PATHOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 4

Apoptosis and autophagy

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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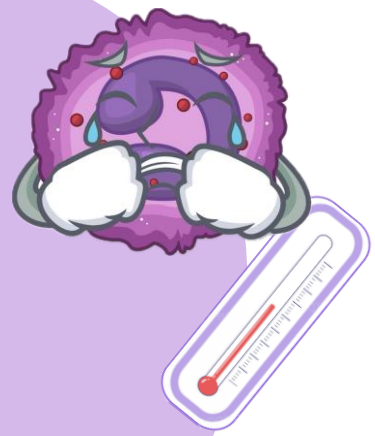


bismillah

Apoptosis and autophagy

Definition

- **“Programmed cell death”**

  It is also called clean cell death or cell suicide.

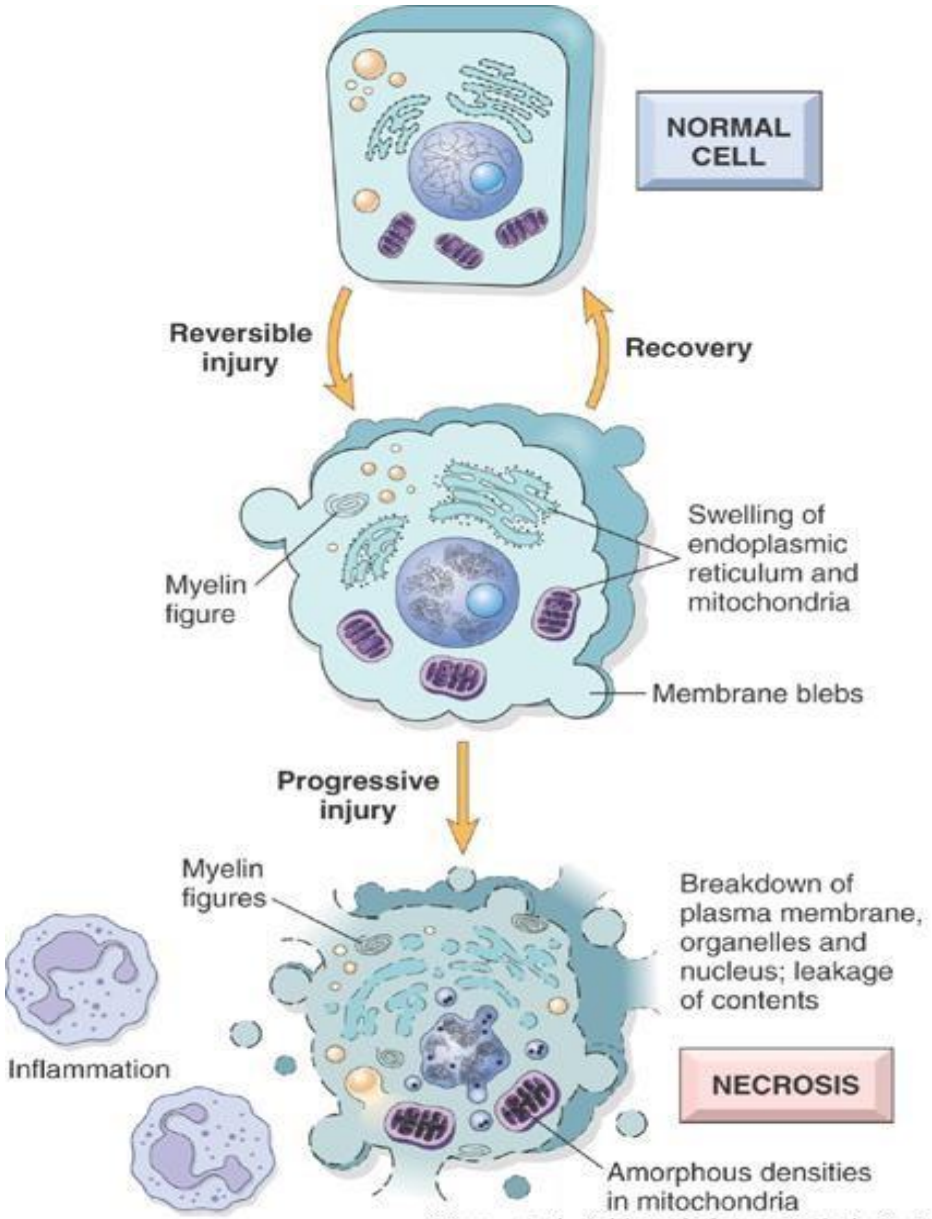
- “a genetically determined process of cell self-destruction”

- “ pathway of cell death in which cells activate enzymes that degrade the cells’ own nuclear DNA and nuclear and cytoplasmic proteins.”

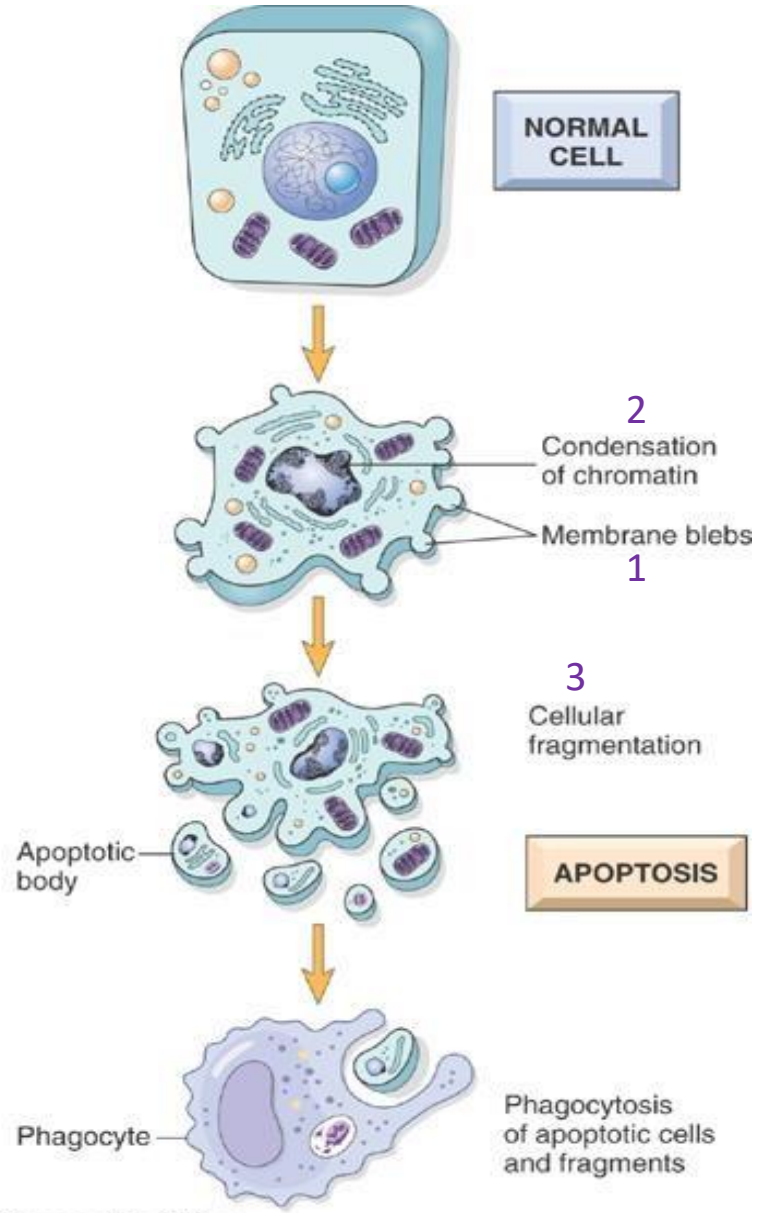
- The dead cell and its fragments are cleared with little or no leakage of cellular contents, NO inflammatory reaction.

  This pathway is highly controlled and predetermined.
  It is governed by genetic factors.

Cell death by necrosis



Cell death by apoptosis



There are many differences between these two pathways of cell death, one of the main differences between them is that the cell and the nucleus in apoptosis will shrink instead of swelling.

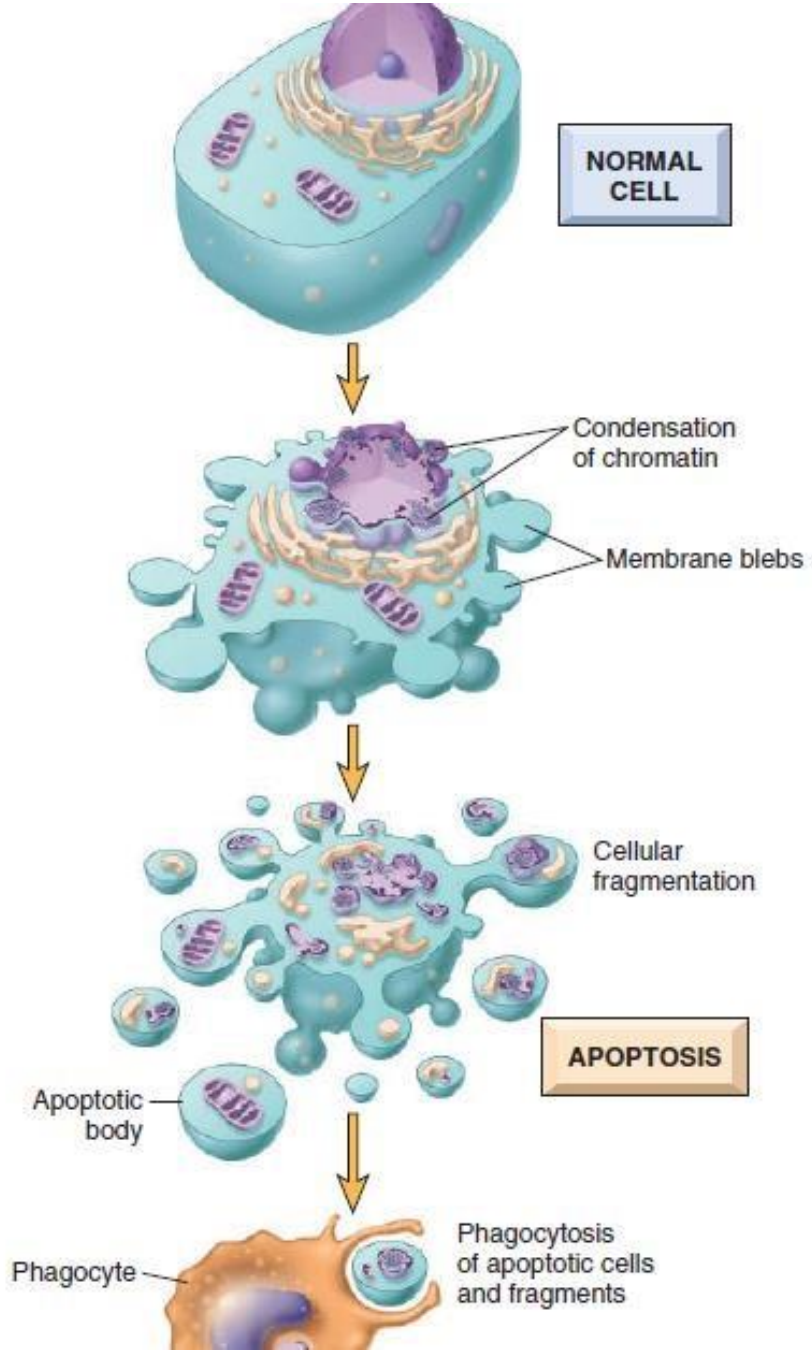
Characteristics of apoptosis:

- The cell membrane will start to have some blebs while it will remain intact.
- Nuclear material starts to condensate (chromatin becomes darker under the light microscope), then the nuclear fragmentation starts (karyorrhexis).
- The cell will start to fall off like (عنقود العنب) (that's why it's called apoptosis), and parts of the cell membrane will enclose some organelles and cytoplasmic proteins separating them from the cell as an apoptotic bodies.
- These apoptotic bodies will send signals to phagocytes which will engulf them to get rid of them without eliciting an inflammatory reaction.

That's why it's called cleans cell death

Apoptotic bodies are membrane bound vesicles.

Notice that every apoptotic body contains parts of the organelles, cytoplasmic proteins, fragmented nucleus or DNA material.



You should know the main differences between these two pathways.

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis, Karyorrhexis, karyolysis.	Fragmentation into nucleosome- size fragments We can say pyknosis and karyorrhexis
Plasma membrane	**Disrupted**	**Intact**, altered structure, especially orientation of lipids
Cellular content	Enzymatic digestion, may leak out of cell we can detect these enzymes in the blood stream.	Intact, may be released in apoptotic bodies.
Adjacent inflammation	**Frequent** Because of the leakage of cell content due to the disrupted plasma membrane.	**No**
Physiologic or pathologic role	Invariably pathologic Almost always pathologic.	often physiologic and may be pathologic
# of dying cells	Many cells will die	Usually not too many

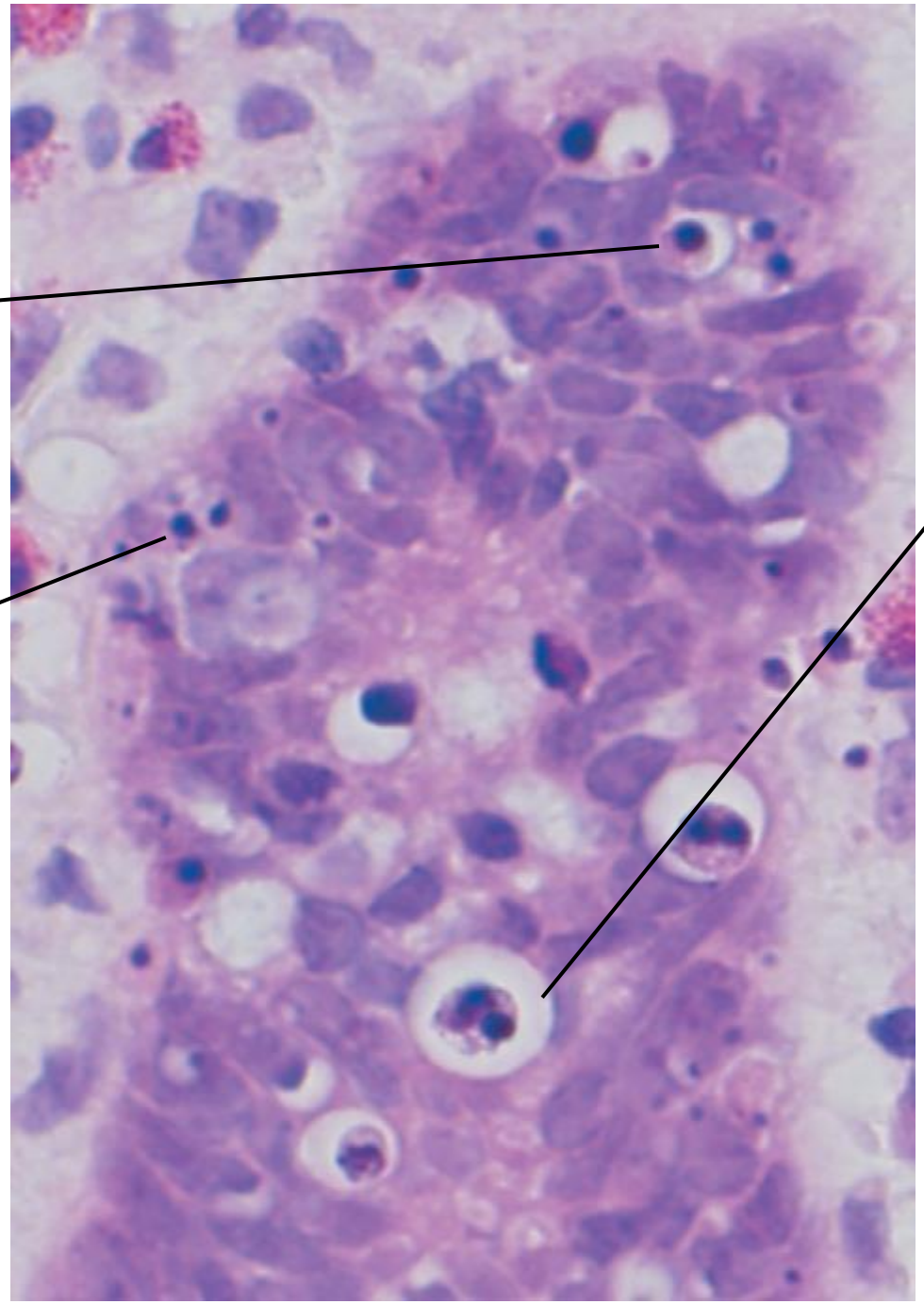
Karyorrhectic debris of the nucleus will start to separate in apoptotic bodies.

"Karyorrhectic debris" refers to the fragmented remnants of a cell's nucleus after it has undergone karyorrhexis.

However, apoptosis and necrosis sometimes coexist, and apoptosis induced by some pathologic stimuli may progress to necrosis, like in ischemia.

H&E Stain section under the light microscope.

A few cells that died with the same pattern.



These cells are shrunk as we can see an empty lacuna around them, the nuclear material is fragmented and condensed (dark color).

Here we can notice fragments of condensed DNA material. Inflammatory reactions are absent.

Causes of apoptosis

- Physiologic apoptosis (normal scenarios)
- Apoptosis in pathologic conditions

Causes of Apoptosis

- Physiologic
- During embryogenesis — During embryo development in the uterus, new cells appear, and old ones die by means of apoptosis.
- Involution of tissues upon hormone deprivation (Cyclic endometrium, lactating breast)
- Steady state population (Gut, Skin) — Gut and skin have rapid turnover, so new cells are made all the time, and the old ones die by apoptosis in a rapid regenerative capacity process.
- End of function/life (neutrophils at end of inflammation) — They have done their job in inflammatory reactions and there is no need for them anymore.
- Self reacting lymphocytes

In the cyclic endometrium, at the end of the menstrual cycle, the endometrial glands are lost and then the shedding of the endometrium will start as menses, this death of endometrial cells is usually mediated by apoptosis.

In the lactating breast, new glands will appear in the process of lactation. When there will be a deprivation of hormone stimulation, the new glands which have appeared at the onset of lactation will involute and die by apoptosis.

During development or maturation process of lymphocytes in the bone marrow or in lymphoproliferative organs, sometimes new lymphocytes could have some activity against the self-antigen these lymphocytes are called self-reacting lymphocytes, if they stay in the body, they will cause autoimmune diseases, so the body will get rid of them by apoptosis.

- **Pathologic:** (damaged cells beyond repair)

- DNA damage (Rx, chemoTx, temperature, UV, hypoxia)

  DNA damage in cells is caused by radiotherapy, chemotherapy, extremes of temperature, exposure to UV light, and hypoxia. These damaged cells will undergo apoptosis if the damage is not repaired to prevent them from proliferation as they will cause cancer.

- Accumulation of misfolded proteins

  Any cell that accumulate misfolded proteins will be directed to cell death by apoptosis.

- Some infections (adenovirus, HIV, hepatitis viruses) Viral infections.

  Those viruses can cause cell death by necrosis or apoptosis.

Condition	Mechanism of Apoptosis
Physiologic	
During embryogenesis	Loss of growth factor signaling (presumed mechanism)
Turnover of proliferative tissues (e.g., intestinal epithelium, lymphocytes in bone marrow, and thymus)	Loss of growth factor signaling (presumed mechanism)
Involution of hormone-dependent tissues (e.g., endometrium)	Decreased hormone levels lead to reduced survival signals
Decline of leukocyte numbers at the end of immune and inflammatory responses	Loss of survival signals as stimulus for leukocyte activation is eliminated
Elimination of potentially harmful self-reactive lymphocytes	Strong recognition of self antigens induces apoptosis by both the mitochondrial and death receptor pathways
Pathologic	
DNA damage	Activation of proapoptotic proteins by BH3-only sensors
Accumulation of misfolded proteins	Activation of proapoptotic proteins by BH3-only sensors, possibly direct activation of caspases
Infections, especially certain viral infections	Activation of the mitochondrial pathway by viral proteins Killing of infected cells by cytotoxic T lymphocytes, which activate caspases

Mechanisms of Apoptosis

- **Activation of enzymes called caspases**
- Two distinct pathways can lead to caspase activation:
- 1) The mitochondrial pathway it's also called the **intrinsic pathway** because it starts at the level of the mitochondria inside the cell.
- 2) The death receptor pathway

Each pathway has its own distinct properties.

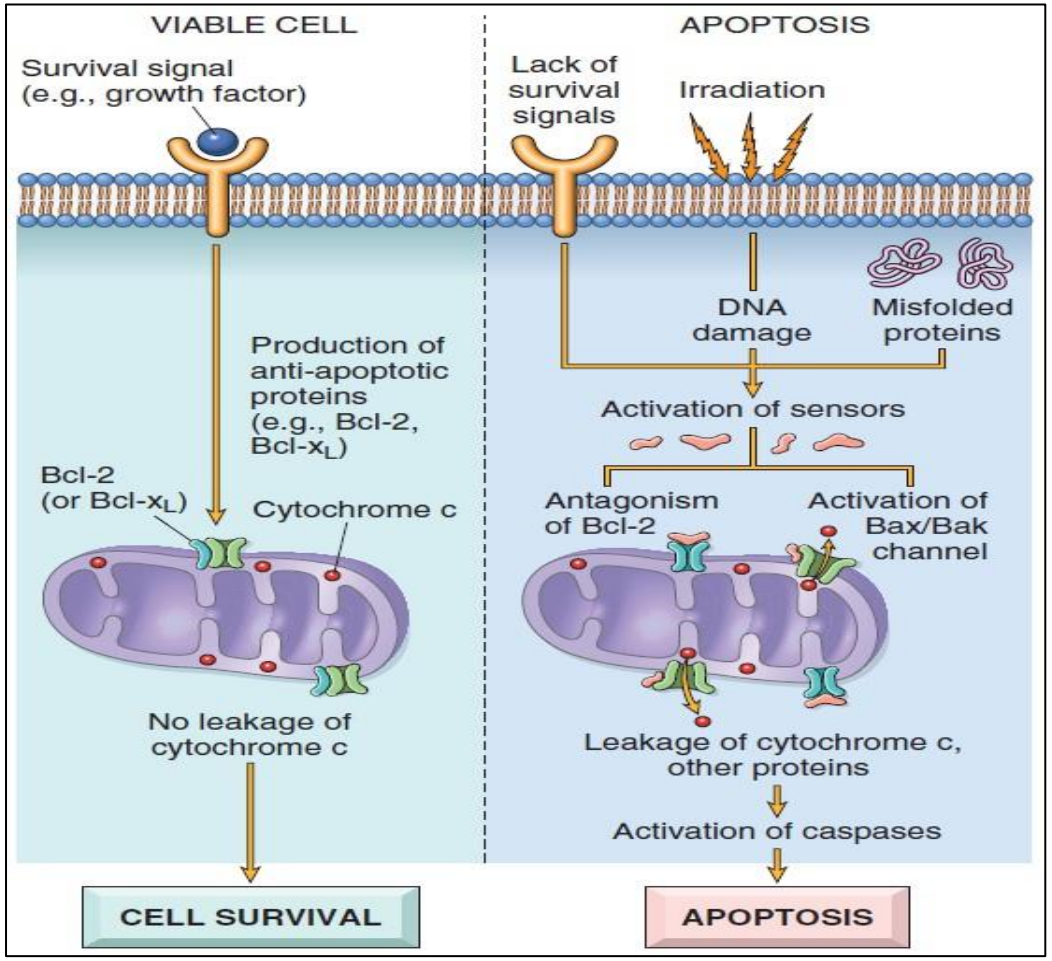
Mitochondrial (intrinsic)

Responsible for apoptosis in most physiologic and pathologic situations.

Bcl2 family of proteins control mitochondrial membrane permeability.

Bcl2 antiapoptotic/Bax/Bak proapoptotic/BH3 sensors.

Cytochrome c activates caspase-9.



Bcl-2 (or Bcl-xL) is an antiapoptotic protein which is coded by antiapoptotic gene, this protein prevents apoptosis by controlling the mitochondrial membrane permeability. Inside the mitochondria, there are certain enzymes and proteins that if they leak from the mitochondria, they will cause the activation of caspases, one example of these proteins is cytochrome c which is present inside the mitochondria away from the cytoplasm, the leakage of cytochrome c will cause the activation of caspase-9 firstly, then the other caspases are activated leading to apoptosis.

In normal scenarios, the cell will still receive survival signals such as growth factors signals, cytochrome c must stay inside the mitochondria, this is done by Bcl-2 proteins which blocks the activation of Bax/Bak channel, so the cell stays viable.

In the case of cell damage, BH3 proteins which are the damage sensors found in the cytosol will be activated, these proteins upon activation will inhibit Bcl-2 proteins and activate the Bax/Bak proteins, Bax/Bak proteins will dimerize making a channel that increases the permeability of the mitochondrial membrane, this will lead to the leakage of cytochrome c through the channel, cytochrome c will activate caspases leading to apoptosis.

Death receptor (extrinsic)

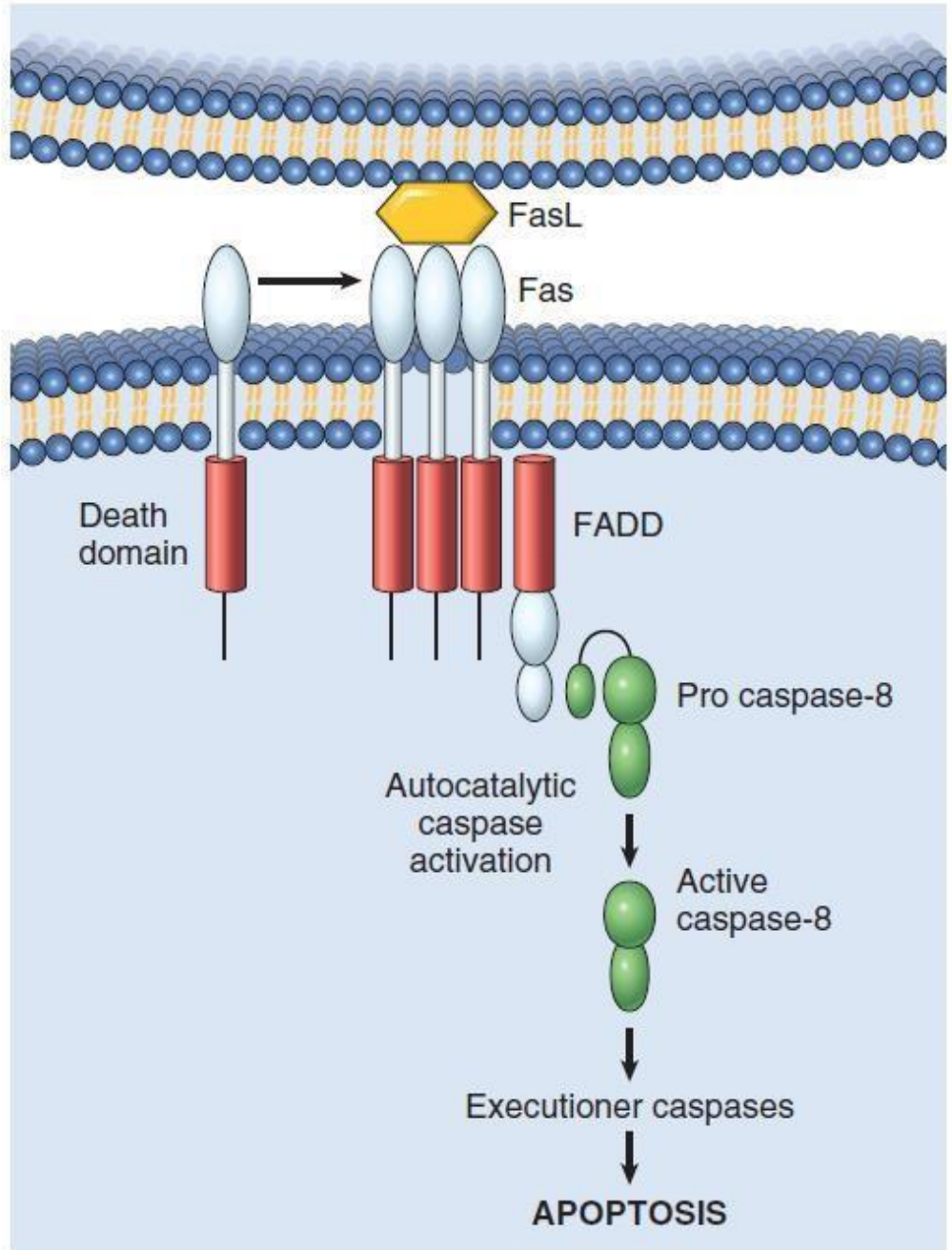
Cell death receptors, which are located in the cell membrane, have a cytoplasmic region known as the **death domain,** this domain interacts with proteins inside the cell, leading to the activation of caspases. **Extrinsic**

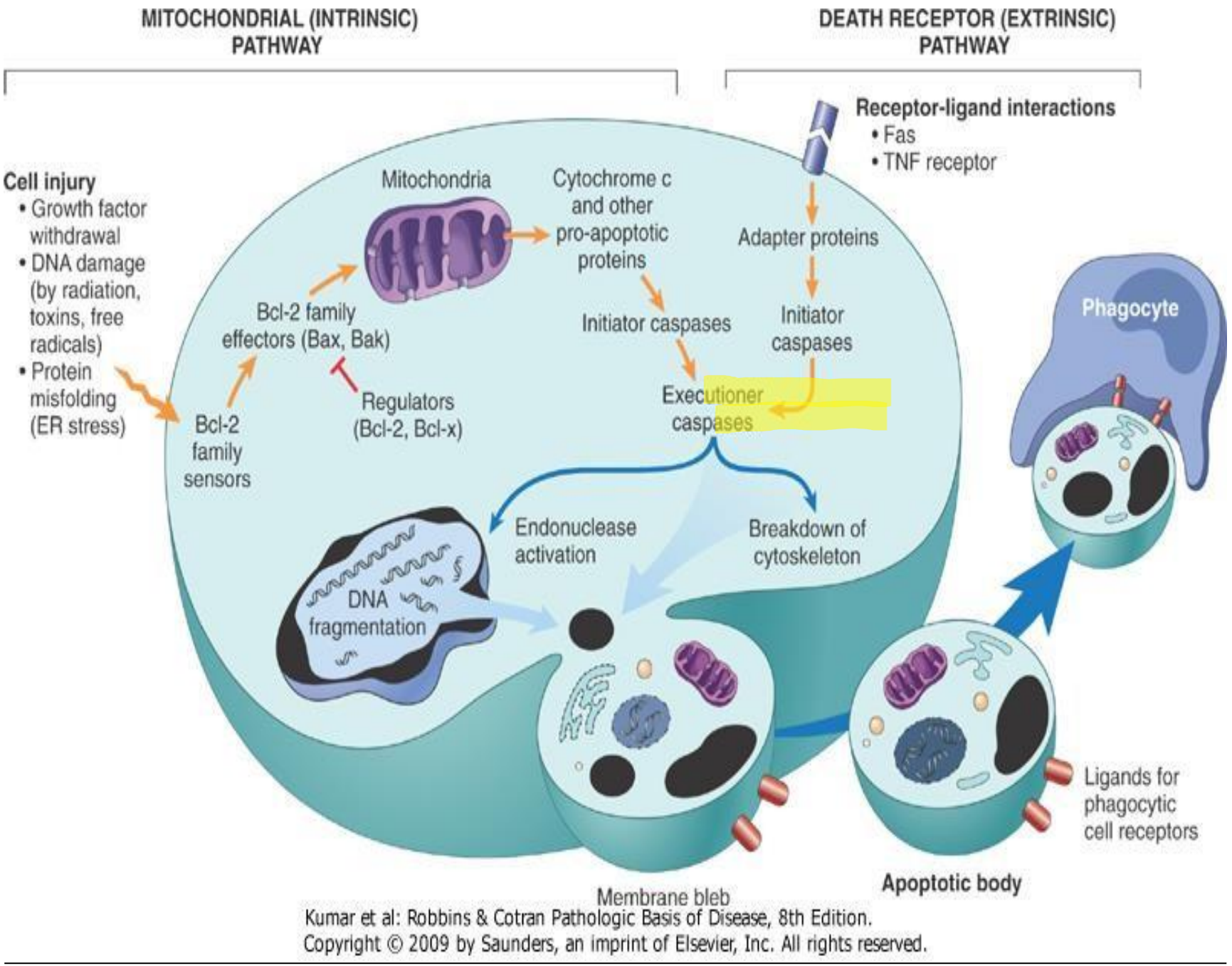
- TNF receptor family, cytoplasmic death domain
- Prototypes: Type 1 TNF receptor and Fas
- Fas ligand on activated T lymphocytes
- Fas –FasL interaction activates death domain which in turn activates caspase 8

These receptors which are called tumor necrosis factor receptors (TNF receptors) has two types: Type 1 TNF and Fas.
The Fas type interacts with a FasL ligand that is present on the surface of T lymphocytes, the interaction between the Fas receptor and its ligand activates the death domain of the receptor, this domain upon activation will activate the cytoplasmic enzyme caspase-8, caspase-8 then initiates a cascade of other caspases, ultimately leading to apoptosis.

- **Used in:**
- Elimination of self-reactive lymphocytes
- killing of target cells by some cytotoxic T lymphocytes (CTLs)

Target cells could be virally infected cells, tumor cells that are recognized by CTLs.


FasL
Fas
Death
domain
FADD
Pro caspase-8
Autocatalytic
caspase
activation
Active
caspase-8
Executioner caspases
APOPTOSIS



Both pathways will lead to executioner caspases, these caspases will initiate the apoptosis process by breaking down cytoskeleton, activating endonuclease.
The cell will be fragmented into apoptotic bodies, these apoptotic bodies will attract phagocytes with certain signal mechanism to engulf them.

Autophagy

It's related to apoptosis and the adaptive mechanism **atrophy.**

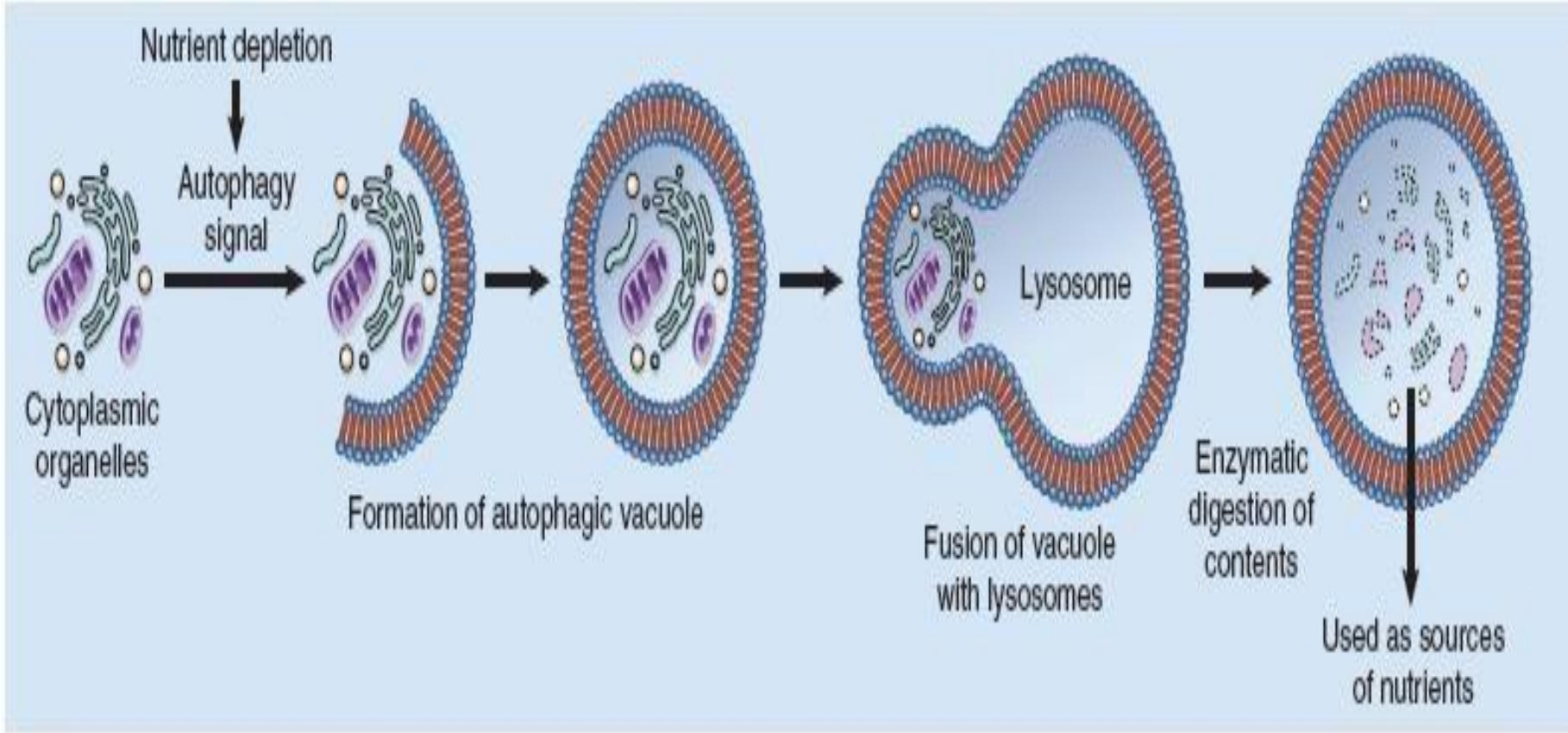
- Self-eating
- Lysosomal digestion of the cells own components
- Survival mechanism in times of nutrient deprivation.
- Recycling cells contents to provide nutrients and energy In times of starvation.

- ER-derived autophagic vacuole
- Vacuole fuses with lysosome >>>autophagolysosome

In autophagy, membrane bound vacuoles form in the cells, these vacuoles are derived from ER membrane, and they are called autophagic vacuoles.
These vacuoles fuse with lysosomes making autophagolysosome, then the lysosomal enzymes will start the process of digestion.

- May lead to atrophy.
- Failure of adaptation >>>apoptosis

The end result will be shrinkage and atrophy of the cell, this is sometimes considered an adaptive mechanism.
When the cell cannot adapt any more in case of continuous state of injury the cell will undergo apoptosis.


Nutrient depletion
Autophagy signal
Cytoplasmic organelles
Formation of autophagic vacuole
Lysosome
Fusion of vacuole with lysosomes
Enzymatic digestion of contents
Used as sources of nutrients

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			

Additional Resources:

رسالة من الفريق العلمي:

اللهم يامن لا يهزم جنده ولا يخلف وعده، ولا إله غيره، كُن لأهلنا في فلسطين عونًا، ونصيرًا، ومعينًا، وظهيرًا.. اللهم احفظ أهل غزة بعينك التي لا تنام، وارزقهم الثبات والقوة والتمكين وبارك في إيمانهم وصبرهم.. اللهم إني استودعك غزة وكُل فلسطين، اللهُم كُن لهم عونًا ونصيرًا يا رب العالمين.. اللهم حرر المسجد الأقصى يا رب اللهم انصر شعب فلسطين ضد المحتلين».