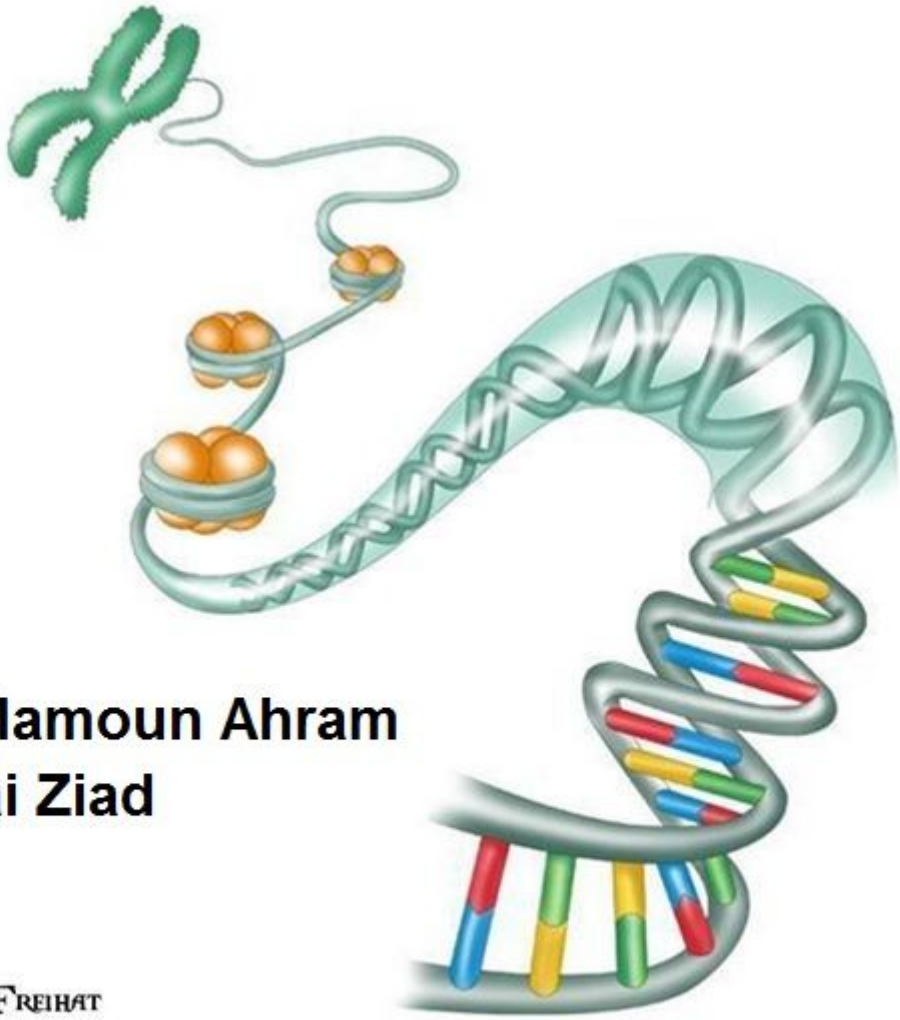
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Sheet#: 13
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Cell Death and Cancer

Hello ☺ This is the last lecture for Dr. Mamoun and by this lecture we conclude Cell biology.

There are some good animations and videos I added I hope you will see them, they are really good ☺

Enjoy the lecture, it's a long one (Two lectures originally) but an easy one *.*

-Cell proliferation, differentiation, and death:

The way we develop as embryos and as adults is all about cell proliferation, differentiation and cell death..It's all under control ☺

PROGRAMMED CELL DEATH:

Remember when we talked about C.elegans (Caenorhabditis elegans)? "first sheet p.7 "

"We said that the Adult worms consist of 959 somatic cells only, starting from 1090 cells (the 131 missing cells in the adult form die selectively by Programmed cell death). That's why C.elegans is used for studies of animal development, cell differentiation and apoptosis.

So what is programmed cell death?

It's a normal physiological process, which plays a key role both in embryonic development and adult tissues with a distinct process known as **apoptosis.**

It's different from necrosis→which is the major pathway of cell death in many commonly encountered injuries like trauma (you hit the cell and it explodes).

This is a really good video for Armando Hasudungan Showing the differences between apoptosis, necrosis and autophagy in a really awesome way ☺. Have a look at his channel it has good videos.

https://www.youtube.com/watch?v=Egy--doiBF0&index=10&list=PL561A1F2B5E7C80AA

→ Programmed cell death is carefully regulated so that the fate of individual cells meets the needs of the organism as a whole. For example:

- About 5×10^{11} blood cells are eliminated daily by programmed cell death balancing their continual production in the bone marrow.

- Elimination of nerve cells with faulty connections.

- In addition, programmed cell death provides A DEFENSE mechanism by which DAMAGED and potentially DANGEROUS cells can be eliminated for the good of the organism AS A WHOLE such as: cells with DNA damage and even mitochondrial or membrane damage and virus-infected cells. In other words; cells scan cells and damaged cells get exposed to be eliminated.

There are two distinct pathways of apoptosis: Intrinsic pathway and extrinsic pathway.

Now what are the EVENTS of APOPTOSIS?

Have alook at this animation ☺

http://sites.sinauer.com/cooper6e/animation1701.html

1. Fragmentation of chromosomal DNA:

(If you analyze the DNA of a normal cell, you'll find it intact or in a single large piece. On the other hand, in cells undergoing apoptosis, DNA will be fragmented: divided into many small pieces rather than one large piece; as a result of cleavage between nucleosomes)→ We will take this in electrophoresis with Dr Saeed *.*

⬇

2. Chromatin condensation

⬇

3. Breaking down nucleus into smaller pieces

- It can be observed under the microscope, and you can see the nucleus fragmented into smaller pieces.

⬇

4. Cell shrinkage

This is one feature that you can differentiate between apoptotic cell from healthy normal cell: differences in cell size :*D*

⬇

5. Cell fragmentation

To which is known as apoptotic bodies.

⬇

6. Phagocytosis by macrophages and neighboring cells.

Now this is a video that shows apoptosis under microscope
→http://sites.sinauer.com/cooper6e/video1701.html

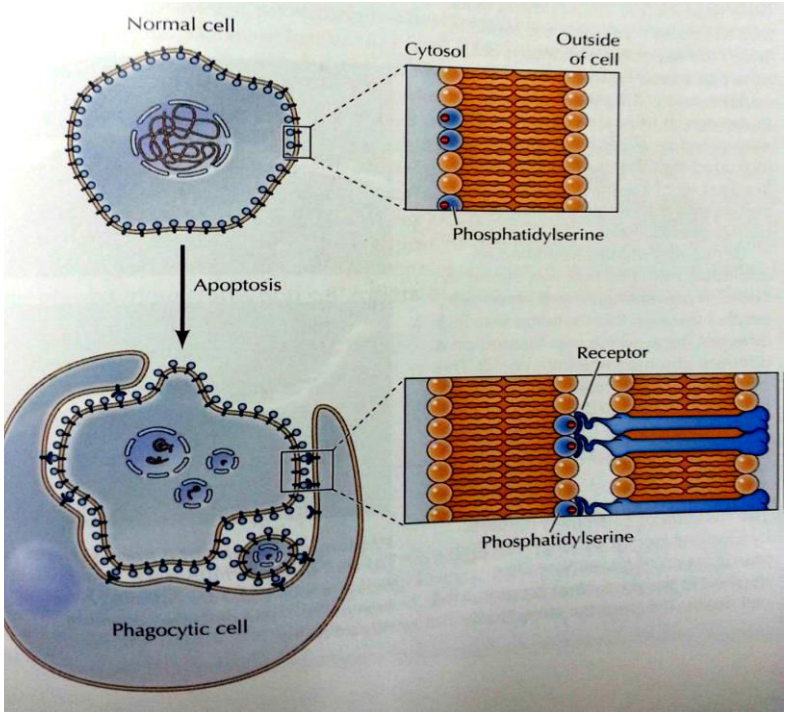
-But in <u>necrosis</u> it's different ; because it results in **membrane damage** → causing enlargement of cells (due to entrance of water and solutions from outside to inside) → and release of intracellular contents → causing inflammation ^_^

In contrast, Apoptosis→ plasma membrane is **intact**, only lipid orientation is altered.

<u>Now what happens to the apoptotic bodies?</u>

The removal of apoptotic cells is mediated by the expression of what so-called <u>EAT ME</u> signals on the cell surface which include <u>PHOSPHATIDYLSERINE.</u>

- Phosohatidylserine (PS) which is one of the EARLY MARKERS of apoptosis, is a fatty acid that exists on the inner leaflet of the membrane, but when the cell is dying and undergoing apoptosis the PS FLIPS to the outside and becomes on the outer surface of the cell..PS is then recognized by receptors of the phagocytic cells and binds to them.



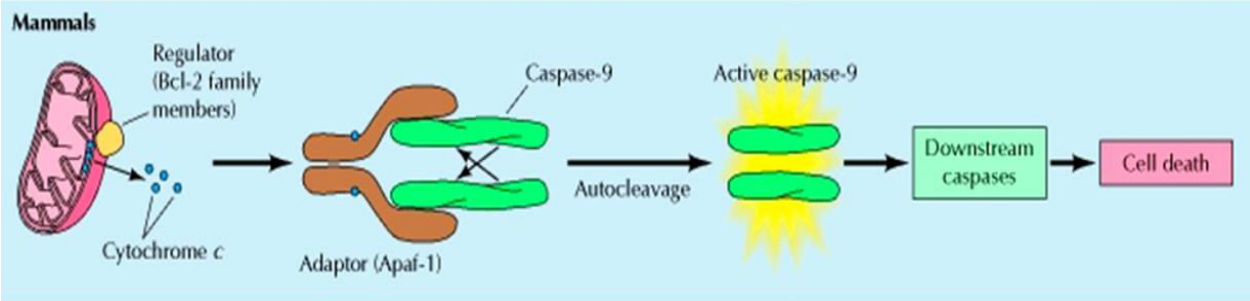
Caspases: The Executioners of Apoptosis:

http://sites.sinauer.com/cooper6e/animation1702.html

Awesome animation :D

- Apoptosis results from the activation of enzymes called caspases. They are the ultimate effectors of apoptosis. They bring about the events of apoptosis by cleaving many different cell target proteins. (Caspases are cysteine proteases that cleave proteins after Asp residues)

Now, how do we activate Caspases!!??



- Activation of caspases depends on a finely tuned balance between production of pro- and anti- apoptotic proteins that belong to a family called BCL-2 family.

- Regulators of the BCL-2 family act at the mitochondria to control the release of CYTOCHROME C which binds to an **adaptor protein** known as Apaf-1. As a result, Apaf-1 binds to caspase-9 thus activating it and the net result is the activation of caspase cascade ultimately leading to cell death.

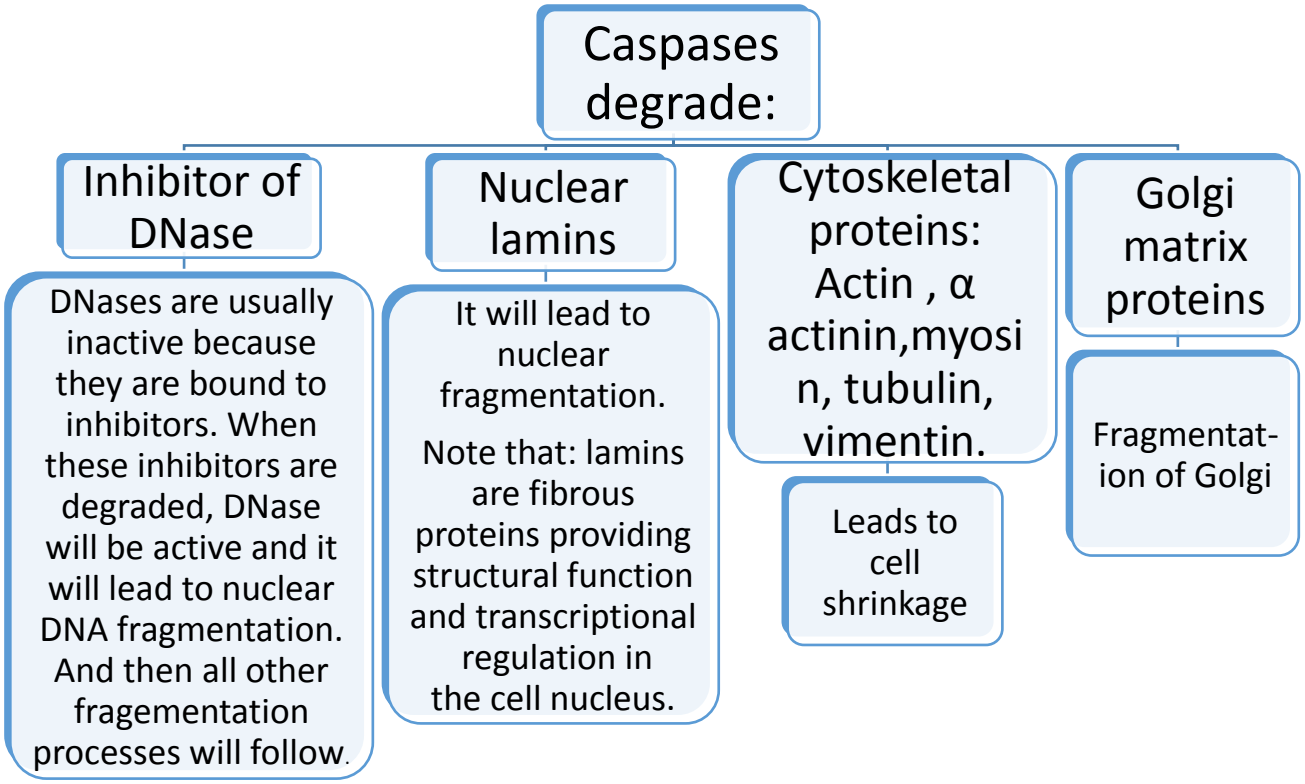
Note: Apaf-1 is an adaptor protein and these proteins are accessory to main proteins in a signal transduction pathway. They tend to lack any intrinsic enzymatic activity themselves but instead mediate specific protein-protein interactions. (In this case: cytochrome C and caspase-9) Exp on adaptor proteins: G-proteins, Ras.

PS: from book:

APAF-1 and CASPASE 9 when they bind together they form what's called Apoptosome which is a multisubunit complex.

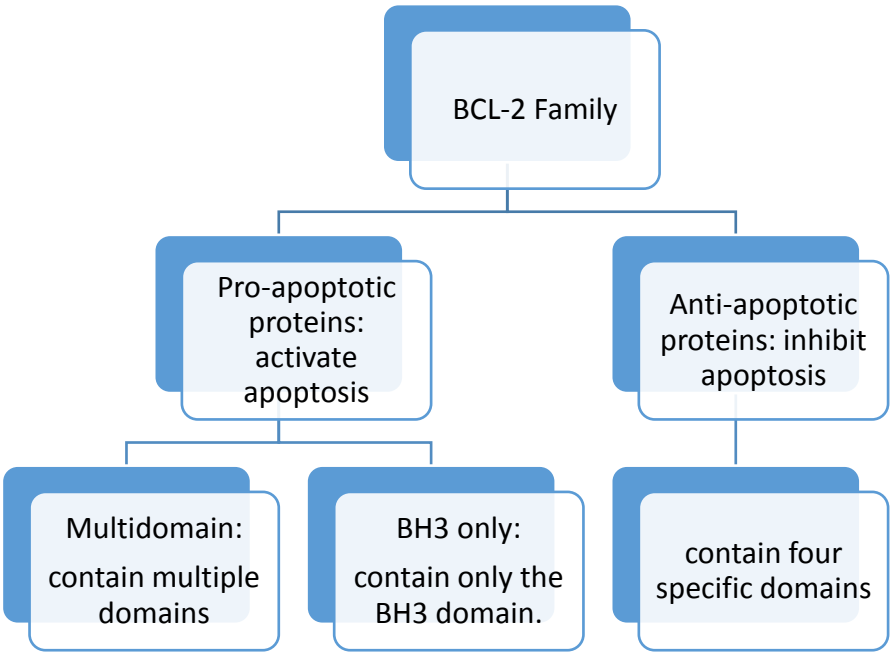
-There are different types of caspases (2, 3,6,7,8...etc) and each one has a certain function. Caspase-9 cleaves other downstream effector caspases (CASP3 and CASP7) which in turn cleave other protein substrates within the cell to trigger the apoptotic process.

<u>Now what are these protein substrates?</u>



BCL-2 family; regulators of Cell apoptosis..:

WELL, these proteins are divided to three classes according to their structure (number of domains) and function (pro- or anti- apoptotic effect).

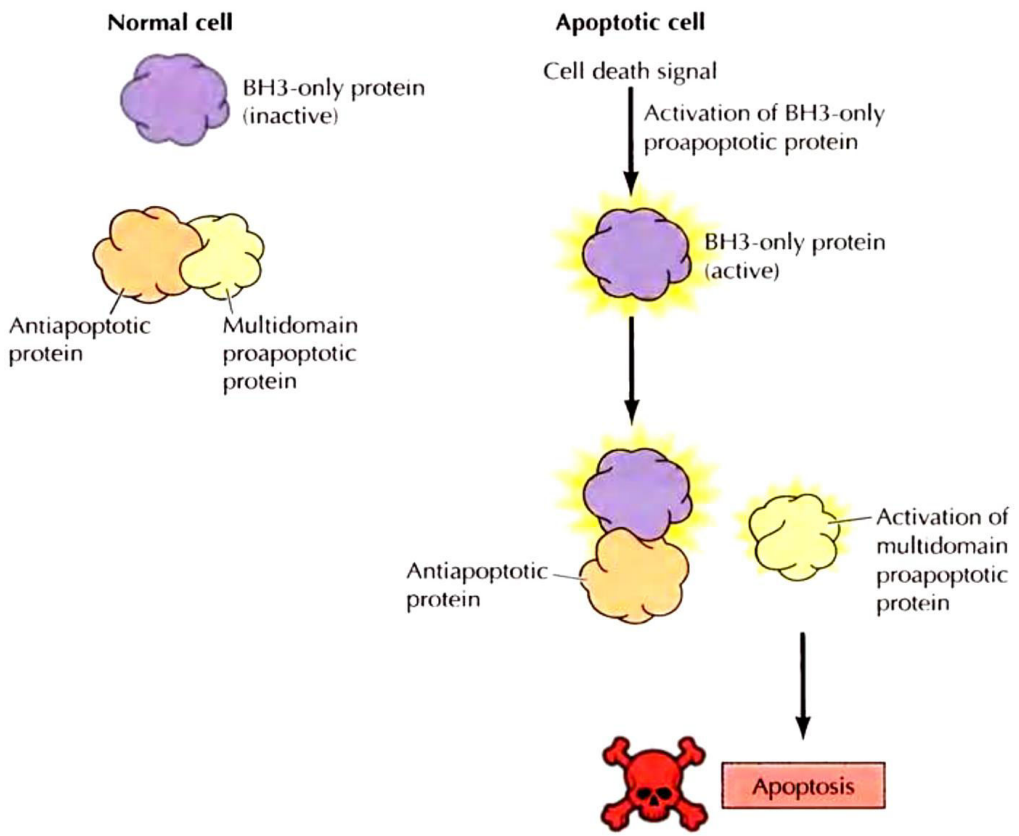


How is apoptosis activated upstream?

→In a normal cell, the multidomain proapoptotic proteins are inhibited by binding to the antiapototic proteins and the BH3 only proteins are inactive.

→ The BH3-only proteins are upstream members of the pathway and they are regulated by signals that induce cell death (DNA damage) or cell survival (growth factors).

When there is an apoptotic signal BH3 proteins are activated. And they bind to the anti-apoptotic protein, degrade it; leading to activation of the multidomain proapoptotic proteins and cell death.



- These multidomain proapoptotic proteins accumulate (act on mitochondrial outer membrane) and they form oligomeric pores (channels) that lead to the release of cytochrome C from the mitochondrial intermembrane space.

*Cytochrome C is an essential component of electron transport chain.

- Cytochrome c then binds to APAF-1 which then binds to Caspase 9 and activates it. After that, caspase 9 activates other caspases. The most important downstream caspase is CASP3.

Signaling pathways that regulate apoptosis :

Apoptosis is regulated by integrated activity of a variety of signaling pathways, some act to induce cell death and others act to promote cell-survival.

Apoptosis can be regulated by two pathways:

1- Intrinsic (internal) pathway:

From inside the cell: stimulated by DNA damage or mitochondrial damage→ all signals take place inside the cell.

DNA damage is one of the principal triggers of apoptosis. It leads to the activation of ATM and CHK protein kinases which phosphorylate and stabilize p53 resulting in rapid increases in p53 levels. P53 is a transcription factor which activates the transcription of genes encoding the proapoptotic of BH3 only proteins leading to cell death.

2. Extrinsic (external) pathway:

As a result of the presence of signals from outside the cell these signals bind to a receptor→ inducing another signal inside the cell.

There are two types of external signaling that regulate apoptosis:

A.Pro-survival signaling pathway (PI3 kinase pathway):

Rather than inducing cell death, this pathway acts to promote cell survival by inhibiting apoptosis. It is mediated by **Akt protein** which is found in the cytosol.

-Growth factor activates receptor→Receptor activates PI3 kinase→PI3 kinase converts PIP2 to PIP3 →PIP3 binds to Akt (protein serine/threonine kinase) and activates it →

Akt then phosphorylates a number of proteins that inhibit apoptosis and promotes cell survival.

***Whenever Akt is activated the cell is healthy and everything is fine: D

Note: One of the proteins that are activated by AKT is **mTOR** which is responsible for inhibiting autophagy. When it is activated autophagy is inhibited.

B- Pro-death signaling pathway (death receptor pathway) :

In contrast to the growth factor signaling pathways, some proteins signal apoptosis by activating receptors that directly induce apoptosis of the target cell. One of the most important pro-death signal pathways is mediated by tumor necrosis factor receptor. When TNF binds to its receptor, it activates it so it can bind **Caspase 8**. As a result, caspase 8 is activated and then it activates BH3-only proapoptotic proteins inducing apoptosis.

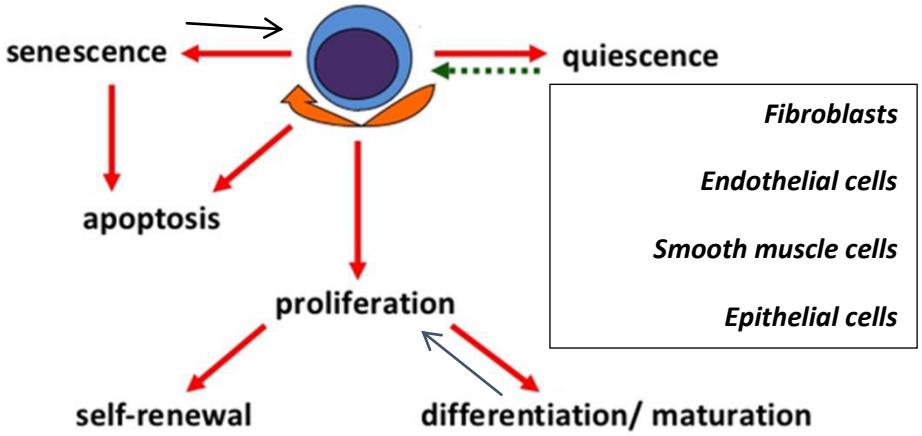
Autophagy:

When the cell doesn't have functional caspases, apoptosis can be caspase-independent and mediated by autophagy through mTOR signaling. As previously mentioned, when mTOR is inactivated it can induce autophagy in which the cell eats its own contents to provide nutrients and energy.

The dying cell does not go through the same morphological features of apoptosis (nuclear, DNA fragmentation..Etc), but accumulate lysosomes.
Autophagy has some advantages over apoptosis like:

- When cells lack molecular machinery of apoptosis it resorts to autophagy.
- It provides cells with an opportunity to repair the damage prior to death; it is a slower process.

Cell Fate→ not explained in section1



→The cell enters the G0 phase which is→ quiescence → meaning the cell is metabolically active but it isn't proliferating → It's not growing and most cells in adult tissue are in G0 ☺.They have a constant morphology .When cells are activated they can enter the g1 phase ..etc.

→ When the cell is damaged it can enter Senescence → case of emergency → metabolism is stopped until the damage is

repaired if not → APOPTOSIS. And it's reversible (According to the doctor)

- A few types of differentiated cells, are able to resume proliferation as required to replace cells that have been lost because of injury or cell death.

<u>And by this we conclude Cell Death</u>

Cancer:

Have a look at this video for Armando about cancer ^_^

https://www.youtube.com/watch?v=M5VM62MvKxM

Tumor: is any abnormal, excessive proliferation of cells forming a mass. It can be either malignant or benign. <u>MALIGNANT tumors are called cancer.</u>

→**Benign tumor**: remains confined to its original location and can be removed surgically.

→**Malignant Tumor**: degrades the basal lamina, and it invades the connective tissue and then spreads throughout the body through the lymphatic or circulatory systems (metastasis).

Tumor \cancer is a:

- ✓ Very slow disease in some cancers and fast in others like embryonic cancers.;
- ✓ It develops from a multistep process involving mutation, with progressively increasing capacity for proliferation, survival, invasion and metastasis. For example:

- Breast cancer takes about 15 or 20 years to develop, Pancreatic Cancer takes 11 years to develop and become

malignant→ therefore your message as a doctor should always be " EARLY-DETECTION-"

Process of Tumorigenesis

In order to form a tumor there are two important steps:

- Tumor initiation.
- Tumor progression.

Tumor initiation:
The hit that makes the tumor, the genetic alteration that leads to abnormal proliferation of a single cell into a tumor under certain conditions.

Tumor progression: accumulation of mutations within cells of the tumor population and these mutations give the tumor cells the selectivity to become invasive and metastatic and eventually cancer.

The mutations sometimes are epigenetic; meaning there is no change in the sequence of DNA, like methylation, and it's reversible ☺

Tumor Heterogeneity:

Tumor heterogeneity makes the practice of oncology VERY hard because it introduces significant challenges in designing treatments.

➔ Let's say you have two patients with breast cancer, medication works on one patient and on the second patient it doesn't, meaning: the cancer comes back to the second patient after five years of stopping the medication for example.

➔ Initiation of mutations in several types of cells, that –under the right conditions- start to proliferate and they form a tumor.

- ➔ As they proliferate, they accumulate more and more mutations and they are still benign.
- ➔ The acquired mutations are different between cells.
- ➔ According to the acquired mutations some of them can be dangerous, as they would allow the cell to become invasive and go to different sites.

For that reason, patients with the same type of cancer (Breast for example) can respond differently to treatment, even in the same patient ;treatment can kill 99% of the tumor cells, but it may not kill this remaining 1%, so the cancer is no longer detectable but it might come back and be very aggressive and more resistant.

In other words, tumor heterogeneity describes the observation that different tumor cells can show distinct morphological and phenotypic profiles and this occurs between tumors and within tumors.

Environmental causes of cancer:

Carcinogens : Substances that cause cancer, and they are of two types :

1. Initiators: induce genetic mutations:

→Chemicals that induce a mutation in the cell:

Eg: radiation,viral and chemical carcinogens :chemicals in tobacco smoke and aflatoxin.

2.Promoters: stimulate cell proliferation.

→Chemicals that induce cells to grow fast and proliferate and accumulate mutations (Tumor progression):

Eg:* the phorbol esters stimulate cell proliferation by activating protein kinase C.

* Hormones(estrogens) increase risk of female cancers.

* Pathogens.

Now here is a question:

If you add a promoter to a healthy cell then remove it and add initiator, does the cell become cancerous ? NO

The initiator creates the mutation and the promoter allows cells to grow, BOTH ARE ESSENTIAL.

What are the features of cancer ?

1. Clonal: They arise from a single cell.

2. Uncontrolled proliferation.

3. Accumulation of genetic mutations:

As they undergo fast proliferation process, there will be higher incidence of mutations as a result. Similar to a situation where you are asked to write every single word in a book very fast; you do more mistakes, or when writing it down slowly; you will do less mistakes.

→ This leads to genetic instability (unstable DNA) that refers to a high frequency of mutations within the cellular genome.

4. Autocrine growth stimulation (more than normal cells):

So there is a cancer cell releasing growth factor and this growth factor binds to a receptor on the same cell inducing cell growth. And therefore, cancers cells are less dependent on growth factors from other normal sources.

5. Reducing cell- cell contact and cell-matrix adhesion:

Remember when we said that E-cadherin is reduced in cancer cells; so they become separated from each other. Along with reduced cell-matrix adhesion, this feature contributes to the ability of malignant cells to invade and metastasize.

6.Density dependent inhibition:

Animation:
http://sites.sinauer.com/cooper6e/animation1802.html

A primary distinction between cancer cells and normal cells is that normal cells display density-dependent inhibition of cell proliferation. Normal cells proliferate until they reach a finite cell density for example in an injury or a cut. This is determined in part to the availability of growth factors. The proliferation of most cancer cells, however, is not sensitive to density-dependent inhibition. They generally continue growing to high cell densities.

7. Contact inhibition:

Normal cells migrate until they make contact with a neighboring cell. Further cell migration is then inhibited, and normal cells adhere to each other. Tumor cells, in contrast, continue moving after contact with their neighbors, migrating over adjacent cells and growing in disordered manner. Cancer cells are characteristically insensitive to such contact inhibition of growth.

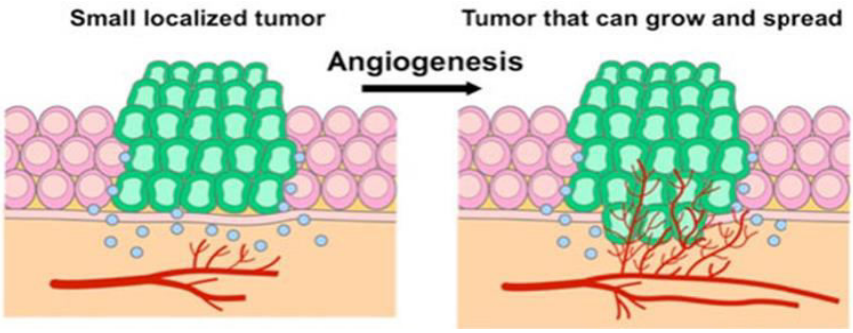
8. Invasiveness and extracellular proteolysis:

Tumor cells generally secrete proteases that degrade extracellular matrix components (basal lamina and connective tissue) allowing the cancer cells to invade adjacent normal tissues. And this plays important role in invasion and metastasis.

Animation about metastasis

http://sites.sinauer.com/cooper6e/animation1801.html

9. Angiogenesis:



Cancer cells secrete growth factors that promote the formation of new blood vessels (angiogenesis: angio- blood vessels and genesis-formation). New blood vessels are required to supply oxygen and nutrients to the proliferating tumor cells. The formation of such new blood vessels is important in supporting tumor growth and also in metastasis.

10. Lack of differentiation:

Another general characteristic of cancer cells is that they fail to differentiate normally and this is consistent with their continued active proliferation.

11.Loss of apoptotic capability:

As already known, there is a balance between cell death and cell proliferation in order to maintain constant population of cells. Many cancer cells fail to undergo apoptosis and therefore exhibit increased life spans compared to their normal counterparts. And this failure **by itself** contributes substantially to tumor development. For example, the survival of many normal cells is dependent on signals from growth factors that prevent apoptosis. In contrast, tumor

cells are often able to survive in absence of growth factors and this is important not only in primary tumor development but also in survival and growth of metastatic cells as well. (Inhibition of apoptosis is sufficient to cause tumors).

11. Cessation of senescence:

Senescence is the condition in which normal cells cease to divide and their metabolic activity is altered. This is due to the gradual loss of telomeres, as cells divide, leading to cessation of replication (senescence) or death. In contrast, cancer cells generally acquire the capability of unlimited replication as a result of expression of high levels of telomerase allowing them to divide endlessly ☺

Oncogenes and tumor suppressor genes (terminology) :P:

Oncogene: A gene capable of inducing one or more characteristics of cancer cells when activated.

Tumor suppressor gene : A gene whose inactivation leads to tumor development.

Proto-oncogene: normal cell gene that can be converted into an oncogene when mutated.

Viral Oncogenes

Oncoviruses are viruses that can cause cancer. They have oncogenes that are very similar to humans' oncogenes and are responsible for cell transformation. They have oncogenes such as: akt, ras, raf, src, and the Her2/ ErbB2-gene that is human epithelium growth factor receptor.

For example, the "rous" sarcoma virus RSV has the src oncogene which encodes a tyrosine kinase that phosphorylates many proteins inducing cell growth, and tumor formation.

The murinsarcoma virus has RAF oncogene that encodes a kinase that will activate the MAP kinase pathway and it will induce cell growth.

Any mutation or alteration in any of these genes will lead to cancer; the ligand (protein) itself might be mutated or we might have over production of the ligand or the growth factor. (Unregulated activity)

Oncogene proteins act as: growth factors, growth factor receptors, intracellular signaling molecules (transducers like raf, ras and G-proteins) and transcription factors and mutations can affect any of these proteins.

Examples of such mutations:

-Oncogenes and receptors: (Breast Cancer)

Mammary_breast epithelial cells have a receptor that is called Her-2 (Human epidermal growth factor receptor 2). It's the normal receptor that induces cell growth and it is limited in number in normal cells.

Development of breast cancer is associated with amplification or over expression of this oncogene on the cell surface. Furthermore, mutation in the active kinase domain of the receptor in the absence of receptor over expression can lead to cancer development by continuous signaling of growth and proliferation.

-Oncogenes and transducers: (Ras)

Ras is mutated in 95% of pancreatic cancer that is usually detected in terminal stages.

Ras activity is regulated by GAPs and GEFs. The ras oncogenes differ from their proto-oncogenes by point mutations resulting in single amino acid substitutions at critical positions, such as the substitution of valine for glycine at position 12 (Val instead of Gly). This will make ras very active transducer and always bound to GTP so it keeps on activating the MAP kinase pathway and RAF and inducing cell proliferation.

-Oncogenes and transcription factors: (Colorectal cancer)

There are a group of proteins called Wnt proteins that were identified as oncogenes. Mutations in these proteins frequently convert the downstream target of Wnt signaling, **β-catenin** to an oncogene in human colon cancers. These activating mutations stabilize β-catenin that stimulates transcription of target genes leading to unregulated cell proliferation. When there is no signal β-catenin (as a transcription factor) is usually degraded in normal cells. While it remains active in tumor cells even in the absence of a signal.

*β-catenin also links the receptors like-e-cadherins- to the actin cytoskeleton. (Plays a role in cell-cell adhesion besides being a transcription factor).

*Target genes include cell cycle proteins, oncogenes and proto-oncogenes.

-Oncogenes and cell survival:

Mutations can also occur in the pro-survival signaling pathway. (Already discussed)

If there was no induction of proliferation, failure of cancer cells to undergo apoptosis is enough to develop a tumor. Mutations in genes coding for PI3- kinase/Akt signaling pathway result in cell survival and cancer development.

-Oncogenes and cell differentiation:

Mutated forms of both the thyroid hormone receptor (ErbA) and the retinoic acid receptor (PML/RARα) act as oncogene proteins in human acute promyelocytic leukemia where the mutated oncogene receptors block cell differentiation and maintain the leukemic cells in an actively proliferating state. (Cells will keep on proliferating hoping to differentiate)

-Tumor suppressor genes and proliferation and survival:

Mutations can occur in tumor suppressor genes that represent the opposite side of cell growth control, normally acting to induce cell death or stop cell growth.

Example:

The tumor suppressor protein PTEN is a lipid phosphatase that dephosphorylates PIP3 into PIP2. It counters the action of the oncogenes PI 3-kinase and Akt (even if they weren't mutated- protooncogenes), which promote cell survival.

The rest of the sheet is done on section2 recording.

TSG and cell cycle:

Rb inhibits progression past the restriction point in G1. If RB is mutated (inactivated) by phosphorylation, Cdk4/cyclin complexes promote passage through the restriction point. Inactivation of the tumor suppressor gene Rb will trigger tumor development by the progression of cell cycle.

Role Of p53:

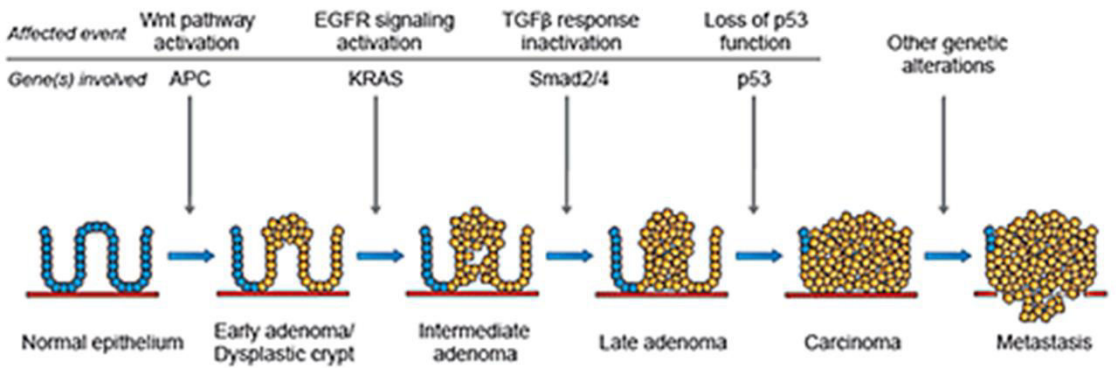
P53 blocks cell cycle progression in response to DNA damage. Loss of p53 prevents DNA damage-induced cell cycle arrest, leading to increased mutation frequencies and a general instability of the cell genome contributing to further alterations in oncogenes and tumor suppressor genes during tumor progression. If the DNA can't be repaired p53 promotes apoptosis.

A mechanism of Viral carcinogenesis

For example human papilloma virus has 2 proteins:

E6 > which stimulates the degradation of P53 by proteolysis.
E7> that binds to "retinoblastoma" and these are the mechanisms by which this virus causes cancer; by inhibiting the tumor suppressor genes specifically ☺

The multi-Step genetic model for the formation of colorectal cancer.



The role of multiple genetic defects is best understood in colon carcinomas which have been studied extensively by Bert Vogelstein.

Colorectal cancer is a progressive cancer, and by following some patients, Vogelstein made a multistep genetic model for colorectal cancer.

1-Inactivation of the APC gene in normal epithelial cells is an early event in tumor development. When APC is inactivated β-catenin will be released and it will act as a transcription factor for target genes that induce cell proliferation→ adenoma (benign tumor).

2- Mutations of rasK. then appear to occur. and they are frequently present in colon adenomas→ more growth of cells (intermediate adenoma).

3- Another mutation in a group of transcription factors arises.

4-Mutations of p53 are associated with later stages of tumor progression→ carcinoma.

THIS MODEL TELLS US : A colorectal cancer develops as a result of accumulation of mutations and these occur in different genes which might be oncogenes , transcription factors and tumor suppressor genes leading to metastatic tumors. ☺

So the most important thing when detecting this cancer is the presence of **rasK** mutation in particular, or receptor mutations and we give drugs accordingly.

One recent way to detect this cancer in very early stages even before noticing it by COLONOSCOPY is by a specialized tool- kit which depends on detection of genetic mutations in rasK in the cells that exist in the stool .

A future look ☺

The whole idea is about personalized medicine, which tends to treat these proteins-based diseases in a smart way depending on a person's genetic sequence and the markers found in it. So we must understand these proteins very well because they are drugs' targets since diseases are proteomics (protein-based).

PLEASE: don't forget to check the slides..

I'm sorry for this long long long sheet :/ but we were supposed to take these subjects in two lectures ☹

Good luck everyone ☺

Dedication to Ansam <3 for helping me with this sheet :D

Dedication to 5C :D

Special thanks for Hiba Mihyar for correcting it ^_^