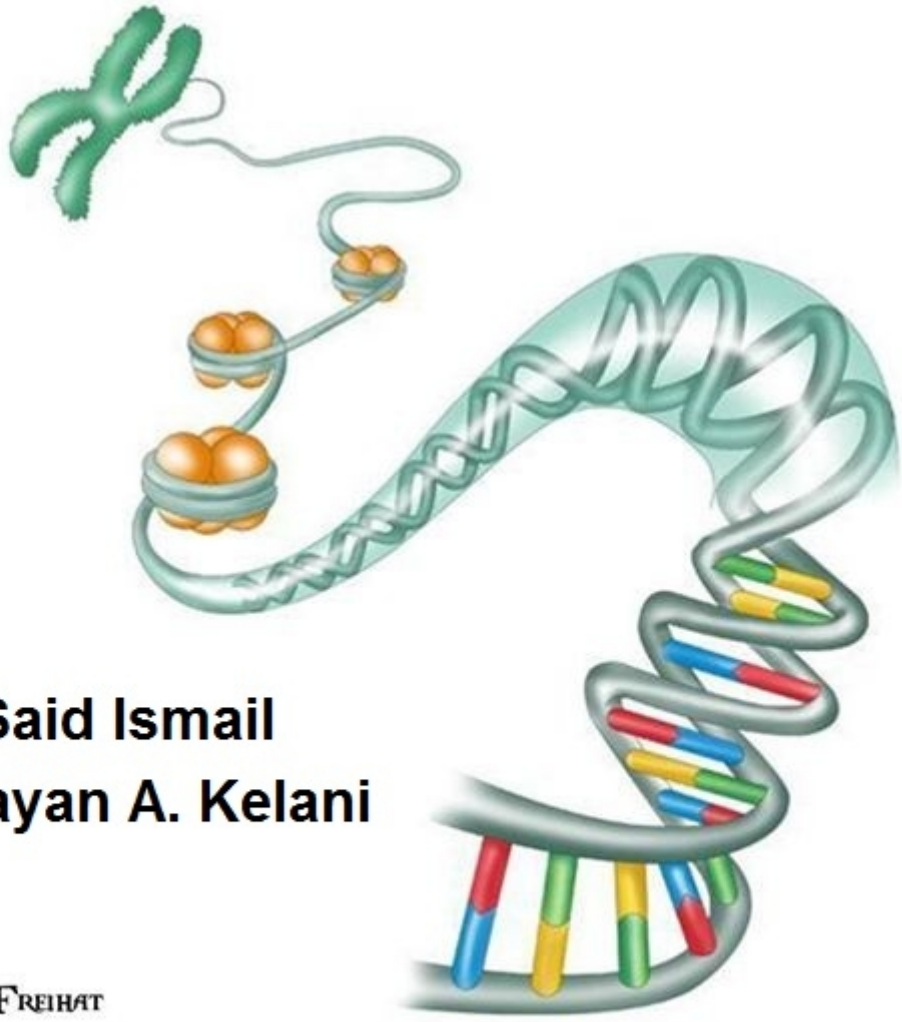
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Sheet#: 25

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Designed by Nadeen Al-Freihat

Apoptosis

I rearranged the information in this sheet ,so don't be confused when you listen to the record. I hope it'll be a very easy and clear sheet.. Good luck doctors ☺

-Introduction

**Apoptosis is a programmed cell death, it isn't a passive process, rather it's an active process by which the cell commits suicide, that means the cell spends energy to die.

**Apoptosis happens normally because of the biological clock which is represented by the telomeres, actually apoptosis happens after a certain number of cell divisions as the telomeres in that cell become short and reach a critical length that can't accommodate further divisions . sometimes, apopstisis happens as a result of mutations in a certain parts of the genome (in oncogenes, to be specidic) where the cell realizes that it's going through transformation, the cell tries to mix mutations and slow down the division but then realize the only option it has is to go through apoptosis.

**Apoptosis also might happen through the cell life when the cell gets injured. One of the injuries that we are concerning about in this chapter is DNA damage represented by mutations in proto-oncogenes and tumor suppressor genes that are beyond repair and may be the cause of the transformation of that cell into a cancer cell .So what is happening here is that the cell commits suicide and sacrifices itself for the benefit of the tissue that it is in.

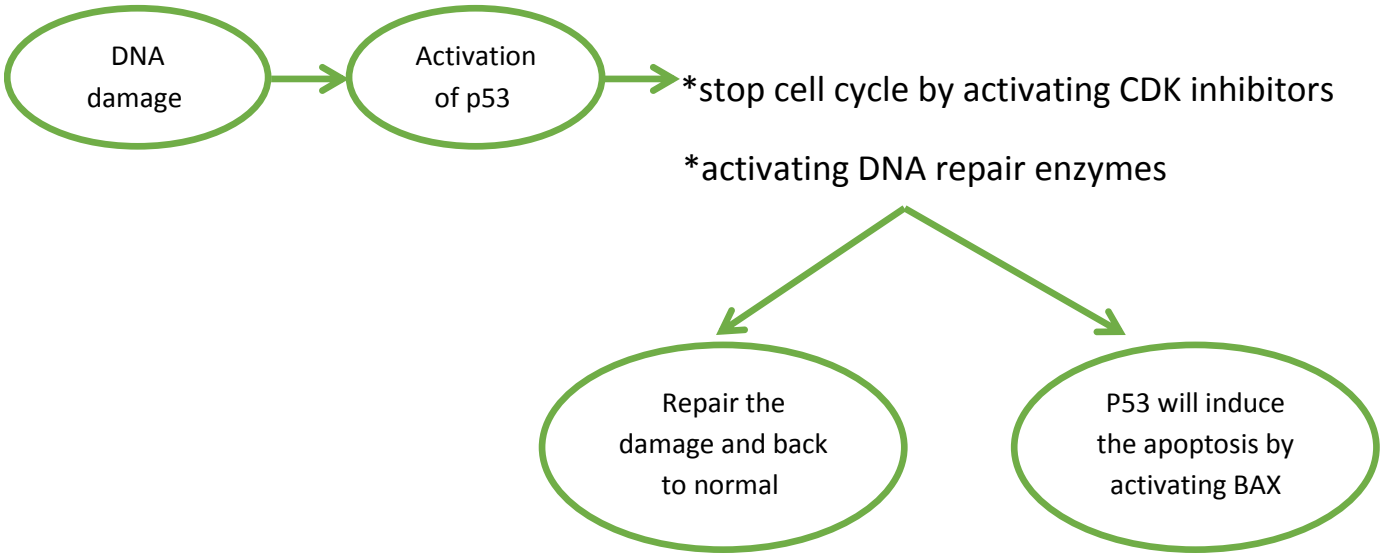
** The cell doesn't go into apoptosis unless It fails in repairing the damage .One of the most important proteins that play a critical role in the repairing process is P53.

**As P53 is activated by other proteins that sense the DNA damage, it will immediately stop the cell cycle by activating CDK inhibitors "like p21", then it will activate DNA repair enzymes and give them a while to repair the damage.

**If they fail, so the damage is beyond repair and that can lead to a cancer transformation so P53 decides that this cell has to go into apoptosis by activating Bax (proapoptotic protein).

NOTE:

P53 activates directly many proteins(Bax,CDK inhibitors….etc) by being a transcription factor of them, so it activates them at the gene transcription level not at the protein level.



**There are 3 phases of apoptosis (because It's such a serious decision between live and death, so the cell has to be sure when taking such a fateful decision) .

1)Initiation of apoptosis (whether it is an external signal or internal event that's so severe that it caused the cell to think about apoptosis).

external signals

-by the death ligand which binds to the death receptor.

internal events

- Affecting mitochondrial integrity (O2 deprivation, radiation, …etc)
- Extensive DNA damage

2)Signal integration phase : by Bcl-2 family

مرحلة الاستئناف

Imagine that after an external signal or internal event happens, the members of Bcl-2 protein family are sitting and discussing the apoptosis, half of them are with and the other half are against. Whoever prevails will lead the cell, either to the excusion phase or to stop that and say that the cell is capable of repairing the damage.

Actually that doesn't happen in the cell, instead each one of them whether it is proapoptotic or anti-apoptotic will do its function. Whoever is more active and can prevail in a practical way will determine the fate of the cell.

3)Excusion phase مرحلة التنفيذ أو الإعدام (by caspases)

- They're cysteine proteases that cleave the peptide bond next to aspartate residue.(This is how they cut and where they cut)

-They are found as procaspases/ zymogens (inactive form with an extra polypeptide that covers the active site, like pepsinogen, trypsinogen etc.) and need to be activated (by cutting this extra polypeptide) to do their proteolytic activity.

-There are two types of caspases:

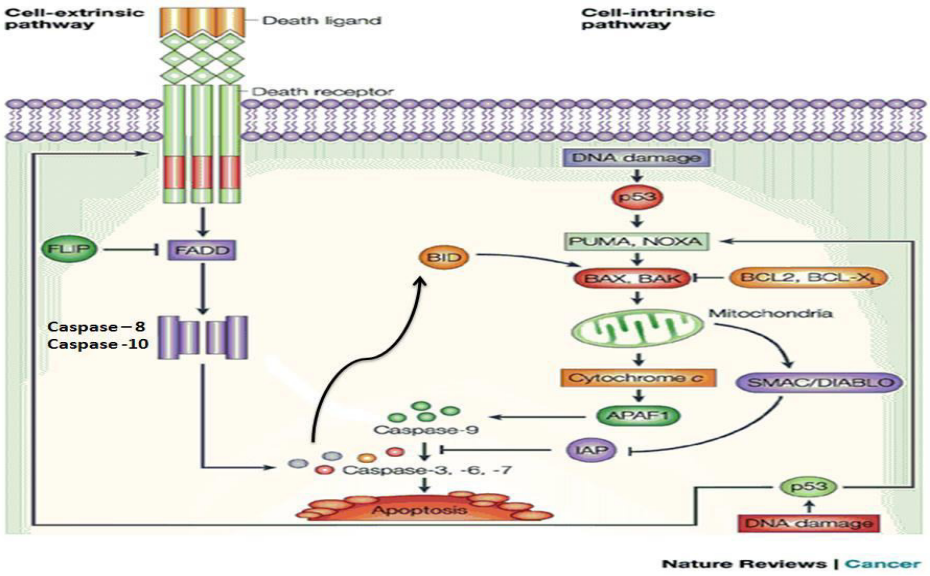
#initiator caspases (the two main ones are procaspase 8 and procaspase 9)

Cleave other procaspases

Other initiator procaspases "Autocatalysis"	Execution procaspases

#execution caspases

Which cleave other cellular proteins involved in maintaining of cellular integrity, actually they will damage the cell.

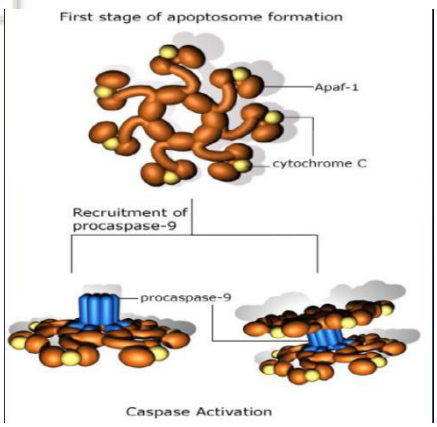


**The mitochondrial integrity pathway to apoptosis(a response to an internal event)

**An internal event "initiation phase" will damage the mitochondria and cause release of cytochrome c then members of Bcl-2 family "signal integration phase" will help in release of cytochrome c or prevent that depending on the severity of the damage.

**The release of cytochrome c from mitochondria to the cytoplasm (important member in electron transport chain that is loosely bound to outer surface of inner mitochondrial membrane).

When cytochrome c presents in the cytoplasm, it will eventually bind to Apaf (pro-A**poptotic **P**rotease **A**ctivating **F**actor) to form a complex called "***APOPTOSOME***" which cleaves procaspase 9 and converts it into an active caspase 9 which in turn activates excusion procaspases "excusion phase".



** Why the cell has chosen the mitochondrial integrity as a parameter of the severity of the damage whether it is repairable "the mitochondria will stay integer" or irreparable "the mitochondrial integrity is damaged and apoptosis is induced"?

Because the mitochondria is such a strong organelle found within the cell (double membrane/small/compact), so it isn't easy for the mitochondria to get damaged . When it is damaged, that means there is a severe condition and the whole cell is in danger because if that happens to a strong organelle, so what will happen to the other organelles which are weaker!!

**How the cell can know that the mitochondria has lost its integrity and is damaged ?

Actually that happens by release of cytochrome c as an indication of losing the inner mitochondrial membrane integrity.

**The death receptor pathway to apoptosis (external signals)

**The classical example to understand the external signal is the following :

A virus infected the cell and that virus is causing a lot of troubles, but that cell isn't even aware that there is a virus in it. Fortunately the immune cells (cytotoxic T-cells) recognize the changes on the surface of the infected cell, so they give that cell an external order to die.

**The death ligand is on the surface of cytotoxic T-cell , so when this immune cell recognize a viral infected cell by identifying some changes on the surface of that cell, it will bind its death ligand to the death receptor on the surface of the infected cell (the death receptor is called that because it can activate apoptosis), that will recruit an adapter protein FADD which eventually activates procaspase 8 and procaspase 10 (procaspase 10 in the latest edition of the book) ,the activated one will cleave the other and activates it . As they become activated (caspases 8 and 10) ,they will start to cleave execution procaspases (3/6/7) , so they will become activated caspases and will cleave cellular proteins and causing damage to the cell.

**__Caspase 3__ has the ability to cleave a Bcl-2 proapoptotic protein (Bid) and convert it into (tBid) which activates the mitochondrial integrity pathway >> t means truncated (cleaved).

**So activated tBid will go and intentionally cause damage to the integrity of mitochondria forming holes in it and cause release of cytochrome c to enhance the decision of apoptosis, then cytochrome c will leak from the inner membrane and bind to Apaf and form Apoptosome which in turn cleave procaspase 9 and convert it into caspase 9. Caspase 9 can then cleave the execution caspases.

**As we can see there will be 3 initiator procaspases working in extrinsic pathway to apoptosis (8,10 and 9).

**Note:

Bid is found in the cell as an inactivated form (like caspases), once it's truncated it will be converted to an active form.

**Bcl-2 family proteins

**The Bcl-2 family members are decision-makers that integrate prodeath and antideath signals to determine whether the cell should commit suicide or not .
**Half of them believe that they can rescue the cell and have a hope that the damage will be repaired, the other half are just so excited about killing the cell :p
**Bcl-2 is antiapoptotic protein, but it was the first one to be discovered so they named the whole family with its name

:P محمد أول ولد فصار اسم العيلة عيلة أبو محمد

Table 18.3 Bcl-2 Family Members

Anti-apoptotic
Bcl-2
Bcl-x
Bcl-w
Proapoptotic
Channel Forming
Bax
Bak
Bok
Pro-apoptotic
BH3-Only
Bad
Bid
Bod/Bim

Roughly 30 Bcl-2 family members are currently known. These proteins play tissue-specific as well as signal pathway–specific roles in regulating apoptosis. The tissue-specificity is overlapping. For example, Bcl-2 is expressed in hair follicles, kidney, small intestines, neurons, and the lymphoid system, whereas Bcl-x is expressed in the nervous system and hematopoietic cells.

***** antiapoptotic proteins***

Bcl-2 /Bcl-w /Bcl-x

They have at least two ways of antagonizing the death signals:

1)They insert themselves into the outer mitochondrial membrane to antagonize channel-forming pro-apoptotic factors and close the holes, thereby decreasing cytochrome c release.

2)They may also bind to cytoplasmic Apaf so that it cannot form the apoptosome complex when there is a little bit release of cytochrome c from the mitochondria.

***pro-apoptotic proteins**

They are divided into two groups:

1- Channel forming proteins: they are channels that can insert themselves in the mitochondrial membrane (outer membrane because cytochrome c is in the intermembrane space and loosely attached to the outer surface of inner mitochondrial protein) , so they make holes in the outer membrane facilitating the release of cytochrome c. They do that by the help of BH3-only proteins which dimirize with them and facilitate the insertion of those channels into the outer mitochondrial membrane.

Bax (which can be activated directly by P53 as previously mentioned)/ Bak / Bok

2-BH3- only proteins
**They aren't channel forming, they don't form channels.
**At the structural level, they share some homology with the channel forming proteins in one domain out of 4 domains that channel forming proteins have and the name of that domain is (BH3),so they are smaller than channel forming proteins.
Bad / Bid /Bod(Bim)

They basically have two functions:
1) They dimirize with the channel forming proteins and facilitate the insertion of them in the outer mitochondrial membrane

2) They inhibit antiapoptotic proteins by removing them from the outer mitochondrial membrane when they are trying to close the holes.

Let's illustrate this figure to make things clearer:

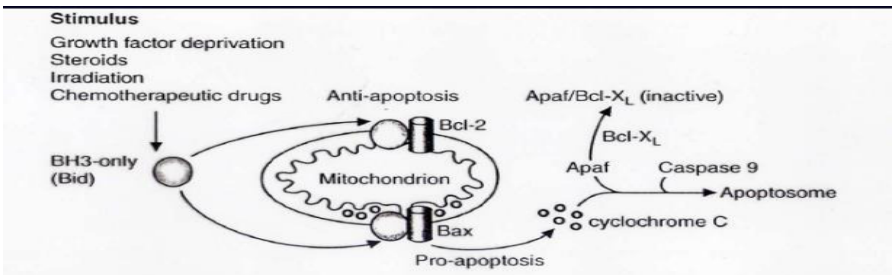


Fig. 18.16. Roles of the Bcl-2 family members in regulating apoptosis. Bcl-2, which is anti-apoptotic, binds Bid (or tBid) and blocks formation of channels that allow cytochrome c release from the mitochondria. Death signals result in activation of a BH3-only protein such as Bid, which can lead to mitochondrial pore formation, swelling, and release of cytochrome c. Bid binds to and activates the membrane ion-channel protein Bax, activating cytochrome c release, which binds to Apaf and leads to formation of the apoptosome.

There is a damage in the cell and that damage causes holes in the mitochondria. Bcl-2(anti-apoptotic) comes and tries to close those holes to prevent the release of cytochrome c also if there is a bit release of cytochrome c to the cytoplasm it binds to Apaf and inactivate it to prevent binding of Apaf with cytochrome c and the formation of apoptosome. Bax (pro-apoptotic) is doing the opposite , it inserts itself in the mitochondrial membrane and causes the release of more cytochrome c. Bid (pro-apoptotic) has a dual function, it tries to remove Bcl-2 and make the holes even larger, other function of Bid is to bring the Bax and encourage it to insert itself in the mitochondrial membrane as a channel protein to release more cytochrome c. So at the end whoever prevails will determine the fate of the cell.

A question was asked in section one about the function of BH3-only protein as we took a different mechanism of its action with Dr. Mamoun in cell biology and the doctor answered that question in section two <3 , so here is what the doctor said :

There are 3 different mechanisms explaining the action of BH3-only protein, 2 of them are important:

-Displacement model

Bax (channel forming anti-apoptotic protein) is held by the Bcl-2(anti-apoptotic protein) then BH3 will come and bind to Bcl-2 and cause the release of sequestered Bax from the grip of Bcl-2.

Remember that both Bax and Bcl-2 share one domain which is BH3 domain and it's the domain that is bound to Bcl-2 protein

-The other model -

Bax is free in the cytoplasm, but it needs to be dimirized with BH3-only protein to be activated and inserted in the mitochondrial membrane.

In away or other BH3-only protein is important of activation of Bax.

There is no regular way to name the proteins in molecular biology. The discoverer of the protein can name it according to his name, his pet name or as abbreviation of the protein's function. When another one discover other protein that has a homology with the first one, he will call it in a similar way to make them one family. Example : 1) Bad /Bax/Bod (Bim) 2) southern blotting "which is a method used for detection of a specific DNA sequence" is named related to its discoverer ***Sir Edwin Southern***, later on others change a little bit in the technique and called the new methods northern and western. As you can see It's a joke :p

In JAK/STAT pathway, there is a protein called JAK which was discovered before the discovering of the whole pathway. JAK is a tyrosine kinase, the discoverer of that protein decided to name it as "Just Another Kinase" because there are several tyrosine kinases within the cell. Later on, others discovered the importance of that protein in JAK/STAT pathway and the relation of that pathway to many cancers, so they decided to give it more valuable name that reflexes its function of controlling many signal pathways which is "Janus kinase". Janus is the roman god for gates and doorways.

**Cancer cell bypass apoptosis

**The critical point here is that cancer cells won't go into apoptosis, different cancer cells have different ways to bypass apoptosis.

**When the cancer cell manages to activate some oncogenes and inactivate some tumor suppressor genes, it will start proliferating in a high rate, but the problem now is with apoptosis which can be induced by the high rate of consuming the telomeres or by P53 which activates Bax.

**Cancer cell has always the solution, for the problem of telomeres most of them can activate telomerase, others cut the link between the telomeres and the proteins that measure the length and tell the cell about it, each cell has its own way to prevent apoptosis.

**A special case in bypassing apoptosis

Some cells are growth factor dependent for survival, once it is deprived of that growth factor, apoptosis will happen. Note that not all cells are growth factor dependent.

تخيل الخلايا كالنباتات بعضها يحتاج للماء بشكل دائم ويموت في حال انقطاعه وبعضها الآخر يحتاج له من موسم لآخر ولا يشكل انقطاعه خطر على حياته.

- e.g PDGF/Akt/BAD pathway:

**A continuous supply of the PDGF (platelet derived growth is needed for the survival of the cell.

When PDGF binds to its receptor (PDGFR), PDGFR will pass the message to PI-3 kinase, which in turn causes the phosphorylation of PIP2 to become PIP3, that's will make Akt to bind to PIP3 and be activated (Akt is a kinase when it is activated), Akt will phosphorylate Bad>> Bad is inactive when it's phosphorylated >> apoptosis won't occur .

**When something serious happens to that cell, it will be deprived of the growth factor, so the proapoptotic Bad will be active (as it isn't phosphorylated) and induce apoptosis.

**So when this cell is becoming a cancer cell it will try to become growth factor independent by many choices of mutations:

1) mutation in the extrinsic part of the receptor that prevents the release of the growth factor after it binds.

2) mutation in the intrinsic part of the receptor to look like the active form even when there isn't a GF bound to it.

3) Activated mutation in PI-3 kinase to be constantly active.

4) Activated mutation in Akt will make it continuously active independent from the upper cascade that leads to its activation.

In a way or other BAD will be continuously phosphorylated and inactive, so apoptosis won't occur.

**Cancer requires multiple mutations:

**4-7 mutations are required for full transformation of a normal cell into a cancer one, some textbooks say 5- 10 mutations. Anyway several mutations must be there to cause cancer.

**Imagine the possibility of the normal cell that want to be a cancer cell to mutate genes which are only 25% of our genome (not a non-coding regions) ,and the possibility of it to mutate a specific gene of the 25,000 genes that we have (proto-oncogene or tumor suppressor gene not any other genes), and the possibility to mutate that gene in one of the exon regions (not introns), and mutate that exon in a region that activate the proto-oncogene and convert it into oncogene while inactivate tumor suppressor gene. These are specific parts of the genome that can turn the cell into a cancer one.

**It's just like winning the lottery 7 times in your lifetime. The doctor is telling us about the lucky guy who won the lottery twice in England couple weeks ago and they calculated the possibility of happening such thing, the number was 1 in 2 hundred billion chances!! :0

**Depending on that, Epidemiologists aren't convinced about the very small possibility of getting the disease and the high prevalence of it:

1) The oncogenic stress that we are exposed to is very high **(oncogenic stress: is the amount of carcinogens that we are exposed to from air/food/radiations/chemicals/phones/microwaves/nose….etc)**, so the number of mutations is huge.

2) There is a theory which tries to explain that called **"Clonal expansion theory"**

This theory tells us that the 7 mutations that are needed by the normal cell to become a malignant one don't all happen in the same cell; when a normal cell is hit by a mutation, it will start to proliferate in a higher rate and becomes 10 cells, then one of those cells will be hit by another mutation so there will be 2 mutations in it which increase the potential of proliferation so it will become 100 cells, then one of the 100 cells is waiting for a third hit of mutation and becomes 10,000 cells and so on until the malignant cell is formed.

3) Even that, some people weren't convinced because of the high prevalence of cancer (1 in every 1500 persons here in Jordan and 1 in every 1350 in developed world), therefore there is another theory which is **"cancer stem cells theory".**

This theory says that not any cell can be transformed; only stem cells have the ability to be cancer stem cells.

Stem cells are more proliferative than other cells, they are in continuous division and the oncogenes are already active there because they're needed for division so the possibility of accumulating mutations and transforming is more and easier.

تخيل الموضوع انه مين أسهل يصير فيها حادث(حادث=cancer) سيارة في المعرض واقفة ويمكن مو معبية بنزين أصلا (any cell in the tissue) ولا سيارة تانية ع الخط السريع وماشية (stem cell)....أكيد طبعا يلي ماشية. لأنه السيارة الأولى لازم نعبي فيها بنزين بالأول ونشغلها وندعس بنزين و رح تتسارع لحد ما توصل لسرعة بتصير عندها الحوادث .

Cancer epidemiology

In Jordan:

**breast cancer by far is number one

Then comes lung and colon cancers then prostate and leukemia's.

**lung cancer is increasing in females because of the increasing number of smokers between them.

**There is a big misunderstanding between people even doctors about smoking, some people say that his grandfather has smoked for 40 years without having lung cancer and his friend who didn't smoke had a lung cancer at the age of 20 and died!!

**Actually these are exceptions and by this example he won't have the evidence as the studies that are conducted on thousands of smokers for long periods of time and concluded that smoking increases the risk of lung cancer by ten folds.

** 4 weeks ago, a study was published in "Nature" or" Science" indicates that chance is more important than carcinogens, healthy lifestyle and diet. The doctor was really shocked by this study and said that not all published studies are correct, that will need more studies in the future to prove that.

**So far healthy lifestyle, healthy diet and quitting smoking will help ☺

Sorry for any mistake

(قال عمر بن الخطاب –رضي الله عنه- : تفقهوا قبل أن تُسوّدوا)

Dedication goes to Farah Bilal, Mai Ziad, Lara Khalefa☺, Sally Zaidan ,Alia Arman and Nadejda Balkizi <3 <3 <3

Done by: Bayan A. kelani