Morphology of apoptosis

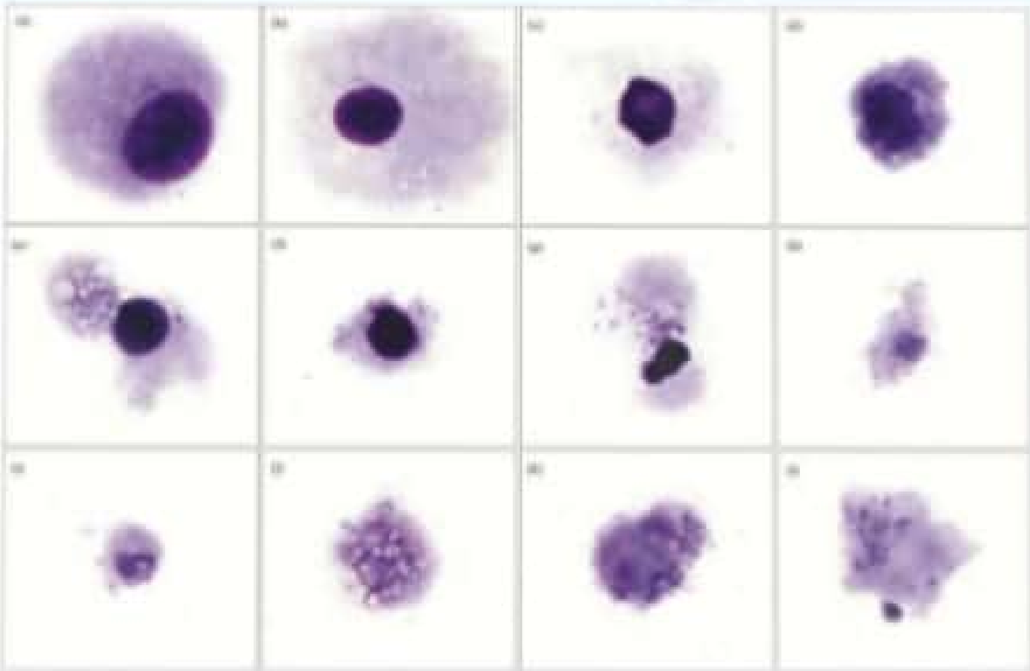


Figure 4. Cells in different stages of apoptosis in treated cultures are easily distinguishable. Most cells had normal morphology (a). Nuclear condensation is evident in cells (dark, condensed and rounded nuclei), as well as vacuolated cytoplasm (b-e). Degradation of nuclei and cytoplasm is also present (f-k). Membrane blebbing and apoptotic bodies are also evident.

Biochemical features of apoptosis

- DNA break down in apoptosis.
- protein cleavage
- phagocytic.

Mechanisms of apoptosis

- **Apoptosis occur in two phases:**

1. **Initiation phase:**

It happen when apoptotic enzymes are getting activated.

1. **Execution phase:**

Activating enzymes are causing cell death.

Mechanisms of apoptosis

- **Initiation phase:**

1. Extrinsic pathway.
2. Intrinsic pathway.

1) Extrinsic pathway

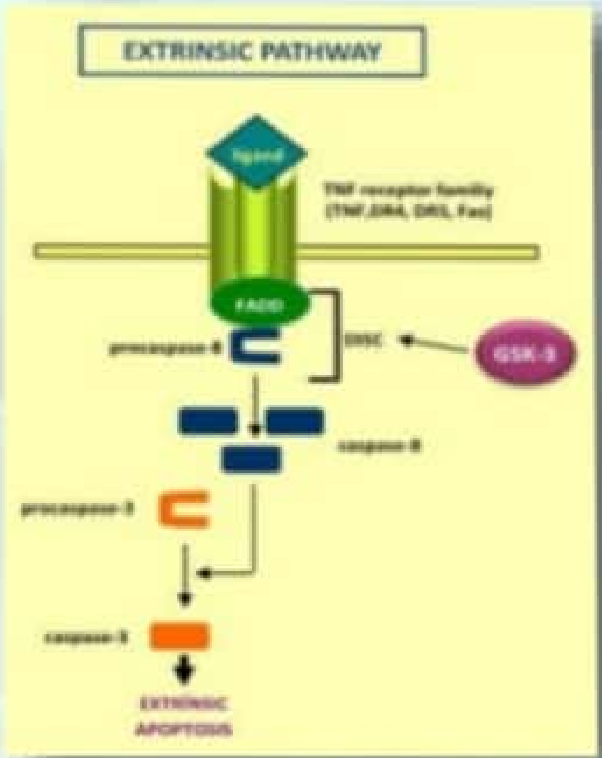
- Called **death receptor pathway**, mediated by:

death receptors.

- **TNF** family protein(tumor necrosis factor) .e.g. TNF R1, TNF fas.
- _**Caspase** are (Cysteine- Aspartic acid) specific proteases that mediates the events that are associated with programmed cell death.

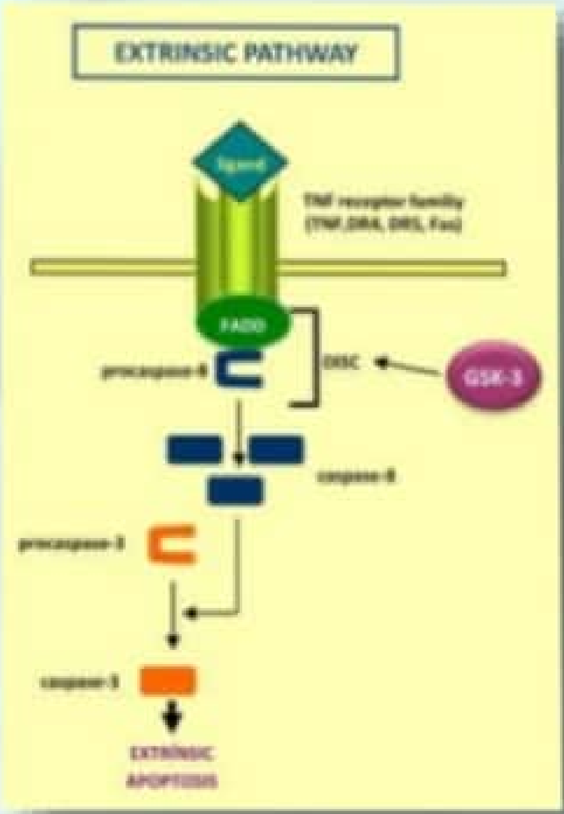
1) Extrinsic pathway

- Apoptosis initiated by extrinsic pathway.
- In cytoplasmic side death receptor has death domains.



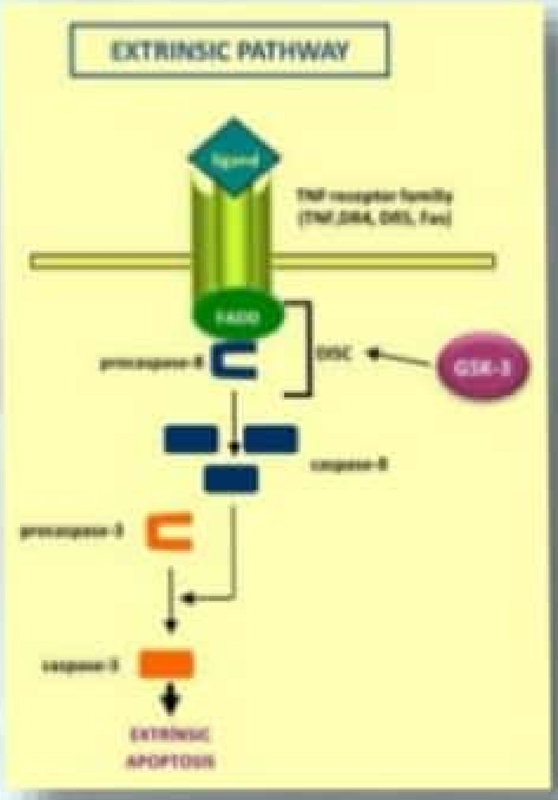
1) Extrinsic pathway

- Fas L(fas ligand) bind to fas receptor, and form cross link between three or more fas receptor then they can form binding site for adaptor molecule called **death domain,** which attract inactivated pro_ caspases and FADD(fas associated death domain).



1) Extrinsic pathway

- **Caspases 8** start to cleave other pro_ caspases to result active form of caspases.
- These caspases go to execution phase and causing apoptosis.

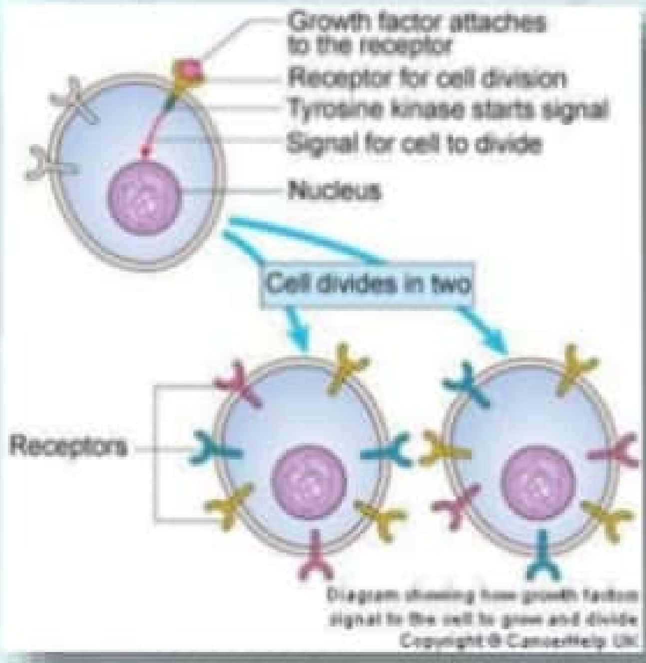


2) Intrinsic pathway

- Known as mitochondrial pathway.
- Apoptosis occur due to increase permeability of mitochondria.

2) Intrinsic pathway

- **In normal situation**,
- growth factor bind to growth receptor in plasma membrane which causing formation of some **anti_ apoptotic protein** .e.g. **Bcl2, Bcl X** in mitochondria membrane.
- Those will prevent leakage of pro_ apoptotic molecule.



ANTI-APOPTOSIS	PRO-APOPTOSIS
Bcl-2	Bax
Bcl-XL	Bad
Bcl-W	Bid
Mcl-1	Bok
A1	Bik
	Bak

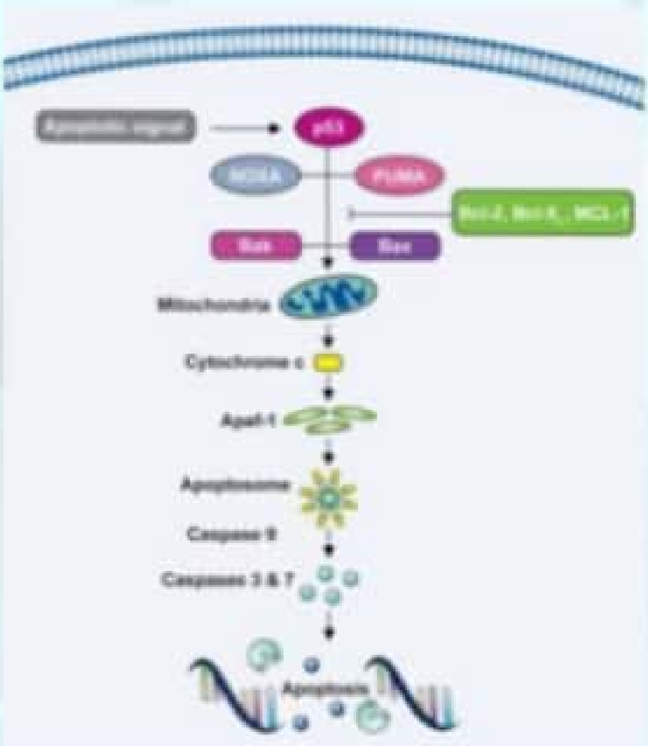
2) Intrinsic pathway

- Radiation and other factors assist to remove these signal e.g. Bcl 2, and BclX via other type of **pro_ apoptotic proteins** such as **Bak, and Bax.**

- Increasing the permeability of mitochondria resulting leakage of pro_ apoptotic molecules from mitochondria to cytoplasm.

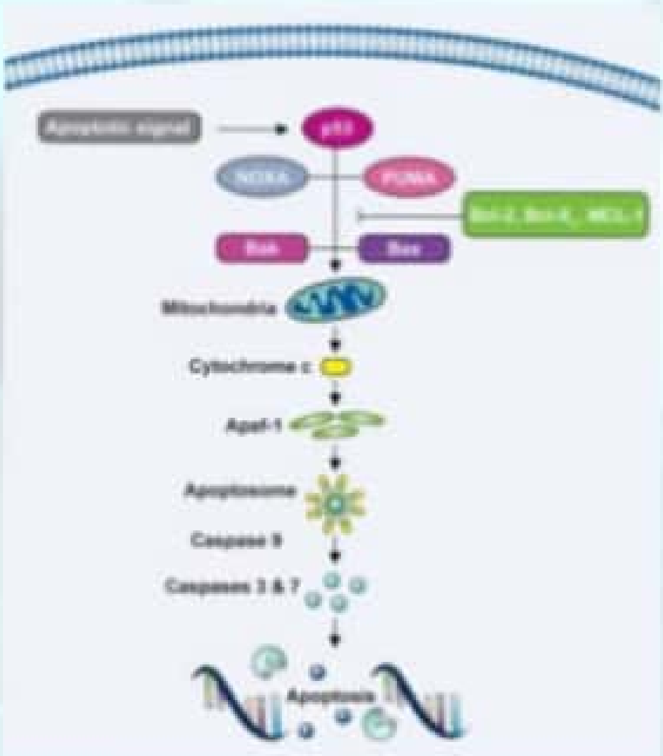
2) Intrinsic pathway

- **In cytosol,**
- Pro-apoptotic Factors Damage to the mitochondrial membrane increasing permeability Entry of **Cytochrome C** into the cytoplasm
- Cytochrome c will bind to another molecule known as **ApaF1** (apoptosis activity factor 1) which activate **caspases 9** resulting apoptosome .



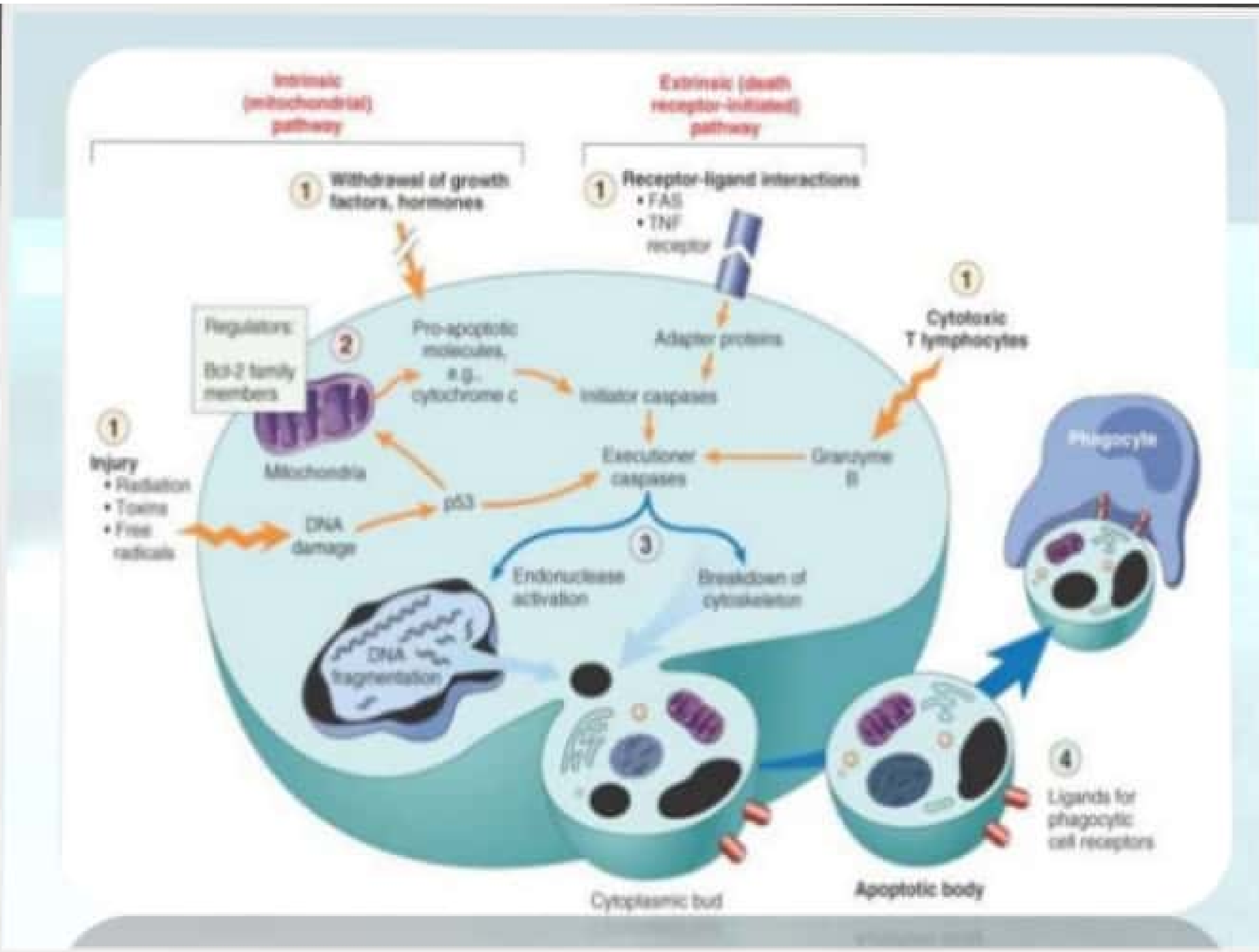
2) Intrinsic pathway

- Apoptosome activates procaspase-9 to caspase-9
- Caspase-9 cleaves and activates **pro_ caspase-3** and **pro_ caspase-7**.
- This executioner caspases activate a cascade of proteolytic activity that leads to apoptosis.



2) Intrinsic pathway

- Other factor releasing form mitochondria is **apoptosis inducing factor** that neutralize anti_ apoptosis factor and promote apoptosis.


Intrinsic (mitochondrial) pathway
Extrinsic (death receptor-initiated) pathway
1 Withdrawal of growth factors, hormones
1 Receptor-ligand interactions
• FAS
• TNF receptor
1 Cytotoxic T lymphocytes
Regulators: Bcl-2 family members
2
Pro-apoptotic molecules, e.g., cytochrome c
Adapter proteins
Initiator caspases
Mitochondria
1 Injury
• Radiation
• Toxins
• Free radicals
DNA damage
p53
Executioner caspases
Granzyme B
Phagocyte
3
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
4 Ligands for phagocytic cell receptors
Cytoplasmic bud
Apoptotic body

Execution phase

- It is mediated by **caspase 3 and caspase 6** , recall caspase 8 and caspase 9(initiation caspases).
- When it's activated, they form sequence chain of reaction that can activate Caspase 3 and 6.
- They **break down cytoskeleton protein**, and **nuclear matrix protein** that resulting **breaking the nucleus**.