PATHOLOGY

◉ Sheets ○ Slides

Number: 3

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Subject: Cell Injury & Death

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The Doctor asked about the major differences between Apoptosis and Necrosis , As a review of the previous lecture :

APOPTOSIS	NECROSIS
1- Organized	messy
2- No inflammation	elicits local inflammation
3- Keeps membrane integrity	membrane integrity is compromised
4- Shrinkage of the cell	increasing the size of the cell (swelling)

- Necrosis → loss of membrane integrity → leakage of proteins to the outside → these proteins have never been seen by WBCs → These WBCs attack the proteins and a state of inflammation occurs.

Apoptosis → the membranes remain intact → no leakage of cellular contents → no inflammation.

- Why there is a swelling (as a part of early response) ?
  ATP depletion → Failure of ion pumps → sodium and calcium will enter the cell causing a net movement of fluids to the interior of the cell → cell swelling.

  - this net movement of water = iso-osmotic gain of water.
  - pump → it must be ATP-dependent by definition of a pump.

- Chromatic Clumping :
  -occurs during cell injury .
  Why ?

Because the PH changes...so when

- When cells lack oxygen (either because of hypoxia or ischemia),they will undergo glycolysis (occurs without oxygen) , and it will result in the formation of pyruvate.
  BUT
  when it comes to Krebs cycle → no oxygen → no oxidative phosphorylation → less ATP
  So
  In order to compensate , the cell produces ATP by anaerobic respiration (Lactic acid fermentation) → Accumulation of lactic acid → pH decrease

  pH decrease is associated with 3 events :
  a- chromatin clumping.
  b- inefficiency in the work of cytoplasmic enzymes , because their optimal pH is nearly 7.
  c- allowing lysosomal enzymes to work efficiently in the cytoplasm after lysosomal membrane damage.

{those were the early stages of necrosis “when it’s still reversible cell injury “ , the late stages occur mainly due to mitochondrial damage and membrane damage}

- So, the membrane gets compromised → leakage of cellular contents → these contents when they are outside the cell , they cause inflammation .
- What happens when we got damaged mitochondria ?
  there will be further depletion of ATP , because they won't be able to produce ATP.

ATP depletion :
- Failure of ion pumps .
- Detachment of ribosomes from the ER and dissociation of polysomes → reduced protein synthesis.
* These ribosomes are attached to the ER when there’s enough energy to synthesize proteins → ATP depletion will cause detachment.

Causes of cell injury :

A-**Hypoxia** ; oxygen deficiency\ Ischemia : loss of blood supply (blood carries oxygen).

*So , hypoxia and ischemia are not the same.
- ischemia is the main cause of hypoxia .

- **non-ischemic hypoxia**:
1- pulmonary problems.

2- carbon monoxide poisoning ;

Because CO will bind to hemoglobin with higher affinity compared to O_2 .

3- anemia .

4- high altitude (if you are climbing Mount Everest for example , you tissues will face hypoxia without having ischemic problems).
- At high altitudes , we have a low partial pressure of oxygen.

B- **other physical causes of damage** (Trauma , radiation , temperature)

*chemical toxins (Water , glucose , sugar , salts).

- All substances can be toxic under certain conditions.

*nutritional defects : vitamin deficiency , protein deficiency ... etc . .

C- **Infections** can cause direct damage to your cells whether it was because of bacteria ,fungi, worms...

The question is : what do our bodies do to overcome these infections ??

(Inflammation)

Note:the inflammatory cells are recruited from the blood to be directed to the infectious agents as well as other normal tissues(that are not necessarily involved in the infection)..... by what do we call it ?? >>> (collateral damage).

- But what will happen if our immune system is activated even if there wasn't any infection (in the case of auto-immune diseases)>>>>> not only collateral damage but also targeting your own cells because your immune system is targeted .
  - Diabetes type 1 and rheumatoid arthritis → auto-immune diseases.

D- **Aging**: is the number one most accurate diagnosis in all diseases , and it's always 100%

(anybody here is not going to die?? Aging is the number one cause of human fertility,as you grow older you are more exposed to diseases :

1- You are more likely to get mutations.
2- Your WBCs are not active as they used to be.
3- Your stem cells are not regenerating as they used to be .
4- You are more sensitive to environmental problems.
5- You're not healing completely as you used to .

*(so cellular aging can cause cellular damage)

10:00

E- **Genetic defects**: some autoimmune diseases can be generated by genetic cause that cause damage ,deficiency in synthesis of vital proteins ,or eliminate vital proteins ...etc.

- Remember we talked about adaptation if the stress is mild or transient , if it was prolonged or severe the cell is no longer going to adapt and cell injury develops .

  The response is not dependent on the injury itself only but also the cell type.

- Example: ischemia causes injury to two different cells and the result is two different response ; ex: liver cells can resist ischemia for a longer time than cardiac muscle cells or brain cells can (because hepatocytes have a source of nutrients which is glycogen stores (high glycolytic activity) while brain or heart cells can't resist ischemia for a long time because they don't have any source of nutrients other than the blood ...

  - Ischemia is more dangerous than non-ischemic hypoxia . Why ?
  Ischemia → lack of oxygen and nutrients (no oxygen + no glucose) .
  Hypoxia → lack of oxygen only → so the cell can produce ATP by glycolysis.

Note:skeletal muscle can resist ischemia for a longer time than the heart can .

Brain → 5-6 min
Heart → 20-30 min
Skeletal muscle → 2-3 hrs

Note : Why does the liver need to break down glycogen in order to generate ATP during hypoxia ?
Aerobic respiration → one glucose molecule is needed to produce 32-34 molecules of ATP .
Glycolysis → one glucose molecule produces much less ATP molecules. → more glucose molecules are needed to have the same amount of ATP → hepatocytes have to break down glycogen.

* ***Genetic variations (polymorphism): Cytochrome P-450***

- Present in the SER of liver cells
- Responsible for metabolism and detoxification of drugs and toxins

Genetic variation of cytochrome P- 450 will lead to different rates of metabolism → different outcomes.

*There are chemicals (not toxins) that go through a particular type of cytochrome P-450 and they become toxins → those people with this particular cytochrome P-450 have **(TOXIC SUSCEPTIBILITY).**

- **Question 1: If there is a polymorphism is the cytochrome P-450 that makes it over-active , are people having this type less or more susceptible compared to a normal person ??**
- **They are less susceptible → you are eliminating the toxins faster .**
- **Question 2: If you give a patient a drug that is not a toxin and it's metabolized by the cytochrome P-450 system >> then it becomes a toxin (toxic intermediates) .**

What do we say?? Does this type of P-450 make the patient less or more susceptible ?

(more susceptible) .

**** Adaptibilty : how capable the cell is to adapt***

- Someone with a normal heart and another with hypertrophic heart → who is going to adapt for a longer period of time ??
- The one with the normal heart ,of course, because hypertrophic heart is beating faster and stronger , there is a lack of oxygen , lack of nutrients (glucose)>> so if you take away any of the remaining oxygen and nutrients >> it's more likely to be damaged compared to a normal heart >>

- ❖ Although , they are exposed to the same hypoxic environment, the diseased heart has gone to the edge of adaptation capability while the normal heart still has the ability to adapt .
  Summary : a diseased heart is going to die faster than a normal one.

- ***Mechanisms of cell injury*** have been divided into 4 major categories:
- But all of these mechanisms are interconnected and tightly interwoven with many intracellular metabolic pathways.

Mitochondrial damage (your powerhouse)

No mitochondria → ***no ATP*** ...
1- ion pumps stop working .

2- we can't produce proteins that are essential

3- we can't produce lipids that are essential for our membranes >> membranes are broken down ...etc.

Note: Normally , through oxidative phosphorylation inside the mitochondria , the electron transport chain is not perfect ??! It produces by-products that are toxic → ROS.

Note : ROS have some physiological roles within the cell in certain signalling pathways and that's why they are produced normally during cellular respiration.
- There are many ways to get rid of these ROS . Why ?
to avoid their harmful effects that are associated with high concentrations of ROS.

- ***ROS*** : reactive oxygen species (oxygen free radicals)
- Free radicals : they have unpaired electrons in their outer orbitals >> extremely unstable and very very reactive >>they will react with any organic or inorganic substances >>they have the ability to producemore free radicals from the substances they react with >> so you are creating a ***chain of damage*** .
- So, damaging in the mitochondria will not result only in depletion of ATP but also producing more ROS that are damaging agents in the cell.

  - ❖ ***Entry of Ca++*** :it's also related to mitochondrial damage >> no ATP>>Ca++ pumps will not work >> influx of Ca++>> activating multiple cellular enzymes that are actually breaking down the cell.
  - ❖ ***(phospholipases\endonuclease\ATPases*** ....)
  - ❖ ***When ATP is already low → Calcium influx → activating ATPase → more depletion of ATP***.

- ***Ca++ also adjusts mitochondrial permeability .***

❖ Mitochondria uses the gradient of the protons(H+) to drive the production of ATP >> an increase in mitochondrial permeability (because of influx of Ca++)>> this is going to ruin this gradient >> and mitochondria is no longer producing ATP as they should even if they have oxygen.
(so Ca++ is interrelated to mitochondria)

Membrane damage

- Again, no ATP → reduced synthesis of lipids →loss of membrane integrity → breaking down of the membranes (plasma membrane and lysosomal membranes)

- Mitochondria are also membrane bound organelles >> so damaging of mitochondrial membranes >> more depletion of ATP.

- So,again they are very interrelated .

Protein misfolding and DNA damage : mostly induces APOPTOSIS.

Note; Necrosis can coexist with Apoptosis (we will talk about it later on)

❖ Actually, the major pathway of Apoptosis is induced through the mitochondria (intrinsic pathway).

*the doctor said that the slide with the **mitochondria** headline reviews everything we were talking about a minute ago .

Note: glycolysis as a compensatory for the loss of ATP→ production of a few molecules of ATP for each glucose molecule.


20:00

- Damage of the mitochondria will increase the production of ROS .
- If you understand the 1st two slides .. the topic is done .

Low survival signals , protein and DNA damage

- Changing the permeability of the mitochondria not only to protons but also to other proteins such as : ***cytochrome C*** (which induces pro-apoptotic proteins but inhibits Anti-apoptotic proteins).
- And then inducing APOPTOSIS (**is a balance between pro-apoptotic proteins and anti-apoptotic proteins)>>** if you push the balance toward one of the ends (pro-apoptotic proteins) you will end up with ***apoptosis*** , if you push toward the other end (anti-apoptotic proteins)you will end up with ***cell survival*** .

Again ; calcium is maintained at a much higher concentration on the outside with regard to the inside (there are two other sources for intracellular calcium concentration >>(1- mitochondria 2- endoplasmic reticulum).

- Entry of calcium , or released from intracellular stores >> activation a lot of enzymes (**ase**s) >> they require ATP >> we already have depletion of ATP >>> and these enzymes will consume more of the ATP >> pushing the cell further down the necrotic pathway .
- Activating these enzymes (phospholipases and protease) will damage the membrane and the ***cytoskeleton*** (which actually maintain the membrane integrity and the shape of the cell.

Endonucleases *: they are damaging DNA and by this we will not synthesize the desired proteins (no vital proteins >> cell death)*

- Ca++ that changes the permeability also attacks the ***mitochondrial permeability transition pores*** >>changes in concentration gradient >> so the mitochondria that were not able to produce ATP are now further unable to produce ATP.

- Experiment : two Petri dishes ... the 1st one with high extracellular Ca++ (has a calcium in it)and the 2nd one didn't has a Ca++ in it .

- If we add a stimulus to these two dishes >>the cells with less matrix Ca conc. are much more resistant than the normal cells (with high extracellular Ca conc.)>>this will lead us to the fact that : ***leakage of Ca from the intracellular stores don't do as much damage as the the ECM. influx of Ca from***

FREE RADICALS (ROS)

They are produced normally by oxidative phosphorylation in small amounts ,and we can get rid of theses amount by :

1- Non-enzymatic systems: ex: antioxidants (vitamins) / beta-carotene

2- enzymatic systems :{catalase \superoxide dismutase\ glutathione peroxidase}

Actually**, these enzymes need ATP too**.

(**damaging mitochondria ... not producing ATP ... induce production of ROS ... no enough energy to activate enzymes that are functioning to get rid of ROS**...we will get accumulation of these ROS >> causing lipid problems , membrane damage)

Oxidative stress : the presence of excess free radicals .
This occurs when :
a- the production of ROS increase .
b- the removal of ROS decreases. (the scavenging systems are ineffective : no vitamins , enzymes are not working due to ATP depletion)

- Membrane damage >>leakage of contents >> induces inflammation >> necrosis.
- DNA damage leads to apoptosis. (pathological in this case)

*some cells are producing oxygen free radicals on purpose (leukocytes , neutrophils , macrophages)WBCs\ through a process called (oxidative burst).

- Leukocytes contain enzyme is called (***myeloperoxidase)***that actually converts these oxygen free radicals into (***hypochlorite... HOCL)***
- **Hypochlorite** :is the major component of bleach >>so neutrophils are actually producing **bleach** in order to get rid of any invading microorganism .

- Again (for the thousand time),mitochondrial damage>> no ATP >>*no energy to produce phospholipids* and this is accompanied with increased *production of ROS* (because of mitochondrial damage) which cause ***lipid peroxidation*** >>and Ca influx *cause activation of phospholipases* which break down lipids >> and all these three factors lead to *phospholipid loss*.
- And the result of breaking down is **LIPID BREAKDOWN PRODUCTS** >> they are going to liquefy the membrane >> changing the permeability or the viscosity>>that mean that we are damaging the membrane .

  Calcium also activates proteases that damage the cytoskeleton>>so now the membranes are not only compromising it's content but also the underlying structure .

  So damaging the mitochondria >> damaging the plasma membrane and lysosomal membrane ,,, so all the way back to mitochondria.

In practice what does this mean :

30:00

* If you have hypoxia or ischemia , your mitochondria is going to be damaged , producing less ATP , exchanging oxidative phosphorylation with glycolysis >>your chromatin will clump , you will not be able to activate sodium pumps >> swelling of the cell , loss of microvillli (all these are still reversible (early) >> in green).

- Massive influx of Ca >>affecting mitochondrial permeability more and affecting cellular enzymes >> accumulating even more ROS>>more injury to mitochondrial memebrane >> irreversible injury (red).

**WBCs: white blood cells.*

Reperfusion injury : the injury the result from re-supplying the ischemic tissue with blood .

- In ischemic injury there will be an inflammation , when reperfusion ; it's going to make **more inflammation**! But why??
- As a part of our immune system , **complements** are going to stick -inappropriately- to the ischemic tissue , and WBCs are going to recognise these complements and this is a signal that this tissue is foreign or pathogen >> stimulating more WBCs and more inflammation .
- While cutting off the blood supply there will be less WBCs , and reperfusion will cause the area to stuck with WBCs and the inflammation get worse .

- ***Another thing that is done by reperfusion***.

*plasma contain electrolytes (ex:Ca++), and plasma is extracellular .

*now, the cell is going through necrotic pathway but still not necrotic cell yet , and this cell – as you know- has a lot of Ca++>>so what happen when resupplying it with blood ??

It's just like opening the tap (recruiting the plasma with its Ca conc.) by reperfusion >> more Ca++>>more injury (irreversible)>> and now it's necrotic cell .

*Note: reperfusion within certain limits of injury can be beneficial , but when mitochondria is already damaged , reperfusion causes disastrous effect .(this is important in the cardiac cells or brain cells(strokes)>>when the mitochondria is already damaged , reperfusion causes excessive production of ROS.

*Note:when reperfusion to transplant organ (in ice),, it's important to reperfuse gradually .

- mechanisms of ischemic-reperfusion injury (ROS+ Calcium + inflammation).

- ***Direct***: 1- cyanide (CN):it can affect all the cells in your body ;because it blocks oxidative phosphorylation and electron transport chain >> so even if there is a little bit of oxygen , you will die of hypoxia.
- 2- mercury chloride (seafood toxin\a common toxin): rather than affecting all the cells in our body , it affects the cells that absorb , use , excrete , concentrate these toxins (kidney and GI{intestines}).

- ***Indirect:*** needs to get metabolized in the liver first (ex;***1- CCL4)***

*CCL4:widely used in dry-cleaning but now they stopped because of its bad effect.

*CCL4 → when it goes through cytochrome P-450 ; it will be converted into CCL3 (with an electron in its outer orbital) >> a free radical >> free radicals damage membranes (mitochondrial, lysosomal, plasma) >> less ATP >> not producing essential proteins .

*one of these proteins (***apoproteins***.....responsible for transporting lipids from the liver >>so when the liver is not exporting fat >> it will accumulate in the liver cells (hepatocytes) >> and cause fatty liver damage + necrosis.

- ***2- acetaminophen = panadol*** =paracetamol
  - This is not contained in the book → stick to what's written in the slides.
- Some people take high dose (as they try to commit suicide) then acetaminophen will over power the conjugation systems- that normally get rid of its toxic effect –
  And start getting detoxified by the cytochrome P-450 >> the intermediate of this detoxification process is a toxic metabolites
  - The only way to get rid of them is through conjugation in the presence of **Glutathione** .
  - We all have a source of glutathione ;however, if we take a dose that is big enough to consume these stores >> we are going to have that damage.
  - Some people have less stores than others ?? who are they ??
  - Alcoholics : they are more susceptible to paracetamol overdose than a normal person.
  - How can we replenish the glutathione ?? by giving them (acetyl-cysteine)
  - Why it's chronically relevant ?? readily available , over the counter , some guys they are stupid enough to take a full bottle of paracetamol for some psychiatric reasons ;) .

*{sorry for any mistakes ,, forgive me for the long interconnected concepts ; I was just trying to make you understand all the details the doctor had mentioned in the lecture} .