PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE

Lecture Number: 4

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Apoptosis

There are several definitions for Apoptosis:

1- It is a programmed cell death.

2- It is a pathway of cell death that cells themselves act through their specific enzymes that degrade the cell's DNA and cytoplasmic proteins.

3- It's a genetically determined process of self-destruction.

4- It's a form of cell death in a programmed sequence of events that leads to elimination of cells without releasing any harmful substances .

NOW, what makes apoptosis different morphologically than necrosis?

- So in apoptosis the membrane will stay intact rather than having the plasma membrane burst and all of the cellular content is spilled out (as in necrosis), the plasma membrane (in apoptosis) buds off into little spheres that contain bits of proteins, organelles, cytoskeleton... etc, with the "eat me" signals on them (the spheres) and phagocytes come and clear these apoptotic bodies from the area.

- SO the membrane is still intact, but the structure have been altered, while in necrosis as we mentioned: the cell will burst and spill its contents to the outside.

- In necrosis there will be enzymatic digestion because the lysosomes will burst , and leakage to the outside will happen also

- Because the contents are released in apoptotic bodies that have the "eat me" signals specifically for phagocytes , and not just bursting to the outside , there will be no inflammation in apoptosis , where in necrosis we are going to have an inflammation due to the ‘foreign’ components which leak outside of the cell and are recognized and attacked by the phagocytes.

- Apoptosis is often physiological but not always, while necrosis is always pathologic.

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- An example of pathological apoptosis is viral hepatitis; this virus induces cell death through apoptosis.

- Another difference is that the cell size in apoptosis is reduced, while in necrosis the cell will swell up because the sodium pumps fails to do its function.

- The nucleus in apoptosis is fragmented into little pieces and is sent off by the apoptotic bodies: this is known as karyorrhexis , while in necrosis you can see all kinds of nuclear changes , one of them is karyolysis : where you no longer can see the DNA in the nucleus because it has been degraded (the DNA is stained with a basophilic dye - blue - so you cannot see the blue colour anymore) .

- The other nuclear change that happens in the case of necrosis is pyknosis , where the nucleus shrinks on itself , and then just because the nuclear membrane has ruptured the nucleus starts fragmenting into bits and pieces and in this case karyorrhexis can occur.

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation (round nucleosome)
Plasma membrane	Disrupted	Intact
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Pathologic	Physiologic and Pathologic

- So rather than being a result of a programmable pathway, it happens because the nuclear membrane has ruptured, so it's not programmed, it's more messy.

- So karyorrhexis can occur in both apoptosis and necrosis, but it's programmed in apoptosis.

Causes & Mechanisms of Apoptosis

- The first example is during embryogenesis : essentially you are creating new organs, some cells grow and some cells die in a programmed manner without inducing inflammatory responses.

- Another example of physiological apoptosis is the menstrual cycle (at the end of the menstrual cycle) in which the endometrial wall begins to shed due to hormonal drops.

- Also during lactation , the breast glands (mammary glands) increase in size so they are proliferating and producing cells , so what's going to happen to these cells after withdrawing is that they will shrink off and eventually they will die (so atrophy is going to occur)

- The doctor gave an example of tissues that are exposed to wear and tear which is the GI tract : in the colon there are always cells that reach a point in their cycle where they have been exposed to enough wear and tear that we don't need them anymore , they die off by apoptosis and they are replaced by new cells , so in many cases apoptosis maintains a steady state population of cells .

- During an infection, inflammatory cells proliferate to get rid of the infection, but after we get rid of it we don't need the WBCs any more so they die off by apoptosis. This is how apoptosis stops a function that was useful at a time and it's not useful any more. (End of life apoptosis)

- Self reacting lymphocytes:

During an inflammation , genes of the immune-system (especially the ones that recognize antigens) are randomly created (antibodies.. etc) , the result is a wide range of receptors (or antibodies) that can identify specific antigens , now if that cell is recognized as being self-reactive , meaning that its reacting while other cells around it are not active , this cell will be recognized as abnormal and its going to be induced to die off.

So self reacting lymphocytes are produced normally, they are recognized and killed off.

- As we mentioned last time : DNA could be damaged due to radiation and ROS , this could send the cells either to apoptosis or necrosis (if the damage was severe enough) .

- Some infections and viruses can induce apoptosis by mimicking a protein in the apoptotic pathway.

- HIV depresses some WBCs by inducing apoptosis.

- Cyto-toxic T cells:

These are T-cells that recognize other cells that are infected with virus or abnormal cells (like cancer cells) , these cells can induce death to these cells through apoptosis , BUT some viruses inhibits the apoptosis by producing proteins that inhibits apoptosis so they can replicate in the cell and eventually cause cell lysis .

- So viruses can induce or inhibit apoptosis.

Also there are certain organs when their ducts are blocked the response is atrophy , for example : pancreas , parotid and kidneys , these are the main examples of pure apoptosis , the rest of the organs are going to be a mix of necrosis and apoptosis .

- For example when the gall bladder is obstructed (cholisis titis) or the liver is obstructed (hepatitis) this will usually cause necrosis , where as for some reason in the pancreas , kidneys and the parotid once they are obstructed they mostly respond by apoptosis . ' the doctor didn't mention the reason J '

The Major Mechanisms of Apoptosis:

1- The mitochondrial (intrinsic) pathway

2- The death receptor (extrinsic) pathway

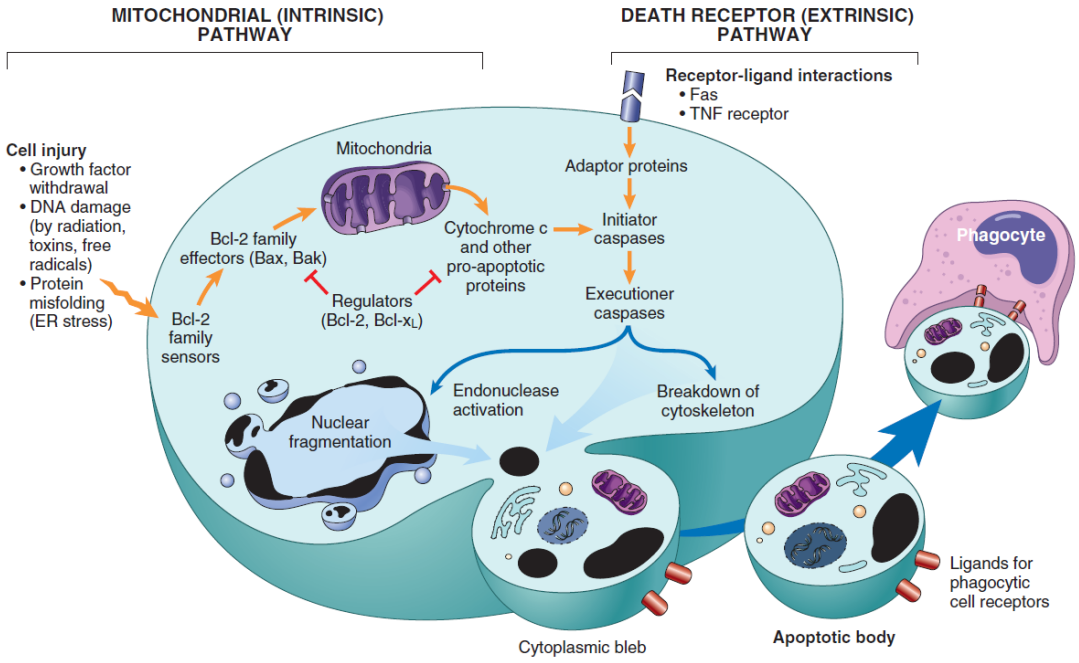
- The following is an introduction for both pathways then each one will be elaborated separately in details J

In the case of intrinsic pathway, if there's growth factor withdrawal or DNA damage or protein misfolding , we are going to get signals towards the bcl-2 family sensors called the BH3 proteins , that signal to the mitochondria through the bcl-2 family effectors BAX and BAK to release pro-apoptotic proteins such as cytochrome C , NOW when these proteins are released (the pro-apoptotic) they are going to activate initiator caspases (which are cystine proteases that cleave the protein after aspartic residues)

The executioner caspases are the caspases that come second in line, they are activated by a cleavage after their aspartic residue, when they are activated they activate a lot of downstream targets like: endonuleases and other proteases that break down the cytoskeleton.

Now also part of this pathway is the extrinsic pathway: rather than signaling through the mitochondria, they signal directly into caspases, BUT the end result is the same è we turn on the specific proteases and endonucleases to break up the organelles and the cell and eventually producing these apoptotic bodies.

Now let's discuss each one in detail J



The mitochondrial (intrinsic) pathway:

So as we said the cell should receive hormones and signals that indicate that this cell is okay and still living , so when it receives these signals the cell will produce anti-apoptotic proteins which are bcl-2 and bcl-XL , these proteins insert in the membrane of the mitochondria, and they inhibit BAX and BAK from being opened, BAX and BAK are membrane proteins in the mitochondria (we will talk about them in this sheet J) , once they are opened there will be a leakage of cytochrome C and other

pro-apoptotic proteins , so essentially bcl-2 and bcl-x will bind to BAX and BAK and inhibit their function.

NOW , if you withdraw these signals or by other factors like damage of DNA or if you have accumulation of misfolded proteins , there are sensors for stopping the signal or DNA damage or misfolded proteins , these sensors are called BH3 proteins , once they are activated they antagonise bcl-2 , they separate bcl-2 from BAX and BAK and they bind bcl-2 so it's no longer active , and also this will reduce the expression of bcl-2 and bcl-XL in some way, which means that BAX and BAK are ready to be opened , also BH3 stimulates BAX and BAK to open further , and the result is as you know is leakage of cytochrome c and other proteins , then caspases will be activated and apoptosis will occur .

There are more than 20 proteins that control mitochondrial permeability besides the ones we mentioned earlier.

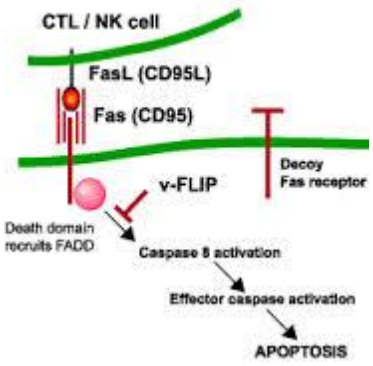
- This is the way that is responsible for apoptosis in most situations

The death receptor (extrinsic) pathway:

- The less common pathway

There are two receptors that participate in this pathway , which are FAS and the TNF type 1 receptors (we will talk about TNF at the end of the sheet).

FAS (CD95) is a part of the TNF receptor family , on its own it can do nothing, it needs a ligand which is FAS-L , which is expressed on the membrane of certain T lymphocytes , the receptor now oligomerizes into 3, comining to the death domain (in the cytosolic side of FAS) , now the death domain recruits FADD protein (fas associated death domain) that then recruits pro-caspase 8 (is caspase 8 that haven't been cleaved yet) , once we activate caspase 8 it activates the executioner caspases , and induces apoptosis .



Caspase 8 can activate BH3 receptors which we discussed in the intrinsic pathway, so if you are signalling the extrinsic pathway , you may activate the intrinsic pathway, so they are connected somehow .

Some viruses inhibit the process of apoptosis by mimicking FLIP protein (a protein produced by some cells and interferes with caspase 8 activation through pro-capase 8 to caspase 8) , we kill virus infected cells by cytotoxic T lymphocytes through inducing apoptosis but these viruses are inhibiting apoptosis !!

- Now this pathway is responsible for getting rid of the self-reactive T lymphocytes , and also for getting rid of target cells by cytotoxic T lymphocyte : tumour cells , viral infected cells

Imagine if there was a mutation in FAS or FASL and we require this pathway to get rid of self reactive lymphocytes, then we cannot get rid of them and they will accumulate and this will lead eventually to auto-immune disease.

Now you know what initiator caspases is, well we have 2 kinds of caspases you should memorize which are 8 in the EXTRENSIC PATHWAY and 9 in the INTRINSIC PATHWAY, and the executer caspases are 3 and 6.

So what causes these apoptotic bodies to be so attracted to phagocytes?

1- Phosphatidylserine is flipped to the outer portion of the membrane (usually its in the inner portion of the membrane) è this is not normal , it's a signal

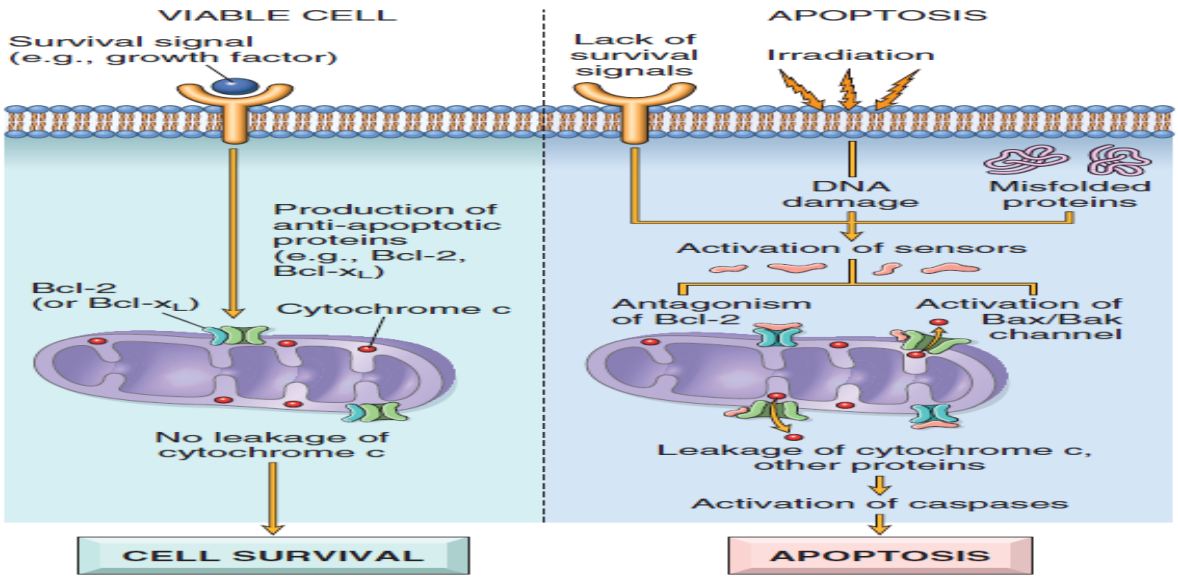
2- Glycoprotein: certain carbohydrate chains are signals for phagocytes for "come eat me"

3- Complement antibodies, they attach to the apoptotic bodies as signals, and are recognized by phagocytes

4- The apoptotic bodies can produce soluble factors that attach to apoptotic bodies that act as "bread crumbs" so the phagocytes can follow these factors to reach the apoptotic bodies eventually.

*Note: Phagocytes also release their own soluble factors which attach to the apoptotic bodies aiding in its final function to consume the body.

Practical examples where apoptosis occurs:



1- ^ this is an example we have talked about, which is the growth factor deprivation

Here the doctor just repeated the bcl-2 thing again :)

2- Hormone sensitive cells: like breast, uterus

3- Lymphocytes: we always produce lymphocytes, if they have never been stimulated and they don't have a specific antibody to recognize an antigen , at some point when they get older and we don't need them anymore , they die by apoptosis

4- Neurons deprived of nerve growth factors will die off

DNA damage

We want to understand how the DNA damage causes apoptosis.

Now DNA damage is not gonna always cause apoptosis , it depends on how severe the DNA damage is , normally when the DNA is damaged the cell will try to repair it , NOW in order to allow the cell to repair its DNA it has to arrest at the G1 phase , before the S phase , because DNA replication occur in the S phase , and we don't want to replicate a damaged DNA , so what will happen is : P53 which is a sensor of DNA damage , when p53 is expressed after DNA damage , it stops the cell from going to the S phase , it gives the cell enough time to repair the damage .

If the damage is still there , and the cell is not able to repair it , or if the damage is so extensive that the cell does not have time to repair it , and the p53 is accumulated in certain amounts in the nucleus , THEN ACTIVATES THE SENSORS .

So if the damage is severe enough or prolonged enough, p53 activates BH3 sensors, it can also act as a transcription factor for the sensors and bak and bax , so you are increasing the proteins that activate the pathway .

If the p53 is mutated CANCER is gonna occur , because you don't have sensors that sense the DNA damage

*Note: Some proteins, during post-translational modification have a little tag on them called 'nuclear localization sequence' , and these proteins are then transferred back into the nucleus to perform their actions. On the other hand some other proteins have tags on them called 'nuclear export sequence' it has the opposite effect to what was previously mentioned. P53 works in the nucleus and thus has a nuclear localization sequence. (not important just for general info)

Chaperones

- Is a protein that helps other protein to fold correctly.

- It's present around the ER to help folding the protein correctly

NOW , if you have metabolic alterations , like : a reduction energy stores , genetic mutations that affect the protein structure , genetic mutations that affect the chaperons themselves , viral infections , chemical insults or aging , you will start to get a loss of this balance between the production of proteins and properly folding them , so you are now going to produce a lot of proteins that are not correctly folded , if this happens , the cell will try first to adapt , by inhibiting protein synthesis , and starts expressing more chaperones , so it tries to catch up .

If the cell fails to adapt, there is gonna be more protein folding demand than the protein folding capacity, so we will get the "ER STRESS RESPONSE"

If we cannot fold the proteins properly, or the ubiquiton proteasome pathway cannot keep up with the amount of misfolded proteins , you

gonna get accumulation of these unfolded proteins , so after that you are going to induce the ER stress , and ACTIVATE APOPTOSIS .

- Ubiquiton proteosome pathway is important in atrophy

- Alzheimer's mechanism that has been proposed (one of the causes) is the accumulation of these misfolded proteins that leads to induction of apoptosis , and these neuron cells die off , when a number of these cells die off , we start getting cognitive impairments .

Cyto-toxic T-lymphocytes

They are the cells that can recognize abnormal cells, we said that they can activate FAS, and activating the extrensic pathway, BUT that's not their main mechanism, they produce ' granzymes ' that can bypass the whole extrinsic pathway and directly activate the executioner caspases.

So these granzymes cleaves proteins at aspartic residue , so they do the same as caspases do.

Necroptosis (not in small Robbins)

We will talk about the TNF receptors J-

So TNF receptors are common in both the extrinsic pathway and the necroptosis .-

TNF receptors (TNFR 1) binds TNF and this complex (RIP) , which is the receptor associated kinase , recruits caspase 8 è so extrinsic pathway , but if we have failure in activating caspase 8 , this same complex is now called the necrosome , and it induces metabolic alteration in the mitochondria that reduce ATP , and increase the production of ROS .

Now necrosis and apoptosis can co-exist in the same pathway.

One of the viruses that encode caspase inhibitors is CMV virus

Necroptosis is important in acute pancreatitis, reperfusion injury,

Also in Parkinson's disease you can observe necroptosis in substantia nigra.

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Sometimes on the way to your dream, you get lost and find a better one. ¬