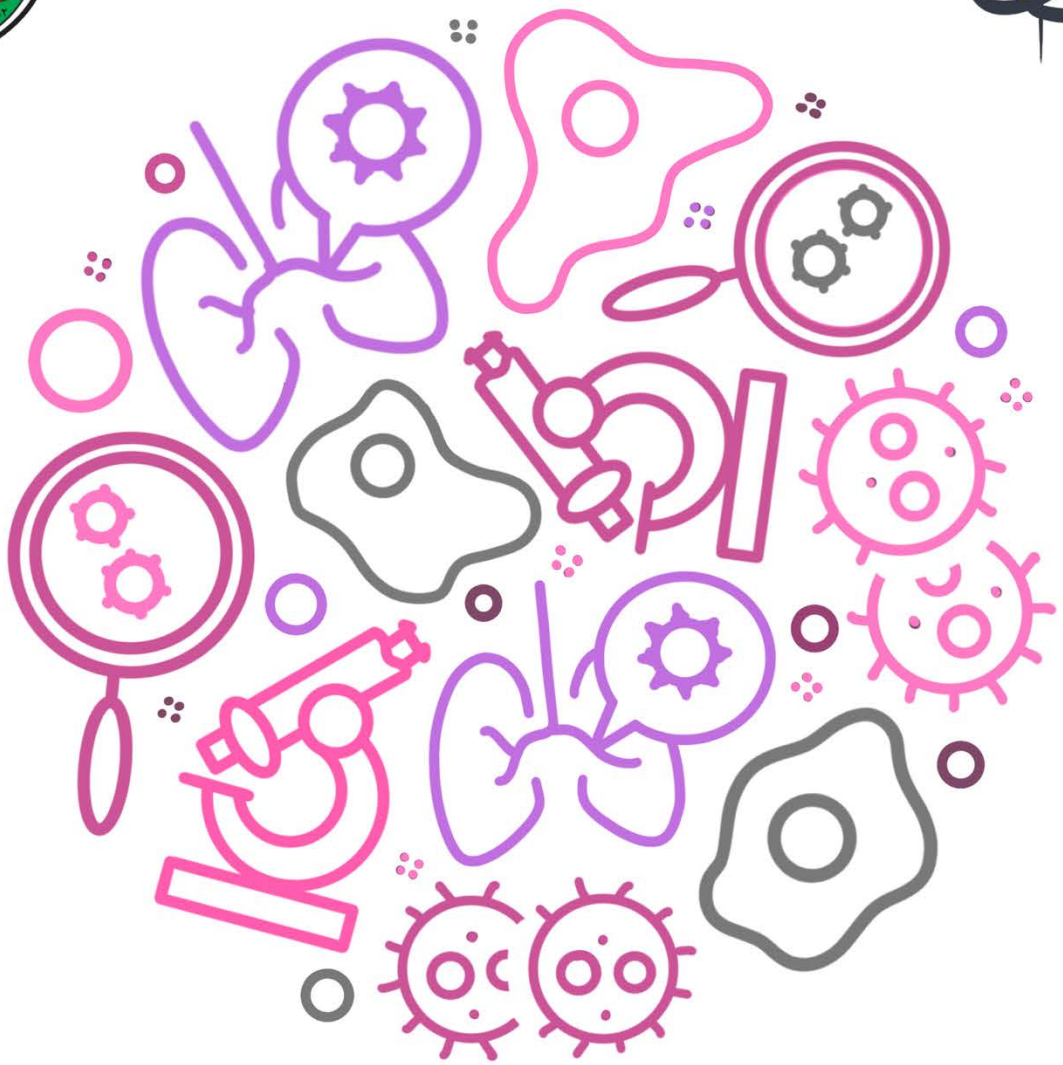
SHEET NO. 3



PATHOLOGY

DOCTOR 2019 | MEDICINE | JU

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بـسم الله الـرّحمن الـرّحيم

During the last two lectures, we have talked about the adaptive mechanisms in response to stress, reversible and irreversible cell injury, and causes of cell injury in general.
In this lecture, we will talk about the mechanisms, and the molecular basis of cell injury.

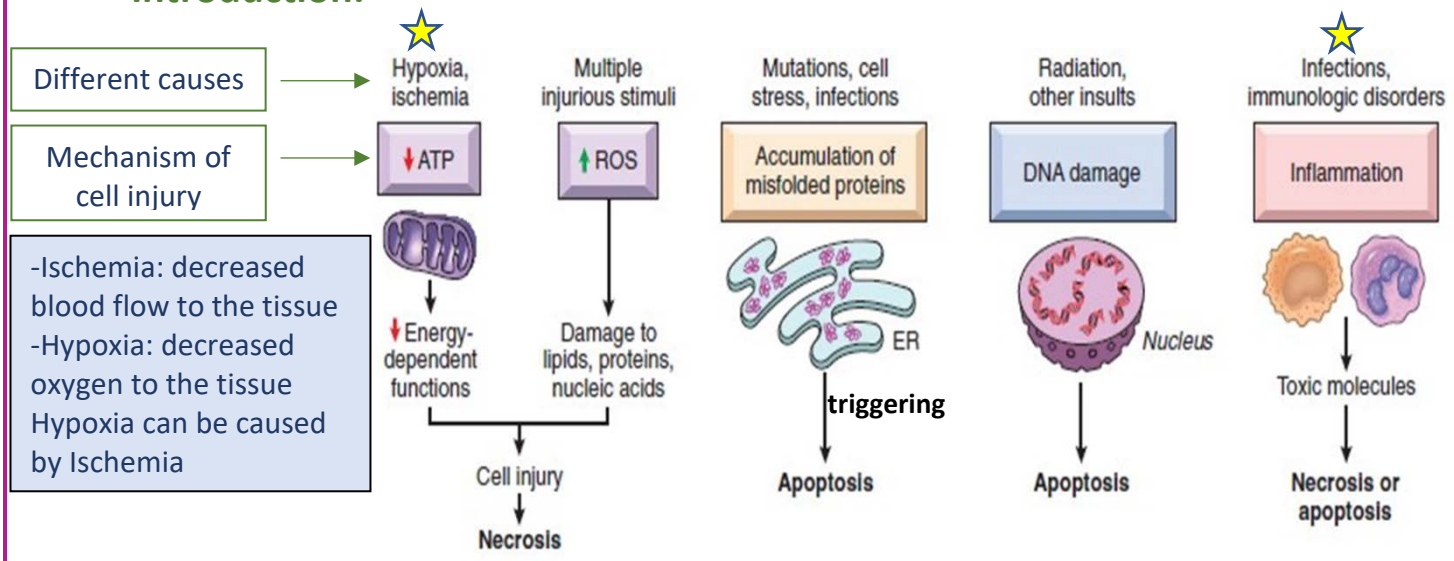
Cell injury disrupts cellular homeostasis. Cells are injured by numerous and diverse causes (etiologic agents) from intrinsic and extrinsic sources; however, all of these causes, activate one or more biochemical mechanisms that may act together at the same time cooperating in the same scenario leading to cell injury.

Principles of cellular response, and consequences regarding injurious stimulus:

- The cellular response to injury depends on:
  1. The type of injury.
  2. Its duration.
  3. Its severity. Ex: low doses of toxins or a brief duration of ischemia may lead to reversible cell injury, whereas larger doses of toxins or longer ischemic intervals may result in irreversible injury and cell death.
- The consequences of an injurious stimulus depend on:
  1. The type of the cell, Ex: a skeletal muscle accommodates ischemia for **2-3 hours** without irreversible injury/necrosis, whereas cardiac muscle **dies** after only **20-30 minutes**, also, in the brain an ischemia for **2 minutes** is enough to cause death.
  2. Its status, meaning that whether a cell is diseased from the beginning or normal looking, determines the injury consequences.
  3. Adaptability, and **genetic makeup of the injured cell.**
     For a period of hypoxia, some tissues can adapt, and some are highly susceptible to it.
     Sometimes cells among different individuals may develop different consequences to the same toxin/drug, ex: when exposed to a toxin, individuals who inherit differences in genes encoding cytochrome P-450 in the liver, may catabolize the toxin at different rates, some could respond exaggeratedly and some respond by the minimal effect. And this is the definition of **Pharmacogenomics.**

So, Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components

Introduction:



A brief discussion about the figure above:

Several causes can lead to the same mechanism of cell injury, ex: Hypoxia lead to ATP depletion (since oxygen is needed in ATP generation in mitochondria) then, every energy-dependent function, like protein synthesis, plasma membrane pumps, will be decreased, leading to necrosis in the end.

Also, Immunologic disorders (ex: allergy, autoimmune disease), cause inflammation to the cell, by the high accumulation of leukocytes, generating toxic molecules, and leading to necrosis "mainly" and Apoptosis "some".

-The stimuli of cell injury with the mechanism of cell injury determine whether the cell will die by Apoptosis or by Necrosis.

Mechanisms of Cell injury

First event: Hypoxia and Ischemia

-As we all know, oxygen is needed to all cells in producing energy in the form of ATP, by oxidative phosphorylation which occurs in the mitochondria.

-So, Hypoxia due to ischemia (which is the most common cause) or any other cause of reduction in oxygen supply to the cell → leads to Defective oxidative phosphorylation → failure of ATP generation → depletion of ATP in cells.

-Depletion of energy source (ATP) in the cell causes failure of energy-dependent pathways **such as**; plasma membrane transportation (Na+/K+ pump), protein synthesis in ribosomes, lipogenesis (production of lipids) and phospholipid turnover (degradation of phospholipids every 1-2 cell division). All of these pathways will get decreased in an attempt of helping the cell to withstand the period of hypoxia, and go back to normal state.

A compensatory pathway of generating ATP, could be followed:
Anaerobic Glycolysis can help the cell to withstand hypoxia for a longer period of time.
This pathway is less efficient than **Oxidative phosphorylation,** since it produces only 2 ATP molecules, and causes muscle fatigue, due to Lactic acid accumulation.

The net effect of hypoxia on the cells explain the ultrastructural and microscopic changes that we see in these cells due to injury, and hypoxia have several effects illustrated below:

1) Reduced activity of membrane ATP-dependent sodium pumps → sodium and water accumulation inside the cell → cellular swelling.
2) Lactic acid accumulation → decreased PH (it's an acid) → failure of enzymes in the cell.
3) Disruption of the ribosomes from the RER membrane → decreased protein synthesis.
4) Accumulation of ROS, (not well understood) and they are more produced in the case of reperfusion after ischemia. Will be discussed later.
5) Damage to mitochondrial and lysosomal membranes and leakage of destructive lysosomal enzymes to the cytosol of the cell.
6) Leading to necrosis.

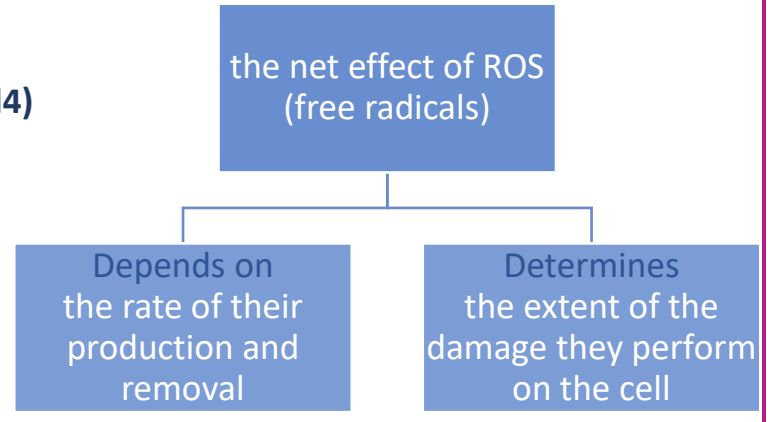
Second event: Oxidative stress

Means: Cellular abnormalities induced by ROS (free radicals), which are chemical species with single unpaired electron (extremely unstable, and have high energy), that if the they bind to any organic/inorganic molecule, they will convert them to a free radical too, and they will induce damage to cellular proteins/lipids and nucleic acids.

Reactive Oxygen Species

when are ROS generated:

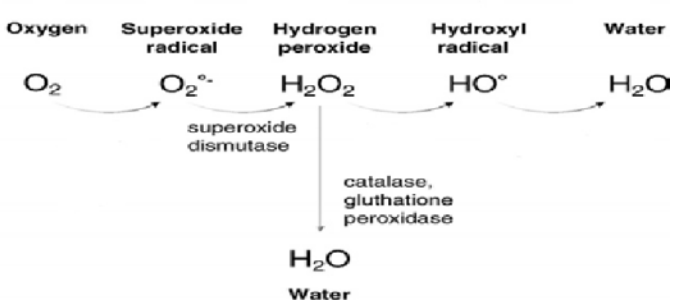
1) **Chemical substance injury (CCl4)**
2) **Radiation injury (UV, Xray)**
3) **Hypoxia**
4) **Cellular aging**
5) **Inflammation**
6) **Ischemia-reperfusion injury**



- Generation and Production

1- Normally produced in small amounts in all cells during the redox reactions, in the mitochondria when oxygen is reduced to produce water. But because these small amounts of highly reactive toxic intermediates are short-lived, the cell is easily able to get rid of them. **Thus,** there will be no net effect of damage on the cell, unless their amount is increased.

Free radicals production reaction:



2- Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation, in an attempt to kill the microbe or to phagocytose the bacteria by phagosomes and phagolysosomes .

The reaction of free radicals production is the same but **H_2O_2** is converted into **Hypochlorite** in phagocytes catalyzed by Myeloperoxidase.

Hypochlorite: bleaching agent, we use at home as a microbicidal agent that can kill a bacterium and often microbes

- Removal

Mechanisms of removing free radicals, limit the damaging effect of free radicals on the cell, and below are some:

- Decay spontaneously,
- Superoxide dismutase, (catalyzes the conversion: **$O_2^{\bullet}$** into **H_2O_2**),
- Glutathione (GSH) peroxidases (mostly GSH type 1), (catalyzes the conversion: **H_2O_2** into water)
- Catalase (one of most active enzymes known, also contributes in catalyzing the previous reaction)
- Endogenous or Exogenous anti-oxidants (e.g., vitamins E, A and C and β-Carotene), antioxidants either block free radicals' production or scavenge them as soon as they are produced

- Effects of ROS:

1-Lipid peroxidation of membranes. (since peroxides are unstable and have damaging effect)

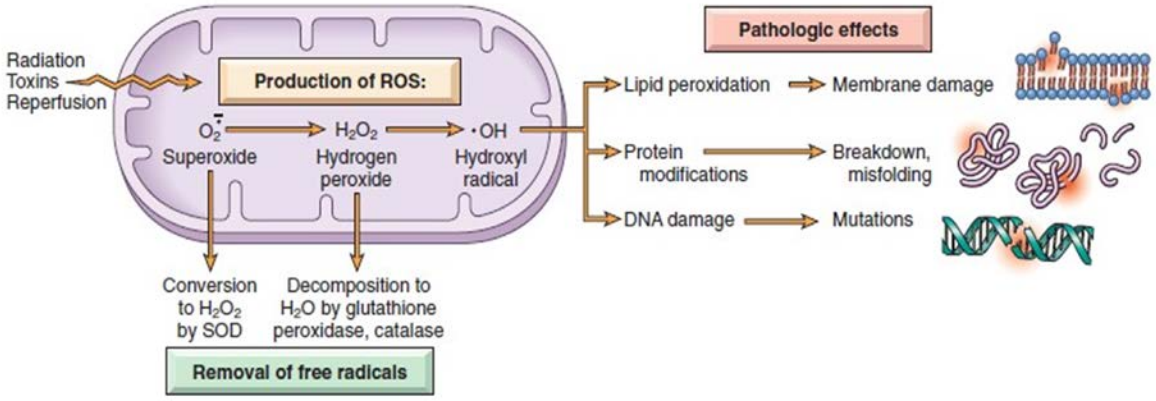
(plasma, lysosomal & mitochondrial membranes)

2-Crosslinking and other changes in proteins.

Causes their (degradation, fragmentation, loss of enzymatic activity & misfolding).

3-DNA damage. How? ⬇
Single strand breaks, mediate: apoptosis, aging, malignant transformation
4-Killing of microbes.

SUMMARY OF ROS



Third: Ischemia-Reperfusion injury

The tissue damage when blood supply returns to tissue after a period of ischemia or lack of oxygen.

Explanation: If there was a blockage in a blood vessel and after a while it was removed, which means the blood flow is back to the tissue (reperfusion), we might think that the tissue will get back to its normal state and nourish immediately, but in certain scenarios it won't. If a tissue undergoes ischemia and it's still viable (didn't undergo irreversible cell injury), Reperfusion will cause Paradoxical cell injury sometimes. An example is: After myocardial or cerebral ischemia.

Further Mechanisms that happen after reperfusion and cause damage:

Increased generation of ROS from 1. injured cells with damaged mitochondria & defective oxidative phosphorylation mechanisms. 2. Infiltrating new leukocytes	Why will there be generation of ROS? due to inefficient use of oxygen by the damaged mitochondria of the injured cell, which will cause incomplete reduction of oxygen in oxidative phosphorylation steps, causing the production of ROS (free radicals)	**Inflammation induced by influx of leukocytes, plasma proteins and complement**

Fourth: Cell injury caused by Toxins

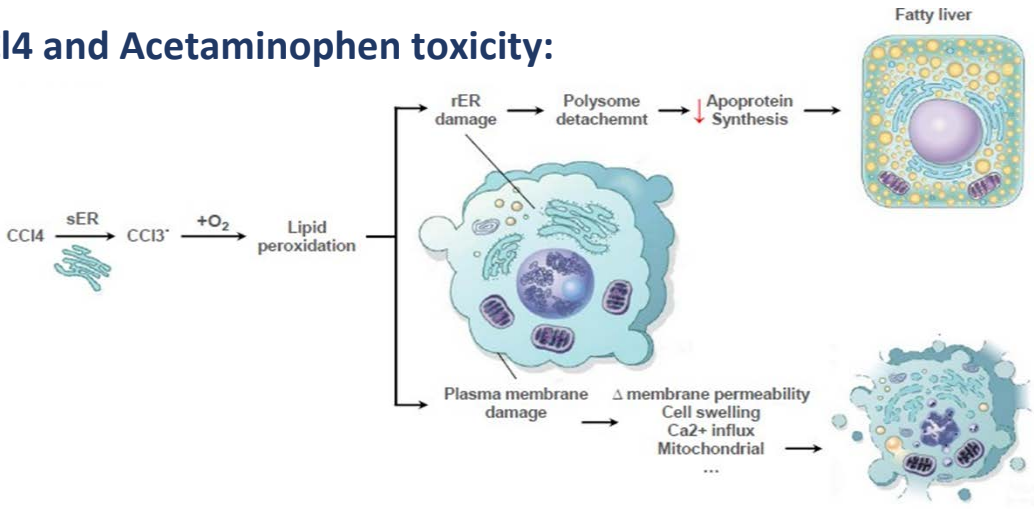
Toxins are mainly: Environmental chemicals & substances produced by infectious pathogens

Their effect could be:

Direct-acting toxin	Latent-acting toxin
• They act directly by **combining** with a critical molecular component or cellular organelle or cellular membrane component leading to change in those membranes Like binding with phospholipids in the brain • Examples: **1. Mercuric chloride poisoning,** occurs due to ingestion of contaminated seafood. Process→Mercury binds to sulfhydryl groups of membrane proteins→inhibit ATP-dependent transport and increase permeability **2. Chemotherapeutic agents,** antineoplastic drugs	• The indirect acting toxins • Not intrinsically active by themselves • Must be converted to reactive metabolites that then act on **targeted cells,** and this conversion usually happens in **cells that contain Cytochrome p450 in SER, mainly in liver cells.** • They cause cells damage by **formation** of free radicals → causing membrane phospholipid peroxidation • Examples: 1. CCl4 2. Acetaminophen (analgesic, found in Panadol) They both cause **membrane** peroxidation, thus damaging the cell. They also cause ER **membrane** damage → decline in synthesis of enzymes and proteins and decreased synthesis of ***apoproteins** → fatty liver They also cause Mitochondrial membrane injury → decreased ATP → cell swelling (due to reduced ATP-dependent membrane function) → cell death

*Apoproteins: they are especially produced in the liver, responsible of carrying lipids and excreting them out of the hepatocytes to the circulation. [Their low concentrations cause accumulation of lipids and triglycerides inside hepatocytes, thus causing fatty liver] one type of CCl4 toxicity, another type is in the figure:

The Mechanism of CCl4 and Acetaminophen toxicity:



**Fatty liver is the consequence of CCl4 and Acetaminophen toxicity

Fifth: Endoplasmic Reticulum Stress

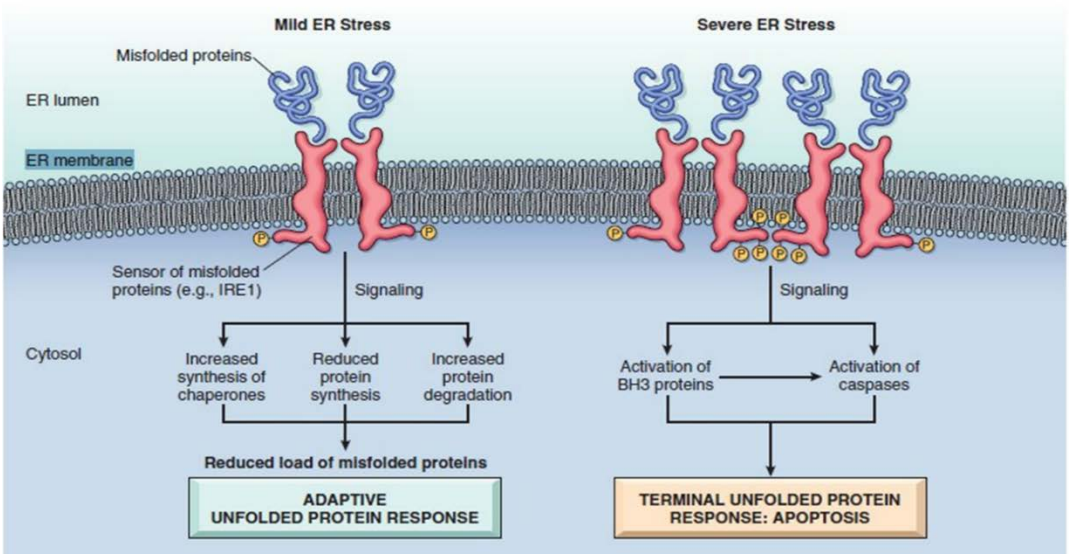
proteins are usually synthesized in the ER, after synthesis they are folded in a proper way to function properly. This folding process needs specific molecules called **chaperones.**

Improper folding can occur due to:

- Gene mutations, leading to production of proteins that can't be folded properly
- Aging, ⬇ cell's ability to produce chaperons, ⬇ cell's capacity to fold proteins.
- Infections, especially viral infections, due to increased production and misfolding of viral proteins causing stress to the cell.
- Increased demand for secretory proteins such as insulin in insulin-resistant patients, the body respond as if there is no insulin, so the pancreas respond by secreting more insulin, which will accumulate in cells, as there is no capacity to fold this insulin properly, so it will accumulate in a misfolded form in the pancreatic cells leading to their death and damage by apoptosis
- Changes in intracellular PH in ischemia and hypoxia.
- Neurodegenerative disease.

Cell's response against misfolded proteins production:

1) If their amount was not high, they will be targeted for proteolysis.
2) If the misfolded proteins were found in high amounts, the cell will try to adapt by the activation of **Unfolded Protein Response** (adaptive response).
   Unfolded Protein Response: increase chaperons' production, decrease protein translation, increase protein destruction.
   →By this, unfolded protein concentration is decreased in the cell.
3) If the previous responses weren't efficient, there will be activation of apoptosis (cell suicide), but, **HOW**?
   By Activation of → 'proapoptotic sensor activation (BH3-protein family)'
   → specific caspases (apoptotic enzymes)
   which will eventually cause apoptosis by the mitochondrial pathway.



We said that misfolded-proteins accumulation may turn on the apoptosis pathway and lead to cell death thus diseases, but not always. Diseases could result from the deficiency of a specific protein that was produced misfolded and degraded, examples of diseases due to such cause are:

1- Cystic Fibrosis:

genetic mutation in a protein (cystic fibrosis transmembrane conductance regulator (CFTR) protein) that function as a membrane transporter
→ production of misfolded protein → degradation of this protein →deficiency of this transporter

2- Neurodegenerative disorders:

Apoptosis of the diseased cell is the result
Ex: Alzheimer, Huntington, Parkinson diseases

3- Type 2 diabetes:

insulin is produced in larger amount but it will accumulate due to low capacity of the cell to fold insulin, causing damage to these cells which causes apoptosis

Mentioned earlier

4- Amyloidosis:

Improperly folded proteins accumulation in EXTRACELLULAR TISSUE

Sixth: DNA damage

causes:

- Radiation
- Chemotherapeutic agents
- Intracellular generation of ROS
- Mutations

Any DNA damage in the cell, is sensed by P53 gene (tumor suppressor gene), leading to its activation.
P53 arrests cell's replication at G1 phase for repair:
→ if Repair is possible, the cell will continue replication,
→ if repair is impossible, apoptosis is induced,
→ if P53 gene is mutated, there will be no stop for the mutated cell from completing replication, leading to neoplastic change. Ex: sometimes cells develop DNA damage after exposure to sun for a long time, and this leads to P53 activation, thus arresting the cell at G1 phase until repair is done
BUT if P53 was mutated, abnormal cells will be produced, leading to skin cancer, like: basal cell carcinoma or squamous cell carcinoma.

Seventh: Inflammation

Normal reaction in the body elicited by:

- Pathogens, ex: microbes

- Necrotic cells
- Dysregulated immune responses (autoimmune diseases and allergies)

When inflammation occurs, there will be accumulation of: Inflammatory cells (neutrophils, macrophages, lymphocytes) that will secrete products that destroy microbes **and damage host tissues, leading to cell injury.**

After studying mechanisms of cell injury , we end up with events that are common in cell injury despite their different causes

So, most of the causes that we have talked about (hypoxia/ischemia/toxins…) lead to :

- Mitochondrial Dysfunction
- Defects in membrane permeability

-Mitochondrial Dysfunction can result from Hypoxia, toxins, radiation, and since it's the energy factory in the cell, its dysfunction causes either Apoptosis or Necrosis.

Consequences of mitochondrial dysfunction:

1) Failure of oxidative phosphorylation, leading to ATP depletion.
2) Abnormal/insufficient oxidative phosphorylation, leading to formation of ROS.
3) Mitochondrial permeability transition pores, leading to loss of membrane potential and insufficient oxidative phosphorylation.
4) Release of cytochrome c which is usually stored in mitochondria, to the cytoplasm which will activate specific cascades >> stimulating apoptosis.

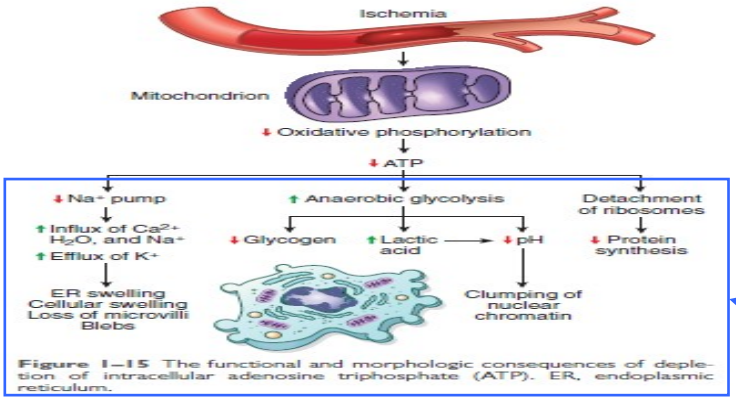


Figure 1–15 The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum.

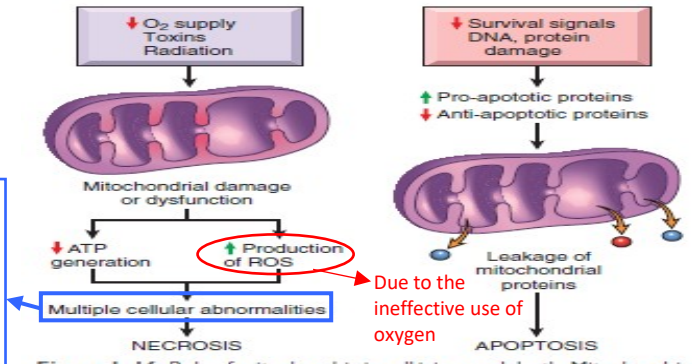


Figure 1–16 Role of mitochondria in cell injury and death. Mitochondria are affected by a variety of injurious stimuli and their abnormalities lead to necrosis or apoptosis. This pathway of apoptosis is described in more detail later. ATP, adenosine triphosphate; ROS, reactive oxygen species.

-Defects in membrane permeability

- Mitochondrial membrane damage: decreased ATP
- Plasma membrane damage: loss of osmotic balance, influx of fluids due to attraction of water, leak of contents
- Lysosomal membranes: leakage of enzymes into cytosol >> leading to cellular digestion.

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Good Luck