PATHOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 3

Mechanisms of Cell Injury (Pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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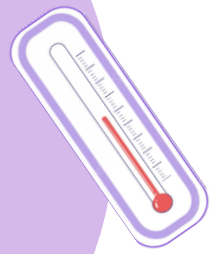

MECHANISMS OF CELL INJURY

cell injury and adaptations

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MECHANISMS OF CELL INJURY

Principles

(***necrosis*** or ***apoptosis*** or both)

- The cellular response to injury depends on:
  1. type of injury (Hypoxia, Ischemia, DNA mutations, Protein misfolding, etc.)
  2. duration 1-2 min of ischemia in the heart usually causes reversible injury, but 20-30 min >> Myocardial Infarction
  3. severity Complete cut (100%) of blood supply will cause (MI), but a small cut (say 70%) will not have a huge damage → may cause Angina instead of MI.

Irreversible

Blockage can occur due to atherosclerosis

- The consequences of injury also depend on:
  1. Type In ischemia, Irreversible brain damage can occur relatively quickly while it needs a longer period in the heart. Skeletal muscles can withstand ischemia for multiple hours.
  2. Status Before the onset of the injury, was the cell already diseased, or did it have any mutations, or was it healthy?
  3. adaptability, and genetic makeup of the injured cell (precision medicine concept)
     Some cells (because genetic factors) can be more susceptible than other cells.

Already diseased cells can have more severe consequences after the injury.

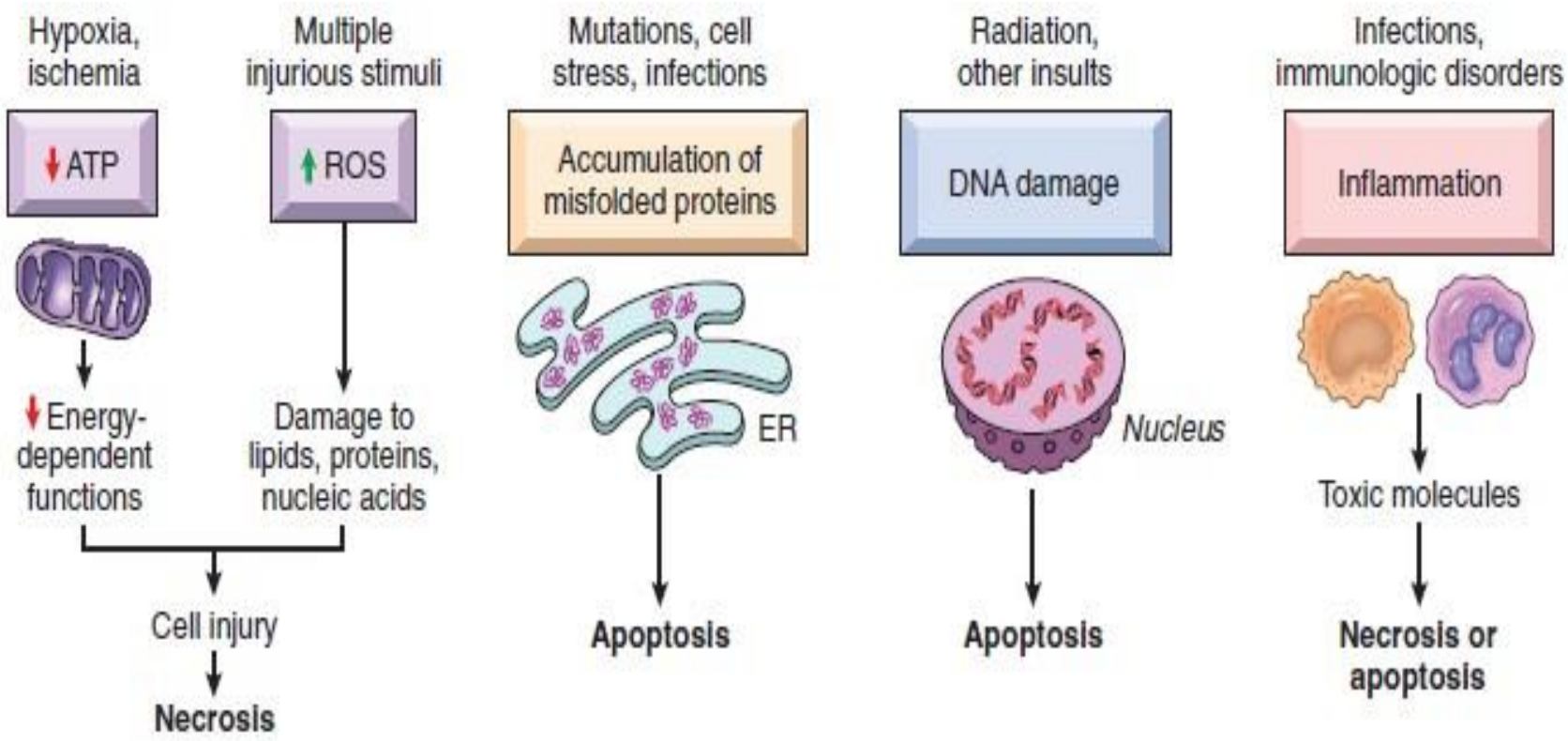
- Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components.
- Same injury may trigger more than one mechanism.

Many causes can cause an injury.
One cause can affect more than one pathway in the cell.

As we said, one cause (e.g.: ischemia) → many responses (decreased ATP **and** increased ROS)

The principal biochemical mechanisms and sites of damage in cell injury

ROS are highly reactive molecules that damages almost all cell components.



Inflammation can be caused by infectious or non-infectious factors.

Inflammation release toxic molecule and leads to tissue damage, necrosis, and in some cases apoptosis.

Further explanation on all 5 in the next slides

Hypoxia and Ischemia

- One of the most frequent causes of injury.
- Defective oxidative phosphorylation >>Failure of ATP generation>>>depletion of ATP in cells
- Failure of energy dependent pathways (membrane transport, protein synthesis, lipogenesis and phospholipid turnover)

Ribosomes need energy to function

Leads to accumulations (myelin figures) in the cell.

- Anaerobic glycolysis.
- Liver cells and skeletal muscle cells **Vs** brain and heart.

They can get benefit from anaerobic glycolysis and make energy even in case of hypoxia.

Whereas these can't do anaerobic glycolysis so will face stronger damage.

Reduced ATP production impairs all ATP-dependent functions.

For example, the NA+/K+ pump gets disrupted, causing Na+ to accumulate inside the cell, leading to swelling and eventually, cell bursting.

Hypoxia effects:

- Reduced activity of membrane ATP dependent sodium pumps>> cell swelling
- Lactic acid accumulation >> decreased PH>> failure of enzymes.
- Disruption of the ribosomes >> decreased protein synthesis.
- Accumulation of ROS → Caused by ineffective oxidative phosphorylation.
- Damage to mitochondrial and lysosomal membranes. → Will further inhibit oxidative phosphorylation.
- Necrosis is the end result.
- Apoptosis can contribute.

Leakage of enzymes → intracellular damage
Or tissue damage if they leak outside the cell.

But necrosis is the main one

Ischemia–Reperfusion Injury

- **<u>Paradoxical cell injury</u>** after restoration of blood flow to ischemic but viable tissues.
- After myocardial and cerebral ischemia.

When ischemia-reperfusion occurs, this will also cause inflammation that moves WBCs to the site, clean the area and add ROS.

- **Increased generation of ROS from:**
- Injured cells with damaged mitochondria & defective antioxidant mechanisms.
- Infiltrating new leukocytes.

Ineffective oxidative phosphorylation in the ischemic cells also generates ROS.

- **Inflammation induced by influx of leukocytes, plasma proteins and complement**

Oxidative Stress

- Cellular abnormalities induced by ROS (free radicals)
- Chemical species with single unpaired electron (extremely unstable)

All these cause ROS injury

- **ROS generated in:**
- Chemical injury (CCL4)
- Radiation injury (UV, Xray)
- Hypoxia
- Cellular aging
- Inflammation WBCs to fight pathogens
- Ischemia-reperfusion injury.

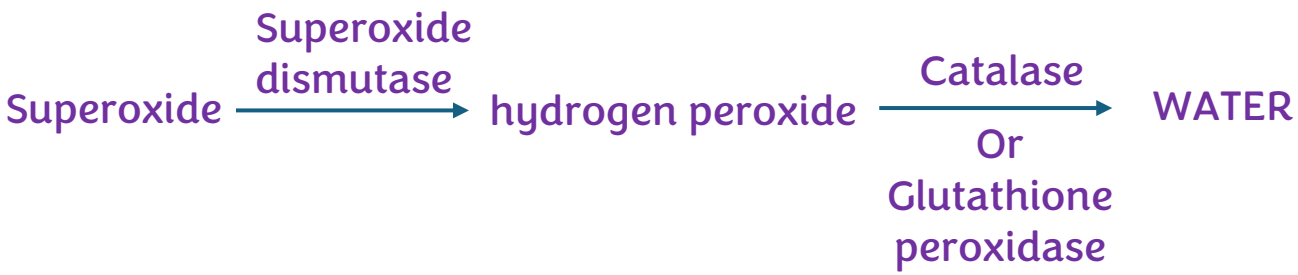
Particles with high free energy:

1. They can directly damage tissues.
2. Make other ROS from normal compounds when interacting with them leading to a “circle” of oxidative stress which damages the surroundings.

Generation and Removal of Reactive Oxygen Species

- **1-Normally produced in small amounts in all cells during the redox reactions.**
- Oxygen is reduced to produce water.
- Small amounts of highly reactive but short-lived toxic intermediates are generated.
- Superoxide (O2 •), hydrogen peroxide (H2O2), hydroxyl radical •OH.

ROS are found in a small amounts and are controlled even when a cell is injured.



During an inflammation They digest any microbes, debris and dead cells. Then release ROS and other mediators to get rid of them.

- **2-Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation.**
- In phagosomes and phagolysosomes to kill microbes.
- O2 >> superoxide >> H2O2 >> hypochlorite.
- Myeloperoxidase (H2O2 into hypochlorite). Kills bacteria.

Phagosomes: a state when a cell engulfs ECF substance and degrades it.

Removal of free radicals

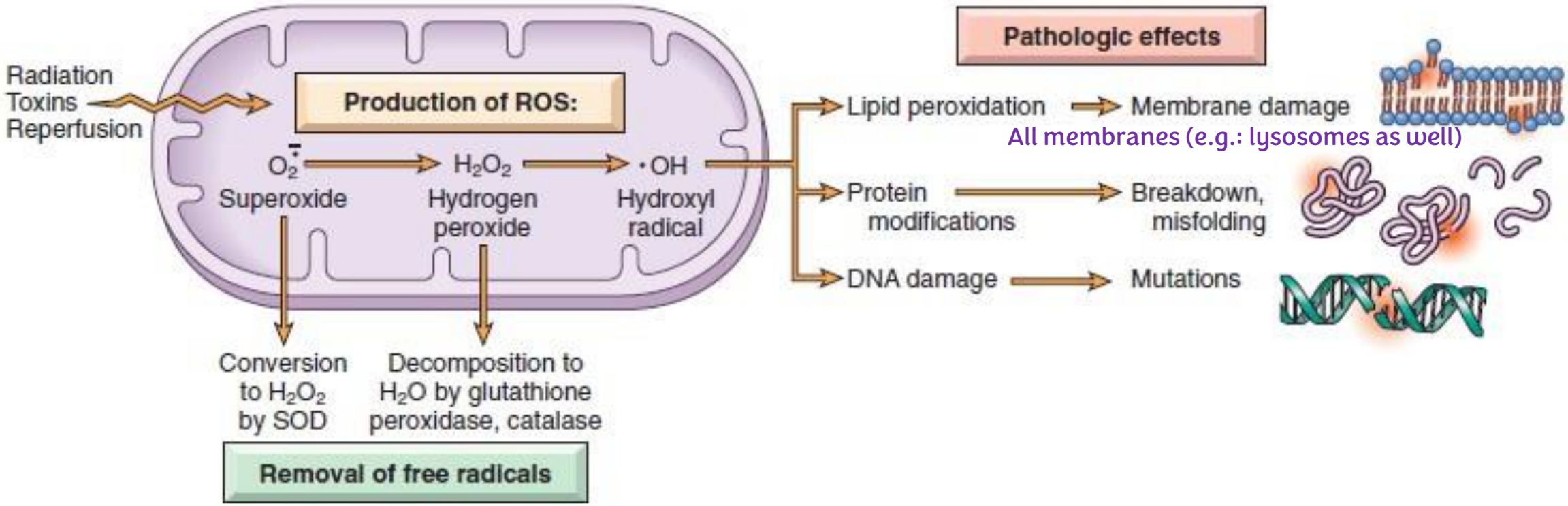
They are short lived.

- Decay spontaneously
- Superoxide dismutase (SOD).
- Glutathione (GSH) peroxidases.
- Catalase (one of most active enzymes known)
- Endogenous or exogenous anti-oxidants (e.g., vitamins E, A, and C and β-carotene)

These vitamins act as antioxidants, providing crucial health benefits.

Catalase and glutathione transform hydrogen peroxide into water.

VERY IMPORTANT



Effects or ROS:

1. **Lipid peroxidation of membranes.**

(plasma, lysosomal & mitochondrial membranes)

2. **Crosslinking and other changes in proteins.**

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

3. **DNA damage.**

Single strand breaks, mediate: apoptosis, aging, malignant transformation

4. **Killing of microbes.**

ROS play a crucial role in the process of inflammation. Every inflammatory reaction involves the presence of ROS.

Reactive oxygen species (ROS) can modify proteins, causing them to break down or misfold. Misfolded proteins lose their function due to conformational changes, leading to the accumulation of non-functional proteins within the cell.

When a cell experiences DNA damage, apoptosis (programmed cell death) may be triggered as a protective mechanism. If the DNA is not properly repaired and the cell continues to divide, it increases the risk of developing cancer.

Cell Injury Caused by Toxins

Toxins are chemical substances produced by microbes or infectious agents.

- Environmental chemicals & substances produced by infectious pathogens.

- **Direct-acting toxins** They will kill the cell immediately.

- **Latent toxins.** They are chemicals that are inactive on their own and need to be converted within the cells into an active substance.

Direct-acting toxins

- Act directly by combining with a critical molecular component or cellular organelle.

1. **Mercuric chloride poisoning**

   Contaminated seafood

   Mercury binds to sulfhydryl groups of membrane proteins>>inhibit ATP-dependent transport and increase permeability.

2. **Chemotherapeutic agents**
3. **Toxins from microorganisms.** As tetanus and cholera toxins.

ATP-dependent transporters, such as the sodium-potassium pump, help regulate cellular permeability by maintaining ion gradients. When these transporters fail, typically due to ATP depletion, the resulting osmotic imbalance can cause the cell to swell.

Latent toxins

- Not intrinsically active
- Must be converted to reactive metabolites, then act on target cells. Via cytochrome P-450 in SER of the liver.
- Damage mainly by formation of free radicals>>membrane phospholipid peroxidation.

Ex: CCl4 and acetaminophen.

Acetaminophen, also known as paracetamol, can be toxic in large amounts. In cases of overdose, such as in some suicide attempts, liver damage occurs due to the buildup of the toxic metabolite NAPQI, with ROS contributing to additional oxidative stress.

- Membrane peroxidation>>>>damage
- ER membranes >> detachment of ribosomes>>decline in synthesis of enzymes and proteins +decreased synthesis of apoproteins >> fatty liver
- Mitochondrial membranes>> decreased ATP >> cell swelling >> cell death.

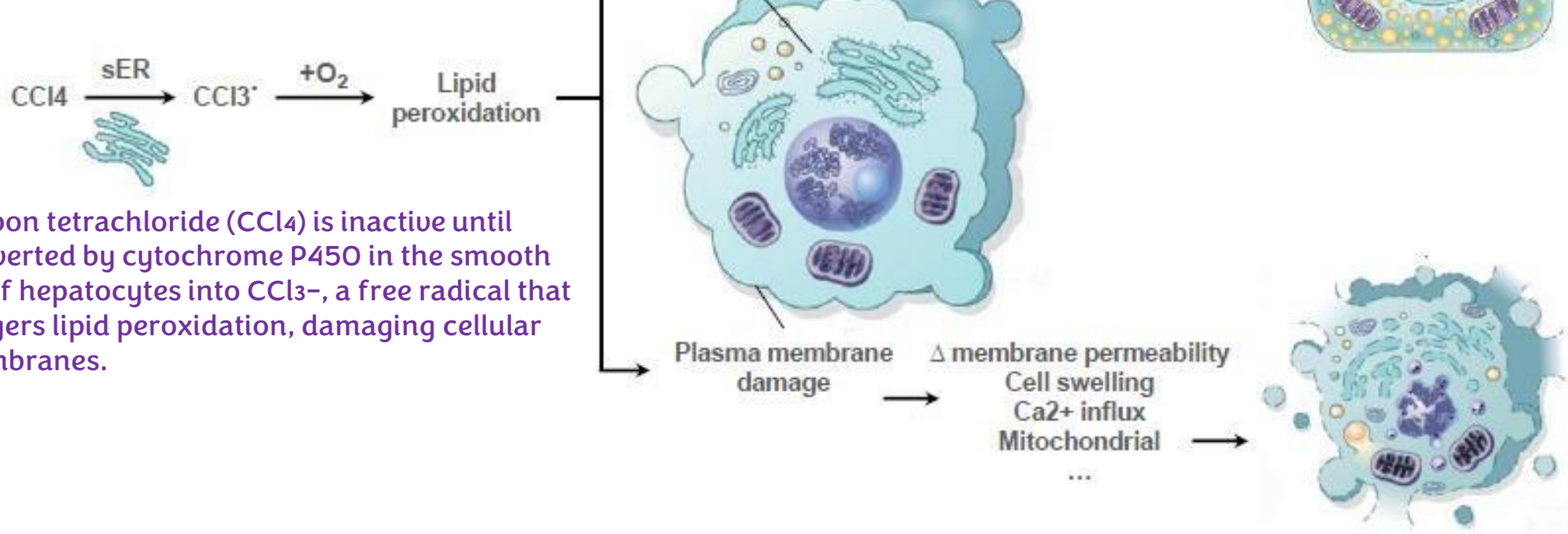
A decrease in apoprotein synthesis causes fat accumulation in cells, as apoproteins are needed to form lipoproteins that transport lipids out of the cell. Without them, lipid export is impaired.

When acetaminophen is taken in overdose during suicide attempts, or in cases of carbon tetrachloride poisoning, the liver becomes enlarged and accumulates fat. This condition is referred to as acute fatty liver. Additionally, liver enzymes are present in elevated levels in the blood, indicating acute liver injury.

Remember: Apoproteins are the proteins of lipoproteins.

CCL4 toxicity

- CCl4



This will lead to decrease in protein synthesis because of the detachment of ribosomes from rER.

Carbon tetrachloride (CCl_4) is inactive until converted by cytochrome P450 in the smooth ER of hepatocytes into CCl_3–, a free radical that triggers lipid peroxidation, damaging cellular membranes.

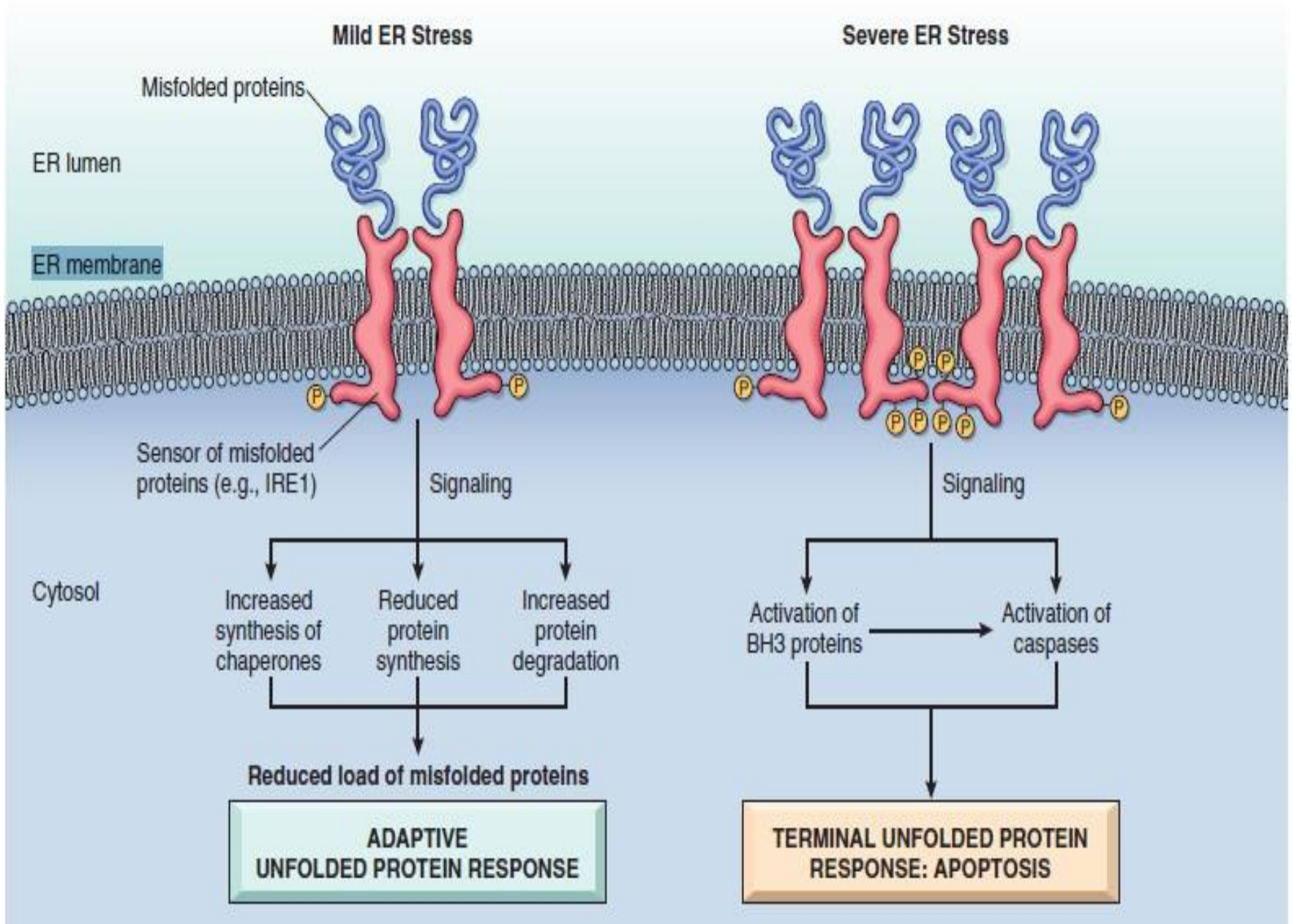
The cell will be dead.

Endoplasmic Reticulum Stress

- Chaperones in ER control proper protein folding
- Misfolded proteins >> ubiquinated >> targeted to proteolysis
- **Unfolded protein response (adaptive response):** increase chaperones production, decrease protein translation and increase destruction.
- If failed >> proapoptotic sensor activation (BH3-only family) + direct activation of caspases >> **apoptosis by the mitochondrial pathway.**

Caspases: apoptotic enzymes.

If chaperones are absent or insufficient relative to misfolded proteins, misfolded proteins can accumulate within cells. Once accumulated, these proteins are ubiquitinated and subjected to proteolysis as an adaptive response, which may involve increasing chaperone synthesis, decreasing protein synthesis, or enhancing protein degradation.


Mild ER Stress
Severe ER Stress
Misfolded proteins
ER lumen
ER membrane
Sensor of misfolded proteins (e.g., IRE1)
Signaling
Signaling
Cytosol
Increased synthesis of chaperones
Reduced protein synthesis
Increased protein degradation
Activation of BH3 proteins
Activation of caspases
Reduced load of misfolded proteins
ADAPTIVE UNFOLDED PROTEIN RESPONSE
TERMINAL UNFOLDED PROTEIN RESPONSE: APOPTOSIS

Causes of misfolding

- Gene mutations
- Aging (decreased capacity to correct misfolding)
- Infections, especially viral infections (microbial proteins) As prion disease.
- Increased demand for secretory proteins such as insulin in insulin-resistant states
- Changes in intracellular pH Enzymes will be non-functional.
- Neurodegenerative diseases As Alzheimer's and Huntington's diseases that lead to the death of neurons due to the accumulation of misfolded proteins within these cells. Ultimately, this process results in brain atrophy.
- Deprivation of glucose and oxygen in ischemia and hypoxia.

Insulin resistance occurs when insulin is present, but receptors fail to respond. Consequently, the pancreas increases insulin synthesis, leading to insulin accumulation in cells. Over time, individuals with type 2 diabetes may experience decreased insulin production due to misfolded protein accumulation in pancreatic cells, triggering apoptosis. This cell death necessitates insulin injections for diabetic patients.

Protein misfolding causes disease by:

- **Deficiency of an essential protein due to degradation**

  Cystic fibrosis

- **Inducing apoptosis of the affected cells**

  Neurodegenerative disorders (Alzheimer disease, Huntington disease & Parkinson disease), type 2 diabetes and prions disease.

- **Inducing both:**

1. Alpha 1 antitrypsin deficiency.

   Improperly folded proteins accumulation in extracellular tissues

2. Amyloidosis

Sometimes, misfolded proteins may become inactive, preventing them from being secreted outside the cell and hindering their proper function.

DNA Damage

- Radiation As UV light.
- Chemotherapeutic agents
- Intracellular generation of ROS
- Mutations

Tumor suppressor gene.

- DNA damage >> p53 activation >> arrest cell cycle at G1 phase for repair >> if repair is impossible >> apoptosis.
- In P53 mutations >> mutated cells replicate >> neoplastic change.

This mechanism will protect us from tumors.

Inflammation

- Pathogens As viruses, bacteria, and protozoa.
- Necrotic cells
- Dysregulated immune responses (autoimmune diseases and allergies)

- Inflammatory cells (neutrophils, macrophages, lymphocytes) secrete products that destroy microbes and damage host tissues.

Inflammation is beneficial for resisting microbes, removing dead cells, and mediating certain immune responses. However, when inflammation exceeds what is necessary, it can lead to necrotic changes and damage to host cells.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

رسالة من الفريق العلمي:

اللهم كن لأهلنا في غزة والسودان معيناً ونصيراً وفرّج كربهم وأزل همهم وارحمهم برحمتك.

وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ.

فريق الليوث العابسة

Additional Resources: