

APOPTOSIS



What makes a cell want to die?

- **Withdrawal of positive signals**

examples :

- growth factors.
- Important cytokines e.g. Interleukin-2 (IL-2)

- **Receipt of negative signals**

examples :

- Increased levels of oxidants within the cell
- Damage to DNA by free radicals or reactive oxygen species.
- Death activators :
 - Tumor necrosis factor alpha (TNF- α)
 - Lymphotoxin (TNF- β)
 - Fas ligand (FasL)



Importance of Apoptosis.

1) Crucial for embryonic development:

-Errors in Apoptosis can lead to Birth Defects

2) Important for maintaining homeostasis:

- Cell death is balanced with mitosis to regulate cell number.

3) Apoptosis helps eliminate cells that are injured:

Such injured cells include:

a) cells with damaged DNA.

b) cells with misfolded proteins

c) cells suffering from certain infections etc.



Characteristics

- It is a process that occurs in almost all living creatures since their early stages of embryological development.
- It is an active cytological process in which energy is consumed (ATP dependent).
- It is programmed or controlled by genetic protocol or program (control of enzymes, cell membrane surface proteins & cytoplasmic molecules, signal transduction, gene expression)
- It may be triggered by intrinsic or extrinsic stimuli



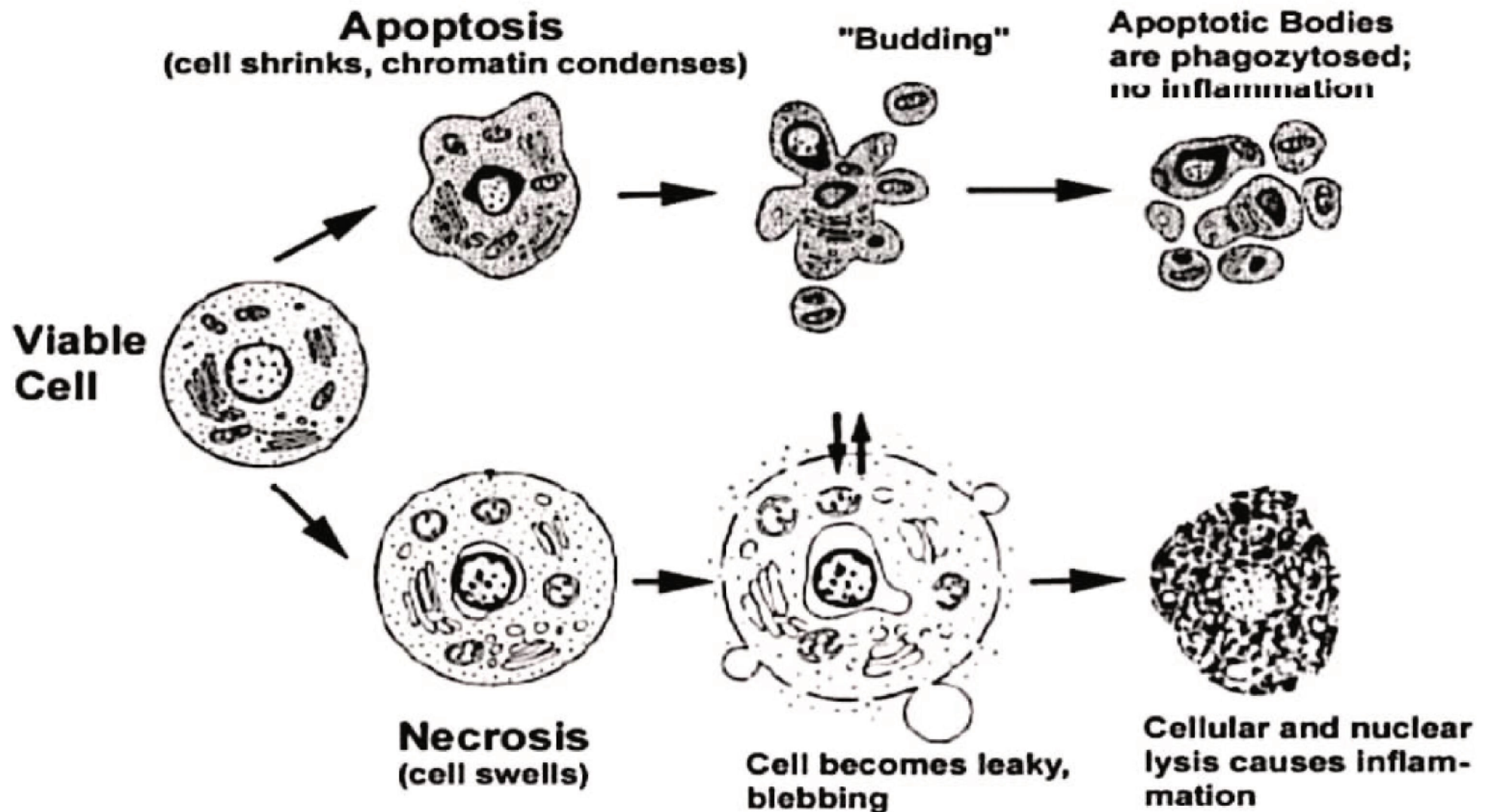
Morphology of Apoptosis.

- The following morphologic features are seen in cells undergoing apoptosis.
- **Shrinkage of cell volume and shape**
- **Chromatin condensation and DNA fragmentation**
(most characteristic feature of apoptosis)
- Formation of surface blebs
- **Fragmentation into apoptotic bodies**
- Phagocytosis of apoptotic bodies by macrophages



Morphologic changes:

Apoptosis vs. Necrosis.



Mechanism of Apoptosis.

- The mechanism of apoptosis is divided into 2 major phases.
- *The Initiation phase:*
- *The execution phase:*
- These 2 different phases, initiated by distinct stimuli and they involve different sets of proteins, although there is some cross-talk between them.
- Both pathways eventually converge with the activation of *Caspase* which are the actual mediators of death.



Intrinsic pathway.

- The intrinsic pathway is the major mechanism of apoptosis in all mammalian cells and its role in a variety of physiologic and pathologic processes is well established.
- This pathway of apoptosis is the result of increased mitochondrial permeability and pro-apoptotic molecules(death inducers) in the cytoplasm.
- Cytochrome c is an important mitochondrial protein necessary for essential life, but when released into the cytoplasm, it initiates apoptosis.



Intrinsic pathway..

- The release of this mitochondrial proteins(cytochrome c) is controlled by the ***balance between pro and anti apoptotic factors*** of the BCL family of proteins.
- There are more than 20 BCL proteins and they function to regulate apoptosis.
- The ***anti apoptotic proteins(BCL-2,BCL-Xl,MCL-1)*** normally reside in the cytoplasm and mitochondrial membranes .
- This anti-apoptotic proteins control mitochondrial permeability and prevent leakage of mitochondrial proteins that have ability to trigger cell death.



Intrinsic pathway...

- When a cell is deprived of critical survival signals or their DNA is damaged or misfolded proteins, sensors of damage or stress are activated.
- These sensors of damage are also proteins that belong to the BCL family of proteins.
- The activated **sensor protein** used in apoptosis include **BIM, BID & BAD**.
- This sensor proteins in-turn activate the **pro-apototic factors: BAK & BAX**.



Intrinsic pathway

- The activation of the proapoptotic molecules coupled with the loss of the anti apoptotic proteins causes the release of Cytochrome-c.
- Once cytochrome-c is released into the cytoplasm it binds with ***APAF(apoptotic protease activating factor)*** to form an **apoptosome**.
- Apoptosome formed causes the activation of CASPASE 9.

Extrinsic pathway.

- This pathway is initiated by the engagement of plasma membrane death receptors on a variety of cells.
- With the extrinsic pathway the cross linking between death receptors and their ligands proteins causes the activation CASPASE 8.

<u>Ligand</u>	<u>Receptor</u>
FasL	Fas (CD95)
TNF	TNF-R



Initiation Phase.

- The initiation phase of apoptosis occurs principally by signals from 2 distinct pathways:
- **The INTRINSIC pathway aka mitochondrial pathway**
- **The EXTRINSIC pathway aka the death domain pathway.**



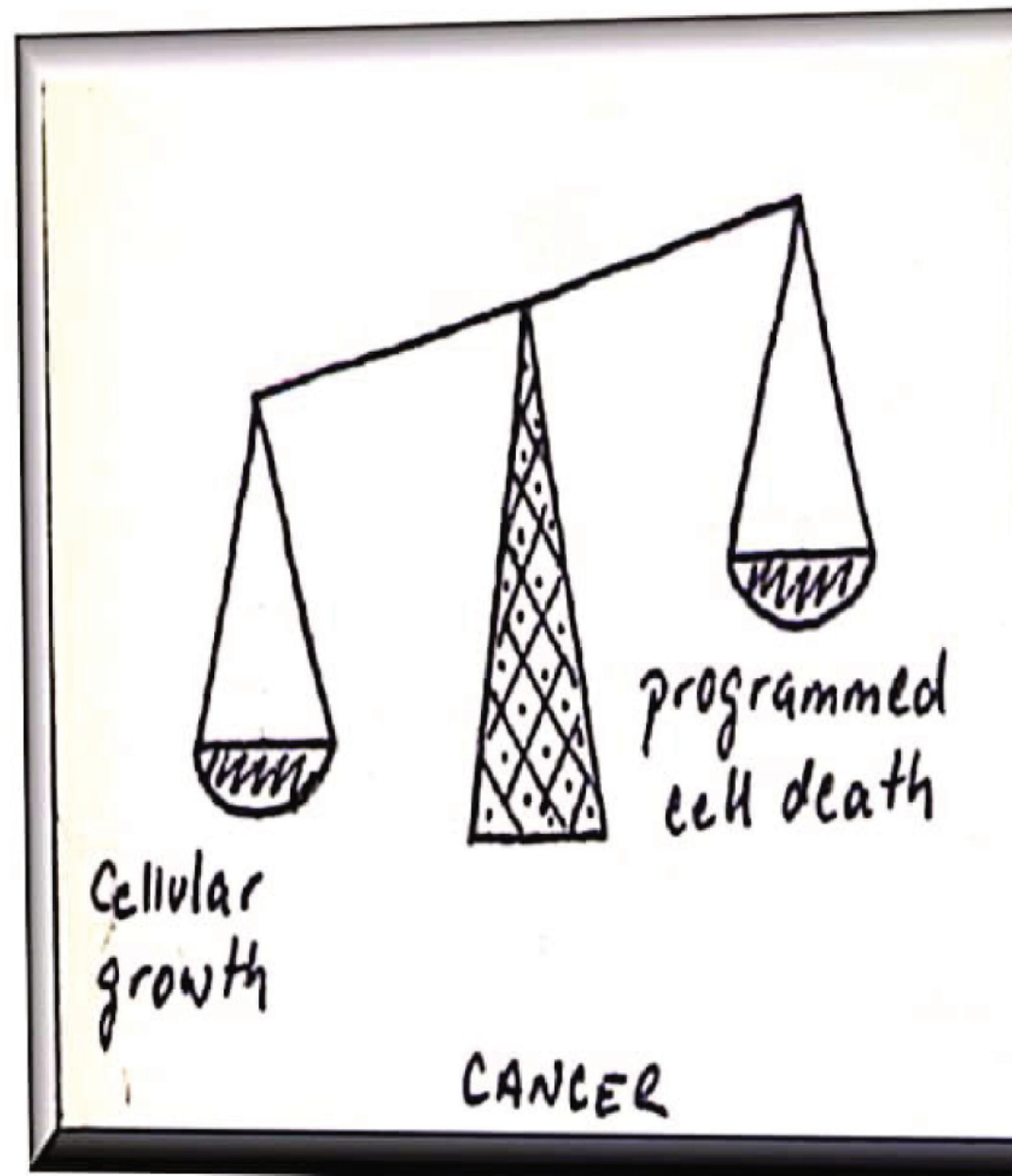
EXECUTION PATHWAY.

- Here the 2 initiating pathways converge to a cascade of caspase activation which mediates the final phase of apoptosis.
- The 2 initiation caspases:
caspase 9 (from intrinsic pathway)
caspase 8 (from extrinsic pathway)
- The activation of executioner caspase (3 & 6).
- Executioner caspases once activated eventually leads to degradation of cellular cytoskeleton, nuclear matrix and promote nuclear fragmentation ultimately leading to cell death.



Dysregulated Apoptosis

- Defective apoptosis and increased cell survival
 - **Cancer**
 - Autoimmune disorders
- Increased apoptosis and excessive cell death
 - Neurodegenerative diseases
 - Ischemic injury
 - Death of virus infected cells



THANK YOU