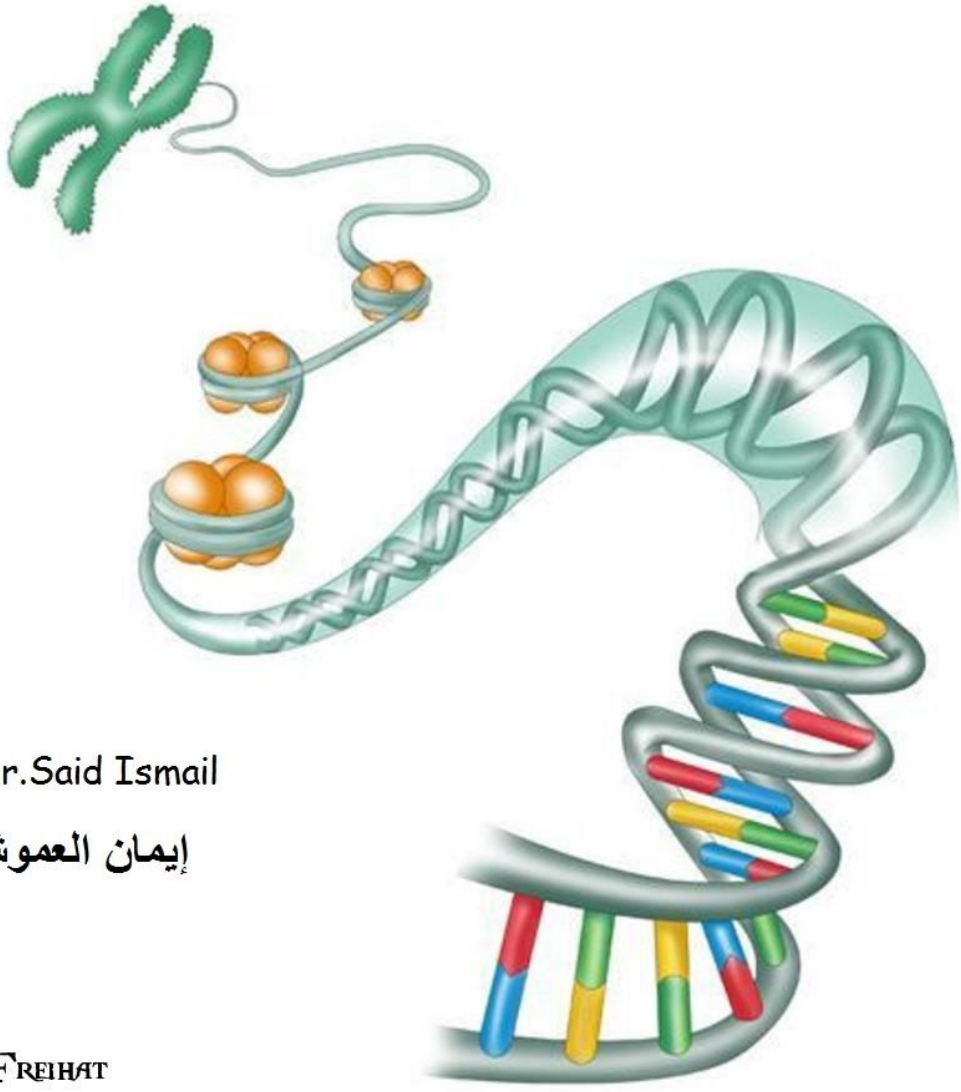
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Sheet#: 24

Dr. Name: Dr.Said Ismail

Done By: إيمان العموش

Designed by Nadeen Al-Freihat

Oncogenes and the cell cycle,

Tumor suppressors and an introduction for Apoptosis

يعطيكم العافية جميعاً، الشيت سهلة جدا والدكتور كتير عاد وزاد ووضّح الشغلات أكتر من مرة فالشيت بتمر معكم بسرعة إن شاء الله، فرّغت الريكورد ورتّبت تسلسل الشغلات متل ما همّا بالسلايد وكل إشي زيادة محطوط بالسلايد حطّيته -وإن شاء الله ما بكون نسيت إشي- ما عدا الصور بدكم ترجعولهم والـ apoptosis عشان الدكتور حكى عنّه مقدمة بسيطة وكمّل عنّه في المحاضرة الأخيرة، ممكن في بعض الفقرات أكون مغيّرة ترتيب الكلام عن الموجود بالريكورد أو بصياغة تانية.
بس تخلصوا الشيت ممكن تلقوا نظرة على السلايد كـ مراجعة عشان السلايد بيعطي الخلاصة من الإشي وما تنسوا تدعولي، موفقين.

-In the last lecture we were talking about proto-oncogenes and we said that proto-oncogenes are not just there to give us cancer at some point, they are normal genes performing normal functions that are cell growth and cell proliferation.

*How do normal cells divide?

-We said that usually the cell receives a massage from outside in the form of growth factor received by a growth factor receptor, then signal transduction to convey the message to the nucleus and in the nucleus replication begins. Any of these guys who got a mutation that made it independent of the control or the cascade or the chain of order, will transform from proto-oncogene into oncogene and it's not a condition to be overexpression, it could be **constitutive activation of the oncogene itself** or **inactivation of the inhibitor**; the proto-oncogeneis still there but the inhibitor which should stop it may not be there.

-And we give some examples on some oncogenes:

Growth factors → PDGF , Receptor → PDGFR

Signal transduction proteins → Ras, Transcription factor (for genes of the cell cycle)→ Myc

-When I say that this oncogene is important it implies that it's involved in many cancers or in the main cancers.

-Ras is involved in lung cancer, breast and maybe colon cancer, so it's an important oncogene.

Other oncogenes are only mutated in very minor cancers affecting one in a million person so these are not very important.

-Myc and Ras for example are also important because they will be a target in the future. These key oncogenes are targets for the new generation of therapeutics. When you identify the main oncogenic event in that cell, then you can specifically target that protein and inhibit it thus the new generation of cancers will be targeting these "heroes" of oncogenes (Ras/Myc/APL). And you will hear in 10 to 15 years about therapies that are almost magic which have zero side effects and 100% effect or at least 99% effective.

-There will always be resistant patients. And such therapies will be expensive once they appear, then the price will come down really quickly and you will hear about a lot of cancers that became chronic disorders, and some of them are actually chronic illnesses, they are not fatal and the infected person will live with them for 10 to 20 years.

→ **Our lecture starts here :**

Oncogenes and the cell cycle

We have mentioned that the purpose of the cascade from Growth factors, receptors, signal transduction and transcription factors is to go out and for the cell to start cycling and dividing.

-Cell cycle is activated by: Growth factors, Hormones & other activators. These activators work through cyclins and cyclin-dependent kinases(CDKs).

*How do cells divide? How does a cell move from G1 or G0 (if it was extended) to S to G2 to M ?

-The main players here are CDKs and cyclins.

Cyclin-CDK complex:

-CDK is the kinase that will phosphorylate proteins starting the cell cycle. But CDKs as the name implies are cyclin dependent kinases, so they are dependent on cyclins; they don't work unless cyclins are produced (they are made throughout cell cycle(constitutive production)but need specific cyclins to be activated). So the cyclins are only produced at specific times for short periods of time.

-Different Cyclins and CDKs control progression from phase to phase. If I want the cell to move from G1 to S I express cyclin D, cyclin D will bind to cdk4 & cdk6 and the cell will enter into the S phase. If I want the cell to move from S to G2 I have to produce cyclin A which will bind to a specific type of CDKs. If I want the cell to move from G2 to M I need cyclin B.

-Most likely when I go back to G1, cyclin D will not be there because if cyclin D was there , the cell will carry on cycling. So I produce for example cyclin D & cyclin A for a short period of time to divide so that the cell divides the number of divisions it should then stop.

Now we will talk about the story of Retinoblastoma protein:

-RB is an example of how important these cell cycle proteins are. RB is a tumor suppressor.

-The first step of the cell cycle to begin is to go from G1 to S phase, and we said that here we need cyclin D, cyclin D is produced from a transcription factor such as Myc.

-There was a growth factor that binds to a receptor , receptor activates signal transduction through Ras and finally we reach Myc which is a transcription factor of cyclin D, now cyclin D is out, immediately cyclin D will bind to CDKs that are already there which are CDK4 & CDK6 and now they are active.

-One of the main targets of CDK4 & CDK6 is a protein called RB protein.

-RB was binding & inhibiting E2F then when it becomes phosphorylated by CDK4 & CDK6 (which have been activated by cyclinD), RB releases E2F,so it is free now.

-So E2F goes to the nucleus, (it is a transcription factor of **S phase** proteins) it activates transcription of genes for entry into S phase, replication happens in S phase so polymerases , hilicases and topoisomerases are released.

-If you want to stop the cell cycle from cycling & the cell from dividing you have to hide cyclin D, so you(as if you are a cell

^_^) will turn off the promoter of cyclin D, but it's too late because already there is cyclin D so you will work on gene level by inhibiting Myc from transcribing cyclinD but you also have to inhibit the already present cyclin D , how ? by CDKIs they will immediately bind the complexes, stop them then they will be degraded.

Inhibitors are divided into two families :

-Specific Inhibitors & broad inhibitors.

-They oppose progression through cell cycle, bind & inhibit different cyclin-CDK complexes.

1) Cip/Kip family (p21, p27, p57): they have broad specificity, they inhibit all cyclin/CDK complexes.

2) INK4 family (p15, p16, p18, p19): they are specific for cdk4 & cdk6.

-When RB gets phorphorylated , E2F gets activated and the cell enter into the s phase, if I want to stop the CDKs from working I have to dephosphorylate RB to let it bind **E2F** again, so one of these inhibitors above such as p21 for example will bind to cyclin complexes and inhibit them so they are no more functioning and they will be degraded.

Tumor Suppressors "Brakes on cell growth"

-Proteins that inhibit cell proliferation in response to DNA damage.

-Act on: cell cycle / signal transduction / transcription / cell adhesion.

-Both alleles of a tumor suppressor gene are mutated in cancer cells. (Oncogenes: one mutated copy is enough)

-A Fact that we must always remember is that if we want to activate an oncogene, it's enough to up regulate one copy, but if we want to inhibit a tumor suppressor, we have to worry about two copies; we have to hit them both.

Consequently, familial cancers, the vast majority of them actually if not all of them, the individual born in that family will be born with a defective copy of a given tumor suppressor in all of his cells. And for him to have cancer, he is waiting for a chance for the second copy to hit any cell of his cells. Whereas in a normal person, two copies must be hit in two fatal spots in one cell but this is very unlikely. For this reason sporadic cancers happen in the 70s for example and it takes so long for this coincidence to happen.

On the other hand, a person who is born with one mutated copy in all of his cells, the chance for the other copy to be hit is very likely to happen. Thus he is going to lose two defense lines, which means he is going to lose his two brakes and this happens in the first or second decades of his age (that is before the age of 10 or before the age of 20).

A. Tumor suppressors directly regulating cell cycle:

1.Retinoblastoma (rb) gene:

-Works on transition from G1 to S phase as it controls the E2F family of TFs. It works on the cell cycle and hits it immediately because it traps the E2F which transfers the cell into the S phase.

-Familial retinoblastoma: A Familial cancer which is very rare.

Most common familial cancers are Breast and Colon cancer (5 to 10% of these 2 types).

-The first time they discovered the RB gene, they found it in the families with Retinoblastoma, and this is why they gave it that name although it is active in most of our cells. The RB gene is available in a lot of more important cancers but they discovered it there (in Retinoblastoma patients) and they gave it that name because they thought that it was only found there.

-Imagine a person who is born with two normal copies of RB gene. For the first copy to be hit (in chromosome 13) in a very fatal spot, then for the other copy to be hit, this is very difficult to happen and it takes 70 or 80 year to happen. But if a person is born with an already hit copy, it is very easy for the other copy to be hit and the cancer will happen faster and earlier.

-Familial cancers always happen at a younger age.

-Yet, at the end of this chapter, we will see that some people are not convinced that it is a coincidence for a Sporadic cancer to happen even after 70 years of age. For a sporadic cancer to happen, we need two mutated copies in fatal spots, but it is not only the RB gene, we need to mutate 4 to 7 or 5 to 10 different genes and all of them in fatal spots, so the issue is very complicated and difficult. And these people explained it as one of the byproducts of cancer stem cell theory and we will be talking about it at the end of this chapter.

-Familial retinoblastoma → first allele is mutated: high probability to gain second allele mutated.

-Sporadic retinoblastoma → two alleles mutated during life time.

2. p53, the guardian of the genome:

(a transcription factor that responds to DNA damage)

Cell cycle arrest vs. apoptosis.

Loss of both alleles in >50% of all tumors.

Loss of function > 90%.

-If you were asked about the most important cancer related gene among proto-oncogenes and tumor suppressors, it is going to be p53.

If we say that a certain gene is important, this means that it is involved in so many different cancers.

-P53 is the only cancer related gene that is involved in over 95% of all tumors. You can hardly find a tumor where p53 is not mutated or neutralized. Pay good attention to your use of terms. It doesn't have to be mutated directly. P53 could be hit in more than one spot in its coding region. We might not hit the coding region at all but rather hit its promoter. We might hit neither the promoter nor the coding region and the p53 gene is 100% intact but we may activate one of its down regulators. P53 is out there trying to work but the inhibitor is immediately stopping it. P53 is called the guardian of the genome. It is the most important tumor suppressor gene. It's multitalented. It can do anything basically. It can stop the cell cycle. It can repair the DNA damage or even it can kill the cell.

-If we reach a point where the cell cannot repair the damage that happens to it or it's beyond repair or the cell needs to stop the cell cycle for a while ,p53 can repair the damage. It can stop the cell cycle and it can even kill the cell so that cell does not

form a tumor. So one of the main enemies or one of the main obstacles against the ambition of a cancer cell to become fully malignant or fully transformed is this guy (p53).

-When the cancerous cells start to do mutations, they hit oncogenes then they hit not very important tumor suppressors but their eyes are always on p53, they worry about p53. That is why they are called malignant.

-Here the cancerous cells show their skills in neutralizing the p53 hitting the promoter, hitting here and there randomly, some activate MDM2 which breaks the p53

-But they have to neutralize p53 otherwise their project will fail for the cancerous transformation.

There are 10s of thousands of researches on p53, because it is one of the most fascinating proteins.

Main mechanisms of action:

1.DNA damage: p53 levels rise.

It can recognize the damage, p53 is always produced in the cell at a very low concentration which scans DNA. If it recognizes any damage, it can stop it. When there is a damage, it can tell. At first it recognizes the damage then it activates p21 which is an inhibitor of CDK complexes. So when p21 inhibits CDK complexes the cell will stop dividing. And it (p53 itself since it is a transcription factor) activates some DNA repair proteins like GADD45 which goes to the damage site and tries to repair it.

2.p53 also stimulates GADD45 transcription: (Growth Arrest and DNA damage).

-This simply means cell cycle arrest. The cell is dividing and if p53 sees a mutation in a sensitive place, it will tell the cell to stop the division because we have to repair the damage, so stop until we see what we can do.

How does it stop the cell ? It can activate a protein called p21.

-P53 here works as a DNA repair enzyme:

a.if repair was successful, p53 down regulates itself**.**

b.if damage is beyond repair, p53 activates 2 apoptotic genes: bax and IGF-BP3.

(bax and IGF-BP3 induce apoptosis.)

P53 gives GADD45 some time to try to repair. If it couldn't repair the damage within the given time, p53 will activate apoptosis for example; it activates Bax and through the coming lectures we will now how bax induces apoptosis.

Now we have known how much p53 is a powerful protein. It is multitalented. It can do so many things.

B. Tumor suppressors affecting receptors and signal transduction:

-They work on signal transduction. And I said that signal transduction is one of the cascade of proto-oncogenes (in their normal state) : Growth factors → growth factor receptors → signal transduction proteins → transcription factors of cell cycle proteins.

-Each one of these proto-oncogenes will be activated by the one before each, it may be by phosphorylation (they are all kinases), but each one of them has a guard on it (tumor

suppressor). If the oncogene itself doesn't stop signaling, the guard will stop it from signaling. So each signal transduction protein for example has one guard that shuts its mouth.

-So these tumor suppressors (guards) are working on the **signal transduction level**.

1- Regulating of ras oncogene:

Eg: GAPs (GTPase-activating proteins), they cause hydrolysis of GTP into GDP.

-NF-1 (Neurofibromin) can silence Ras, it belongs to a family of proteins called GAP proteins (this means the proteins that activate the GTPase domain in proteins like Ras).

-Ras is a signal transduction protein and it is very important (it is a G-protein). It takes the signal immediately from the receptor. Ras is activated by binding to GTP , so it will keep signaling to the protein under it until one of the 3 phosphates of GTP is removed. When ras has a GTP with 3 phosphates it is active, when they become 2 phosphates it is inactive. Who will detach the third phosphate ? Ras itself, because it has a GTPase domain.

-Ras activates Raf which activates Map then the cascade will continue. Here the GTPase domain of Ras could remove one of the 3 phosphates, but it needs something to wake it up which is **NF-1**. So from the beginning what happens?

There is a receptor that gets activated when it binds to a growth factor and this in turn activates Ras, by putting GTP on it. If GTP stays, Ras will keep signaling, so we have to remove one of the three phosphates. Who will remove it ? Ras itself,

but it needs someone to remind it to remove a phosphate, who is this guy ? Nf-1. So NF-1 is a tumor suppressor of that oncogene which is Ras.

-Mutation may happen in Ras in the place where NF-1 gets attached, so NF-1 won't be able to wake Ras up and inactivates it. Or there could be a mutation in NF-1 itself so it won't be able to recognize ras and in both cases the same result will happen.

-Ras-GTP → active in signal transduction.

-Ras-GDP → inactive.

2- Patched and smoothened:

-Good example of close oncogene-suppressor interaction. Encode receptor for hedegehog class of signaling peptides. Normally control growth during embryogenesis.

-Mechanism: Patched receptor bind & inhibits Smoothened; its co-receptor. Binding of Hedgehog ligand to Patched releases Smoothened. Smoothened transmits an activating signal to nucleus activating transcription.

-Patched is the tumor suppressor and smoothened is the oncogene. Patch may escape from the control of smoothened or smoothened may get a mutation and couldn't be able to control patched.

C. Tumor suppressor genes affecting cell adhesion:

-cadherin family:

-Recently, they called them tumor suppressor genes because traditionally this name (tumor suppressors) was for those only affecting cell cycle such as : RB, P53, but they have broadened

it to include every single protein that has a relation in blocking the final cancerous transformation which is the fully malignancy metastatic cell.

-Cadherins attach cells to each other, so the cancerous cell after it hits the first oncogene then the second oncogene and the first tumor suppressor then the second tumor suppressor then p53 is now fully malignant and its ambition won't stop here, it wants to make the same thing in some other place.

-Who is holding the cell from spreading ? Proteins like cadherin, so the cancerous cell wants to go and mutate cadherin so it can detach easier.

-Cadherin is embedded in the membrane and is attached to the actin filaments by catenins.

-**Catenins** :

-B-catenin is a very interesting guy, it is an intracellular cell adhesion molecule. It can be a tumor suppressor on the cell surface (where it works in anchoring cadherins to cytoskeleton) and it can be an oncogene down in the nucleus (where it works as a transcription factor).

-Why does it work on the cell surface as a tumor suppressor ? Because it is attached to cadherins.

-So the cancer cell may not make a mutation in the cadherin. It may make a mutation in the catenins that are attached to cadherins . So in the case of the mutation in catenins, cadherins are attached to each other from outside but from inside they are not fixed because they are not attached to actin filaments because b-catenin went into trouble.

- When b-catenin it reaches the nucleus there will be a problem because it works there as an oncogene, why ? because it activates transcription of Myc and cyclin D1 causing cell proliferation.

-There is a protein which is always stimulated for the b-catenin when it reaches the nucleus, which is APC (Adenomatous Polyposis Coli, a tumor suppressor).

-Here APC takes over and breaks down b-catenin. If a problem happens to APC, such as if a mutation happens to it, B-catenin will then leak to the nucleus and it will perform its function which is activating Myc which in turn will activate cyclin D which will take us into the S phase.

It APC is active → B-catenin levels rise → proliferation.

If APC is inactive → low B-catenin levels → No proliferation.

-Mutation in APC: FAP (familial Adenomatous Polyposis; colon cancer)

There are two types of common cancer that are familial:

1-FAP which is less common and more sever(mutation in APC).

2-HNPCC (hereditary non polyposis colorectal cancer) which is the most common (mutation in MMRs (mismatch repair genes).

APOPTOSIS

This is a brief introduction about apoptosis.

-Apoptosis is a programmed cell-death. It is not a passive sort of cell decay or damage. It is a process in which the cell spends energy and ATP in order to commit suicide. It starts with normal and abnormal ways. In the normal ways, the cell consumes the length of its telomere. It lived a normal life and it continued to divide until it consumed its allotted number of divisions, or it may undergo damage, hit by a virus then commits suicide, or it may have mutations in its oncogenes so the p53 will kill it.

-So, apoptosis could happen naturally due to shortening of telomere or by p53, which causes death for the cell before its time. P53 discovers that the cell faces a big damage so it's preferable that the cell dies in favor of the group which is the tissue in which the cell is a part of.

-Apoptosis is a serious decision for the cell to make. And here I am talking about apoptosis in relation to cancer not the apoptosis that happens in an injury or something else. I want to talk about the one that happens in cancers resulting from accumulating of mutations in sensitive fatal spots which are the oncogenes and the tumor suppressor genes.

-This decision is not easy for a cell to make. This decision will be thought about time and again before the cell makes it and commits suicide. Therefore, there is an **initiation phase** in which something has caused the cell to think of suicide because of external or internal factors. In addition there is the

execution phase which we can translate into تنفيذ → to carry out a task.

or it could be translated into إعدام → hanging (capital punishment).

-In both cases, it works for apoptosis because the proteins that are produced here (called caspases) will execute the orders or they will hang the cell at the same time.

-Between the initiation signals and the execution phase, there is a **signal integration phase**.

بالعربي بنسميها مرحلة الاستئناف، صدر القرار هون وقبل أن ينفذ الحكم صارت مرحلة الاستئناف.

-There is a family of proteins called **BCL-2,** half of which are pro-apoptosis and the other half is anti-apoptosis. They will sit together on the table and discuss the issue: shall we commit suicide or shall we wait? Can we repair the damage or can't we?

-Consequently, whoever prevails over the table dinner will determine the future of that cell.

"Listen to your inner genius. Those who do, often end up by changing the world."

أودُّ شكرَ والدي الذي أُثقِل كاهله دوماً، وأهدي هذه الشيت لِـ ثلاثةٍ قد اكتملتُ بهنّ : ندى الشّريف، تقى الغزاوي، فرح زيادة.

كل الشّكر والامتنان لِـ لجنتنا المميزة، ولِـ فريق الكوريكشن الأفضل، ولِـ نادي قرائيميا بأعضاءه الرائعين تحيّة إكبارٍ وإجلال :)

ولدفعتنا الجميلة الدافئة مني كل الحُب ~ دمتم بخيرٍ جميعا.

زميلتكم إيمان أحمد العموش.