ATM phosphorylates MDM2→ MDM2 itself (because it now has a phosphorylation marker) is susceptible to degradation through the ubiquitin proteasome pathway→It can no longer ubiquitylate and sends P53 off for degradation, which means the t-half life for P53 increases.

**End result DNA damage → P53 accumulation. **

**That's step 1 of inhibitory pathway **

Recap of step 1:

What does P53 do to induce quiescence, apoptosis, and senescence? Once it is no longer sent off for degradation and is phosphorylated by ATM, it will affect transcription. P53 transcriptional targets are vary, first how does it stop the cell cycle? It induces transcription of CDKN1A (P21) which is a cyclin dependent kinase inhibitor, so you stop the cell cycle late in G1, it also induces transcriptions of miRNA (microRNA), that target the mRNA for cyclins, so not only are you producing more inhibitors, you're stopping the production of things that allow the cell cycle to continue (decreased production of activators)!

** Step 2 **

-The whole point of P53 to stay in the cell cycle is to repair DNA

-Important transcriptional target of P53: DNA repair enzymes -

Remember: GADD45 (Growth Arrest of DNA Damage 45) is important for DNA repair.

So what happens if we fix the DNA? The cell cycle continues. *And If we don't?* APOPTOSIS. So depending on the outcome of how GADD45 is able to do one of two things.

If we don't repair the damage → when the damage is too severe:

- Activation & increased expression of the sensors
- Activation & increased production of BAX/BAK channels

- Antagonize & reduce production of BCL2

[Note: BAX is one of the transcriptional targets of P53 → **More BAX = more apoptosis.**]

PUMA (a Bh3 sensor) and also a BCL2 antagonist, so you do not only activate the sensors, but you increase the expression of one of those sensors that further antagonizes BCl2, also out of the transcriptional target is miRNA that reduces production of BCL2. So thereby releasing inhibition of apoptosis and inducing has not been able to repair its DNA. ***However, if DNA repair is successful,*** none of this will occur, and P53 will actually induce transcription of its own destroyer of MDM2. So now we produce new MDM2, there's no longer phosphorylation, it goes back to destroying P53 and P53 no longer stops the cell cycle, so the cell cycle continues. In a normal cell, DNA damage or hypoxia, P53 is activated and acts as a transcriptional factor, inducing either quiescence or senescence (as mentioned in the earlier pages. It will also try to repair DNA through GADD45 and if the DNA repair is successful, it brought happy normal cells ☺, if not successful through induction of BAX among other things we end up with apoptosis.

So what happens if you have a mutated or absent p53? Answer: DNA damage, P53 dependent genes are not activated; there is no cell cycle, no DNA repair, no quiescence, no senescence. No apoptosis and therefore allowing those mutations to continue to make

essentially a malignant tumor. More then 70% of all tumors have an abnormal P53/ the rest have abnormalities in the downstream pathways of P53.

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*Third of the inhibitory pathways (*TGF- β *Pathway Signaling):*

Where we see **TGF-** β before? Repair. What does it do in repair? Stop proliferation of lymphocytes and fibroblasts (inhibitor), induce production of collagen, and turn them into a synthetic phenotype and start producing a cellular matrix and end up by inhibiting inflammation and stimulating fibrosis.

So early on in the cancer, do you think TGF- β is going to be activated or lost? **LOST.** So if you inhibit TGF- β and there are mutations that turn off the type 2 receptor mutation in the (colon, stomach, and endometrium) you end up with a proliferative problem, there is no longer inhibition of proliferation using **TGF-** β. Now you already know the pathway, but some additionally added details there are two types of receptors. (Type 1 and Type 2) and when a ligand binds, you bring two type 1 and two type 2 together and turn on the pathway. Now depending on the ligand, you will bring together different types of receptors so each organ has its own different types of receptors and its own ligands

Result of activation of **SMAD** proteins:

Transcription of cyclin dependent kinase inhibitors = Stopping the cell cycle ----the inhibitors

- MYC, cyclin dependent kinase (2 and 4), and cyclins (A and E)---the stimulators

Actually In pancreatic cancer, you frequently find SMAD-4 mutations, and in 100% of all pancreatic cancers you will find a **TGF-β** pathway mutation (*very important*). Whether were talking about receptors, SMAD, or transcriptional regulators. **All pancreatic cancers→ TGF-β pathway are affected,** in more then 80% of colon cancers, **TGF-β** pathways is mutated. Not only does it inhibit proliferation, it can also help with cancer cells evade your immunity. Remember what does it do in repair? In inhibits the cycle proliferation, induces angiogenesis, makes cells behave more like mesenchymal cells. **AS A DOCTOR** if you activate **TGF-β** pathways, yes you may inhibit proliferation, this is good for **early** on in cancers. **Later** on the cancers are already grown (late cancer) , turning off the **TGF-β** pathway is very important, because what do you want to inhibit? Angiogenesis, epithelial-mesenchymal transitions, and immune evasion →which all are important for metastasis. Later on in the cancer, the cancer may become independent of that **TGF-β** mutation and has already inactivated P21 for example from cyclin kinase inhibitors or MYC is no longer transcriptionally regulated by **TGF-β** because there is some type of translocation or mutation and it has become independent of the **TGF-β** pathway. Even if you somehow restore **TGF-β** activity you are not restoring its inhibitive activity, only immune evasion activity of epithelial-mesenchymal transition and angiogenesis. This is what I

meant by this nasty pathway can do two different things in the same cell at two different times. Simply: early on its good to activate **TGF-β**, later on its better to inhibit it .

Step 4 of inhibitory pathway: contact inhibition.

When you take normal cells and you put them in a petri dish they will proliferate up ***until*** they have filled the whole dish and then they will stop proliferation (forming a monolayer). However, if you take transformed cancer cells and put it in a petri dish they will keep growing and they will also create a monolayer and they will start piling on top of each other. Now the reason normal cells stop proliferating is not fully understand but one of the major things that we know goes on is that contact inhibition is mediated by E-Cadherin (e= epithelial). So if you were to think that an epithelial mesenchyme transition requires that you lose a cadherin you would be correct, your cell cannot go from an epithelial to a mesenchymal shape or function so what does a cadherin and NF2 do?

Lets start with NF2 (aka Merlin)

- Autosomal dominant diseases
- Effects transcription of oncogenes
- When it receives two signals, either from the cadherin or from integrins (bound to the ECM), there is a bounce of these two

signals that either allows merlin to go inside the nucleus or stay outside of it.

When there is contact inhibition (E-cadherin is present at normal) the overriding signal is to prevent merlin from going into the nucleus and turning on other genes.

What about cadherins?

If you lose a cadherin and mostly the cell is just bound to the ECM and goes into the nucleus and turning on oncogenes. So that is partially what we know about a cadherin, the other thing we know about cadherin is its involvement in the b-catenin pathway. B-catenin is part of the APC pathway (remember the Adenomatous Polyposis Coli gene we mentioned in the past first lecture) . And if you have a mutation that is inherited, you are a familial adenomatous patient. And you have hundreds of adenomatous polyps in your colon and actually if you were to look at these neoplastic rows we find that these neoplastic rows have already lost the other APC. So even though it's an autosomal dominant syndrome you have to lose the second copy for the tumor to occur. So why? What's the deal with APC and NF2? As it turns out it is one of those pathways that does not follow the traditional signaling pathways, yes, there are ligands and receptors, but the signal transduction mechanism is unique. In the absence of the ligand that receptor does nothing. The signal transduction molecules create what is called a destruction complex. They gang up on beta catenin and send it off to be degraded. Why do they do that?

Because there is no signal and beta catenin is what transmits the signal to the nucleus, (we have two transcriptional factors: b-catenin and TCF). When a signal is received, then the structure complex is disassembled and you allow the beta catenin along with TCF to induce transcription of genes such as cyclin -b1 and you know they also activated these transcriptional regulators **(SLUG, SNAIL, and TWIST)** and end up reducing cadherin as well as loss of contact of inhibition and this is why its involved in epithelial-mesenchyme transition too!

To make things more complicated turns out that most of the beta catenin you have in your self is bound to membrane E-cadherin so if you lose a cadherin→more release of beta catenin→ more proliferation. Recap: So going back to APC, if APC is part of the destruction complex, we will have *proliferation*. So essentially you have become independent of growth signals. (Remember RAS, we we have a mutation that can cause RAS to be permanently bound to GTP) you are independent of the signal so in this case a mutation in APC becomes insensitive to growth signals (independently active).

Note:
APC mutations: more then 70-80% of the Colon Cancer patients
B-catenin mutations: the rest (30-20%)

TGF-B pathway: in pancreatic cancer
APC/ B-catenin: in colorectal cancer

What about the epithelial mesenchymal transition?

May go in one of two directions

- We either mutate b-catenin (becomes released) → turn on the transcriptional pathway
- You lost APC and b-catenin goes in → turns off transcription of the cadherin→ you lose a cadherin→ the cells are no longer stuck to each other and you get → proliferative signals (which is part of the epithelial mesenchymal transition process).

Result: you down regulate the E-cadherin expression and down regulate to zonula occludins, and end up with dissolution of cell-cell junctions, lose of apical-basal cell polarity (which means the cell is no longer bound to the basement membrane and does not know which way is down and which way is up)→ becomes more like any other fibroblast or mesenchymal cell. After it is reorganized in order to allow that cell to move normally epithelial cells do not move about and you up regulate metalloproteases (recall: found in repair and TGF-beta pathway) and we said in order to create new blood vessels we have to make way for the cells to move and they took one of the repair pathways that is most important for getting your body to heal and use it for their own purpose to move the cells through your ECM→finally this all results in: migration, invasion and metastasis. That is the growth inhibitory hallmark.

Next Hallmark: Evasion of Cell Death (not much new information starting now☺)

*Two types of deaths we took: necrosis and apoptosis. *Which one do you think cancer will really screw around with?* Apoptosis, because it is one of the protective cell responses that we use.

****Revision of apoptosis****

Intrinsic pathway*:* Growth Factor withdrawal, DNA damage, protein misfolding all activate the sensors→the sensors then turn on the effectors (BAX/BAK) proteins channels that allow things to leak out of the mitochondria (ex: cytochrome c)→ this turns on the initiator caspases of this pathway (caspase 9) and this turns on the executioner caspase → (we have A CASCADE!) → activation of endonuclease AND proteases that leak out of the cytoskeleton → results in release of the cytoplasmic contents into apoptotic bodies that does not end in inflammation.

Extrinsic pathway: the only difference is that it is directly acts on the caspases through a receptor like FAS or TNF by binding a ligand and you end up turning on initiator caspase 8 and remember FLIP (inhibitor), some viruses can mimic it and we end up with the same thing. If we have overexpression of FLIP or mutation of FAS or ligand → results in: *autoimmune disease* and even *tumors* (**exam questions**)

Main Q: how can cancer cells screw with this?

1.overexpression of BCl2→ so even if there is DNA damage and activation of extrinsic pathway we end up with NO apoptosis.
2. Imitation of the receptors
3.overactivity of FLIP because its an inhibitor of the pathway

Overview:
DNA damage pathway: P53 accumulation → induces cell cycle arrest→ allows the cell cycle time (breathing space) in order for the cell to repair and now the transcriptional factors is GADD45, if GADD45 over other DNA repairing enzymes do not finish there job in the amounted time it ends up activating the sensors→ increasing the expression of those sensors → increasing expression of the BAX/BAK channels, and antagonizing BCl2 which results in apoptosis. *You screw with the pathway, it will be P53, or MDM2, or ATM*

Apoptotic Abnormalities:

- Mutate Fas
- Alter the P53 response
- Overexpression of FLIP and Bcl2/ Bcl-xl,
- IAP (inhibitors of apoptosis proteins) that act as the balance on the initiator caspases like caspase 9 → One of the proteins: **surivivin (acts as a checkpoint before apoptosis)** it's over

expression = down regulation OR inhibition of apoptosis. → Found in a wide variety of cancers.

Think LIKE A CANCER CELL guys and you can apply information we already took to the new info with ease

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Autophagy

-Survival mechanism during nutrient degradation that we use in organelle turnover and it has a role in cancer
-It can have a pro or anti tumor growth depending on internal or external factors

Early on the cancer: the cell has plenty of nutrients and oxygen. If we activate this pathway it wont be good, because it can induce a certain unique type of cell death (a type that isn't apoptosis or necrosis). There are receptors called *Beclin-1 (BH3-sensor)* that can induce apoptosis and induce autophagy. There are also current medical trials if we detect cancer early on where we induce autophagy.

Later on when a cancer is more established: not enough nutrients and not enough angiogenesis. If we activate autophagy, we are *helping* the cancer, the cancer has already turned off several of the pathways that are activated by autophagy so by turning on the pathway you are allowing the cancer to survive during difficult times and you are not, unfortunately, inducing cell death by apoptosis because the cancer has already circumvented those pathways.
DONE!