# E-content for Programme: M.Sc. Zoology (Semester - IV)
# EC - 1A Elective paper: Cell and Molecular Biology

## Unit II: (B) Apoptosis

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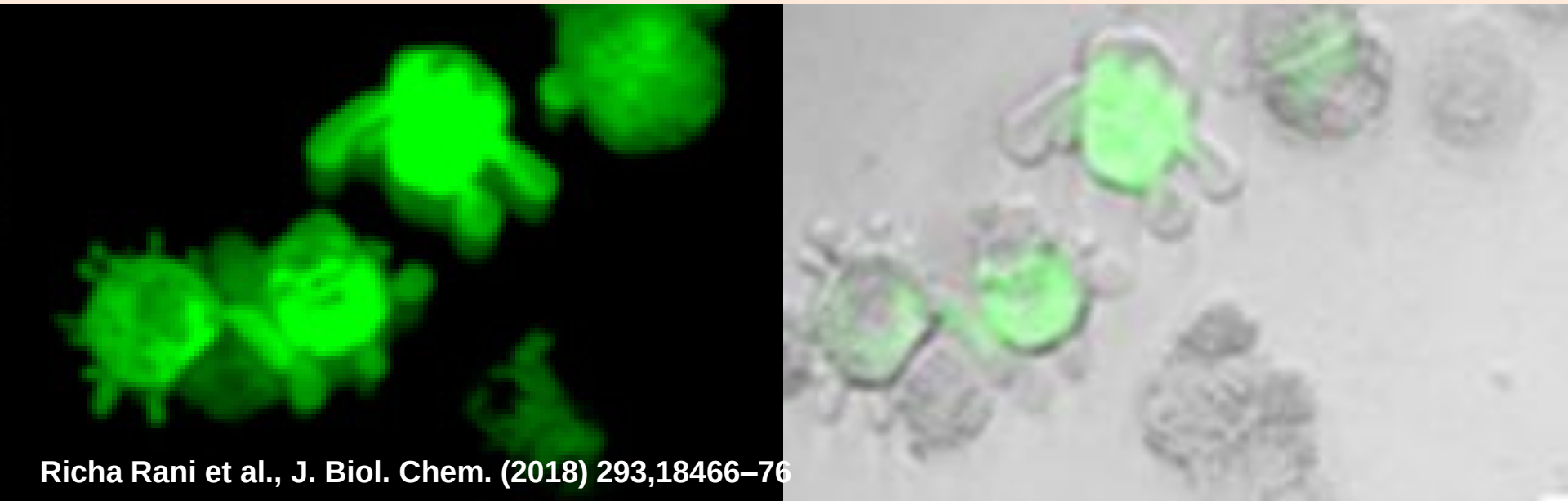
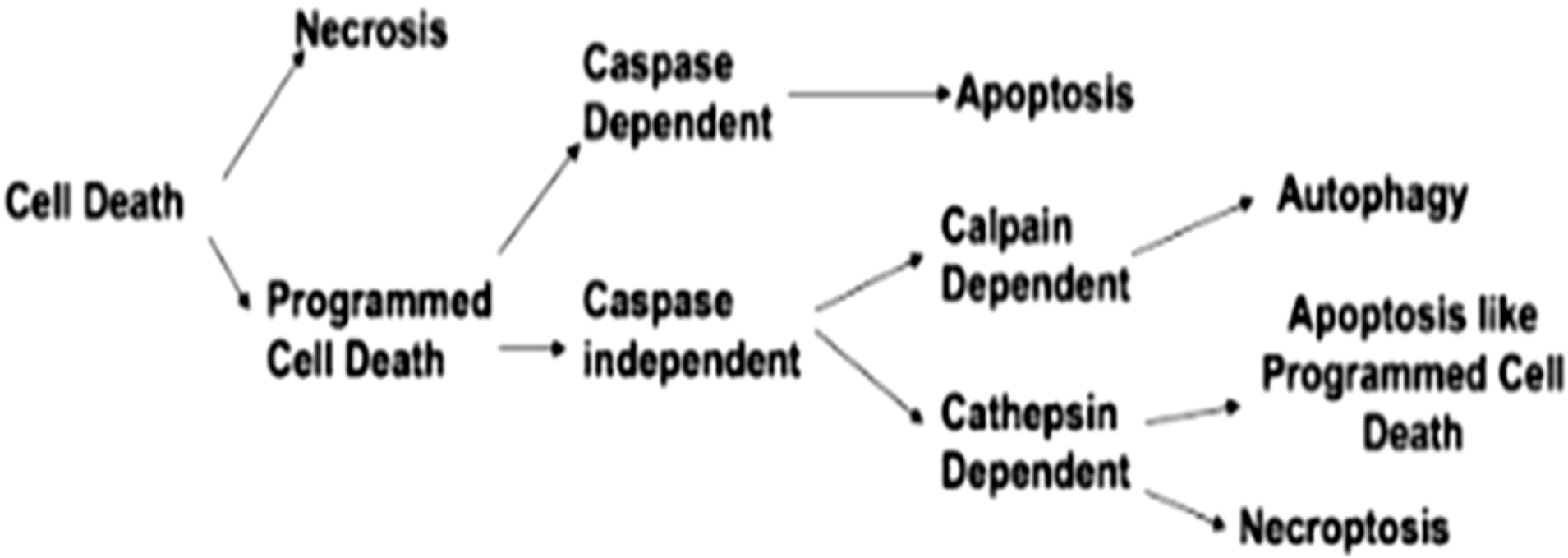


Richa Rani et al., J. Biol. Chem. (2018) 293,18466–76

# Overview of Cell death




Seminars in Cancer Biology, 35, 2015, S78-S103

# Classification of Cell death

According to the nuclear morphology of the dying cell, cell death can be classified into four subclasses:

**1. Apoptosis (Type I cell death):**

Apoptotic morphology includes chromatin condensation into compact figures, which are often globular or crescent shaped. Furthermore, shrinkage of the cell, membrane blebbing, and the formation of apoptotic bodies are important features. Apoptotic cell death is dependent of caspase 3 and caspase-activated DNAse.

**2. Apoptosis-like PCD:**

It is characterized by chromatin condensation that is less compact but which gives more complex and lumpy shapes and is caused by apoptosis inducing factor, endonuclease G, cathepsins, or other proteases. Combination with other apoptotic features can be seen.

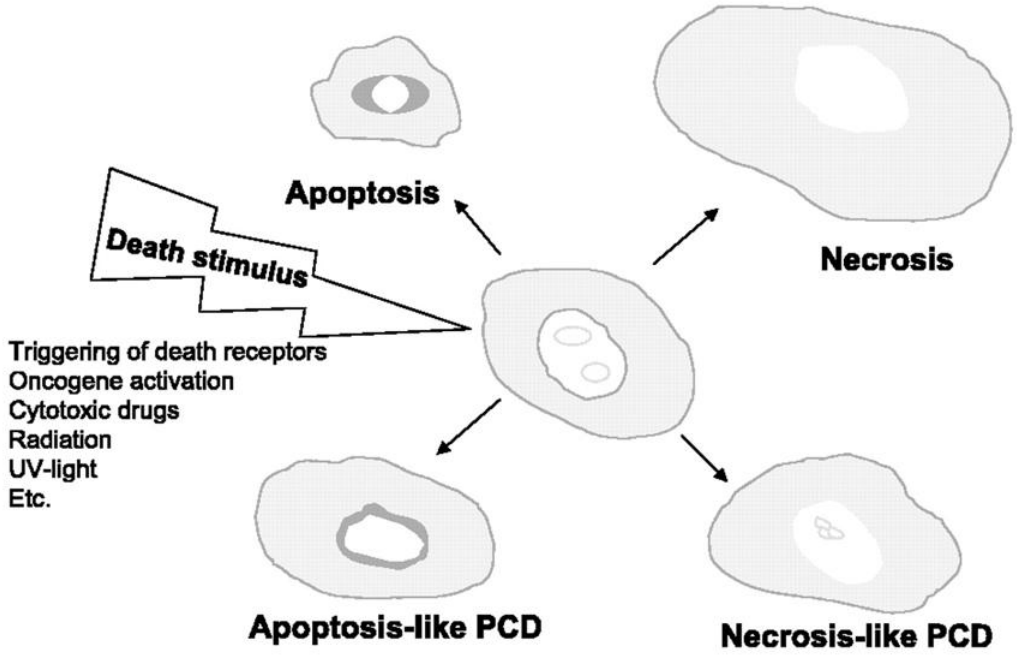
# Classification of Cell death (Cont'd)

**3. Necrosis (Type III cell death):**

Necrosis represents passive cell death without underlying regulatory mechanisms. Key features of necrosis include cytoplasmic swelling, cell membrane rupture, and inflammation.

**4. Necrosis-like PCD:**

No chromatin condensation is observed, but at best, chromatin clustering to




Clin Cancer Res 2005;11:3155-3162

# Apoptosis

- It is a fundamental cellular mechanism to eliminate abnormal cells that are potentially detrimental.

- Apoptosis or programmed cell death (PCD) is energy-dependent biochemical mechanism that plays critical role in the regulation of physiological growth control and tissue homeostasis.

- Mechanisms of programmed cell death in mammalian cells was transpired from the study of apoptotic mechanism during the development of the nematode *Caenorhabditis elegans* (Horvitz, 1999).

- Apoptosis dysregulation contributes to several important diseases, including cancer.

✓***However, apoptosis is not the only mechanism in controlling tumor cell proliferation and cell death, other modes of cell death, such as, necrosis, autophagy, mitotic catastrophe etc. have also been reported.***

# Apoptosis: Characteristic features

| Biochemical features | Morphological features |
| --- | --- |
| Activation of caspase | Cell rounding |
| DNA fragmentation | Nuclear condensation |
| Mitochondrial transmembrane potential (ΔΨm) dissipation | Plasma membrane blebbing |
| Cell surface phosphatidylserine exposure | Apoptotic body formation |
| Plasma membrane blebbing | |
| Apoptotic body formation | |

# Apoptosis *vs* Necrosis

| Apoptosis | Necrosis |
|---|---|
| Known as Type I cell death | Known as Type III cell death |
| Energy-dependent process | Energy-independent process |
| Affects individual or small clusters of cells | Often affects contiguous cells |
| Cell shrinkage and convolution | Cell swelling |
| Pyknosis and karyorrhexis | Karyolysis, pyknosis, and karyorrhexis |
| No loss of membrane integrity | Loss of membrane integrity |
| Cytoplasm is retained in the apoptotic bodies | Cytoplasmic contents are released into the surrounding interstitial tissues |
| No inflammatory reaction | Inflammation usually present |

# Major organelles involved In PCD

- Each organelle senses specific stimuli such as oxidative stress, TNF-α, chemotherapeutic drugs, etc., activates signal transduction pathways and emits signals that facilitate inter-organellar cross-talk.

- Organelle-specific death responses are ultimately coupled to either mitochondrial outer membrane permeabilization (MOMP) or the activation of effector caspases.

- **Mitochondria:** Upon apoptotic stimuli, mitochondria releases cytochrome c in the cytoplasm, and in the presence of ATP, “apoptosome” is formed together with Apaf-1 and caspase 9. This triggers the classic apoptotic cascade, leading to apoptotic cell death.

- **Lysosomes:** Upon membrane destabilization, lysosomes release cathepsins that are endowed with the capacity of triggering MOMP.

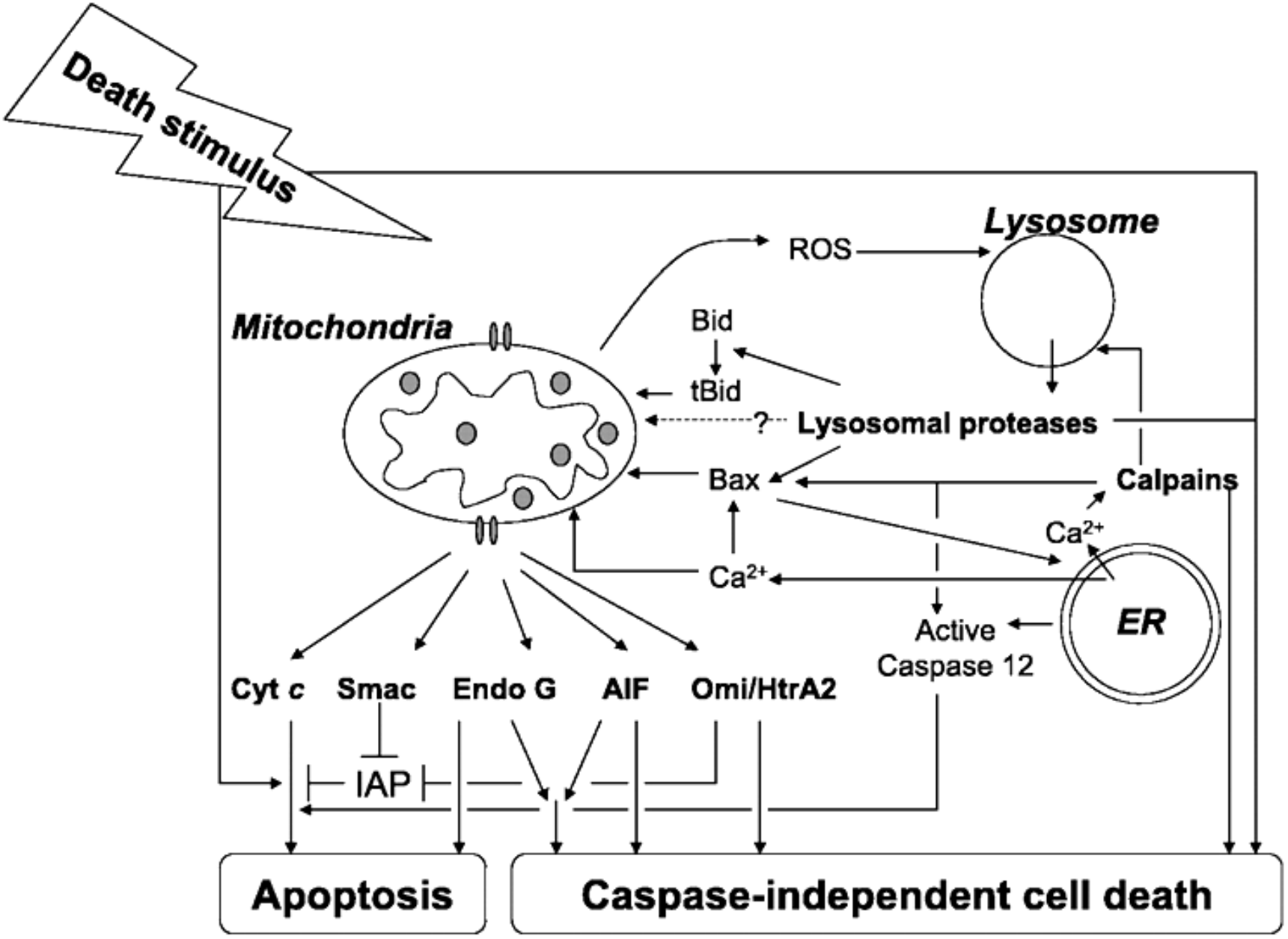
Ferri, K., Kroemer, G. *Nat Cell Biol* **3,** E255–E263 (2001).

# Major organelles involved In PCD (Cont'd)

- **Endoplasmic reticulum (ER):** ER-mediated cell death can be initiated through the unfolded protein response or through mobilization of calcium, leading to the activation of cytoplasmic death pathways. ER stress can induce MOMP and thus activate the classic apoptotic pathway as well as other mitochondrial death pathways. Bcl-2 family proteins as well as intracellular calcium influx caused by ER stress (via activation of a family of cytosolic proteases i.e. calpains, calcium-activated neutral proteases) orchestrate the cross talk between the mitochondria and the ER.

- **Golgi apparatus:** The Golgi apparatus constitutes a privileged site for the generation of the pro-apoptotic mediator ganglioside GD3, facilitates local caspase-2 activation and might serve as a storage organelle for latent death receptors. Under the influence of an apoptogenic stimulus, the Golgi apparatus undergoes fragmentation and redistributes in the cytoplasm, sometimes forming dense aggregates.

# Major organelles involved In PCD (Cont'd)




Clin Cancer Res 2005;11:3155-3162

# Regulation of Apoptosis

**Apoptosis is regulated by two major pathways:**

1. **Extrinsic (death receptor-mediated) pathway:** The extrinsic pathway facilitates apoptosis by activation of effector caspases through the cell-surface death receptors, which respond to cognate death ligands expressed on immune-effector cells.

2. **Intrinsic (mitochondria-mediated) pathway:** The intrinsic apoptotic pathway engages caspases via members of the Bcl-2 protein family and the mitochondria in reaction to severe cellular damage or stress.

*Cysteine proteases called caspases are the key effector molecules in apoptosis and are potential targets for pharmacological modulation of cell death.*

# The mechanism and consequence of Extrinsic pathway

- In the extrinsic (or death receptor-regulated) pathway, extracellular pro-apoptotic signals leads to engagement of the ligands and their respective receptors. For ex- FAS/CD95 ligand binds to CD95, TNFα binds to TNFR1, TNF-related apoptosis inducing ligand (TRAIL) binds to TRAILR1–2.

- Binding of ligands to death receptors initiates the formation of multiprotein complex called death-inducing signaling complex (DISC), resulting in activation of initiator caspase-8.

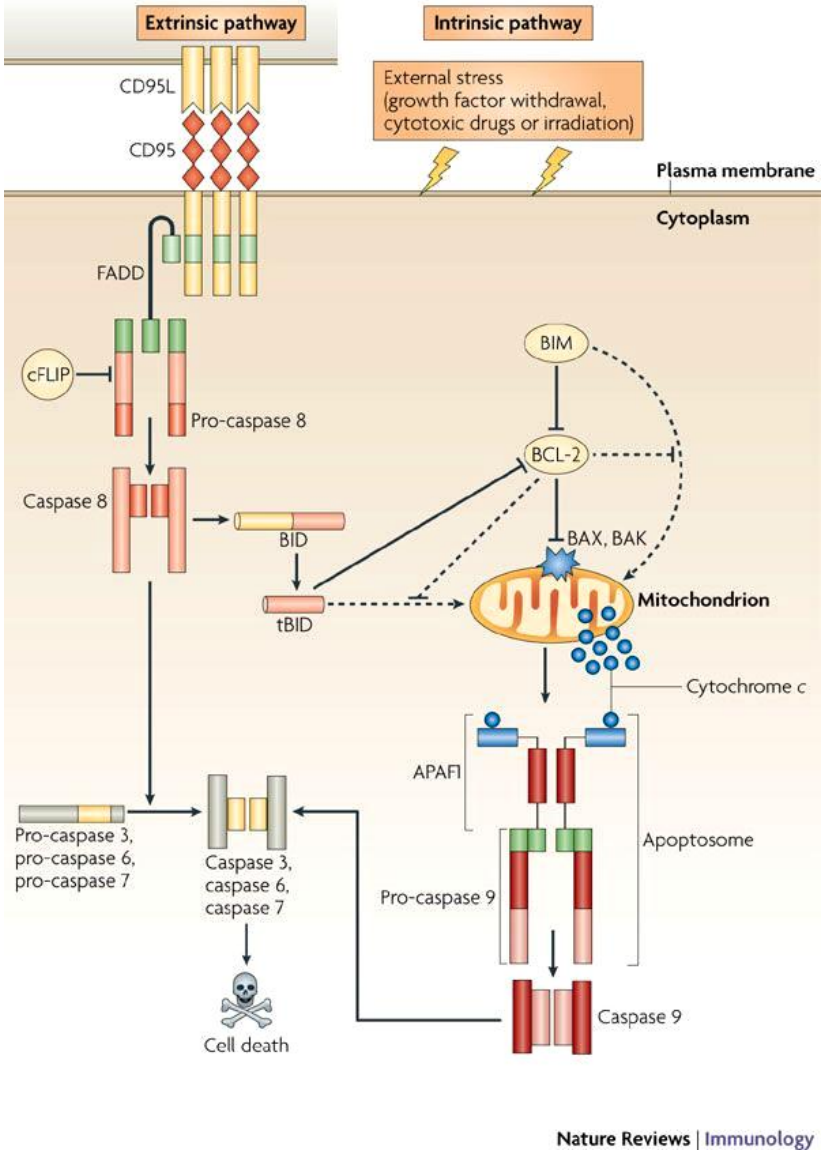
- Active caspase 8 then activates caspase-3, thereby triggering caspase-dependent apoptosis. Alternatively, caspase-8 mediates the cleavage of Bcl2-protein family member BH3-interacting domain death agonist (BID), resulting in a pro-apoptotic truncated BID (tBID), inducing subsequent mitochondrial outer membrane permeabilization, release of CytC from mitochondria and triggering caspase9-dependent apoptosis.

# The mechanism and consequence of Intrinsic pathway

- In the intrinsic (or B cell lymphoma 2 (BCL-2)-regulated) pathway, external stress signals such as growth factor deprivation, exposure to cytotoxic drugs or radiation mediate the activation of Bcl-2-homology domain 3-only proteins such as Bcl-2-interacting mediator of cell death (BIM), which in turn activate BCL-2-associated X protein (BAX) and Bcl-2 antagonist/killer (BAK) either directly (dotted line) or indirectly (solid line) by interacting with pro-survival members of the Bcl-2 family, such as Bcl-2.

- Oligomerization of proteins such as BAX and BAK induce changes in mitochondrial membranes permeability which causes the release of pro-apoptotic proteins such as cytochrome c (Cyt C), second mitochondria-derived activator of caspases/direct IAP-binding protein with low pI (SMAC/DIABLO), high-temperature requirement protein A2 (HTRA2/Omi) and others.

# The mechanism and consequence of Intrinsic pathway (Cont'd)




*Nature Reviews Immunology* 9, 514–519 (2009)

- Cyt C, once released into the cytoplasm, forms apoptosome (by binding to apoptotic protease activating factor 1 (Apaf-1) and dATP) and triggers the activation of initiator caspase-9, which in turn activates effector caspase-3.
- HTRA2/Omi and SMAC/DIABLO antagonize inhibitors of apoptosis proteins (IAPs), thereby activating caspases.
- Cleavage of the BH3-only protein BH3-interacting-domain death agonist (BID) by caspase 8 after CD95 induction links the two pathways in some cell types (for example, in hepatocytes).

# Genes that regulate Apoptosis

- The Bcl-2 family of proteins consists of both anti-apoptotic proteins as well as pro-apoptotic proteins.

| Anti-apoptotic proteins | Pro-apoptotic proteins |
|---|---|
| Bcl-2: B-cell lymphoma 2 | Bax: Bcl2-associated protein X |
| Bcl-$X_{L:}$: B-cell lymphoma extra large | Bak: Bcl2 antagonist/killer |
| Mcl-1: Induced myeloid leukemia cell differentiation protein | Bid (tBid):(truncated) BH3-interacting domain death agonist |
| BCL2L2/Bcl-w: Bcl-2-like-protein-2 | Bim: Bcl2-interacting mediator of cell death (Bcl2-like 11) |
| BCL2L10: Bcl-2-like-protein-10 | Puma: p53 upregulated modulator of apoptosis |
| A1/Bfl1: Bcl-2 related protein A1 | Noxa: Phorbol-12-myristate-13-acetate-induced protein 1 |
| | Bad: Bcl2-associated agonist of cell death |

# Ions and Ion channels also regulate Apoptosis

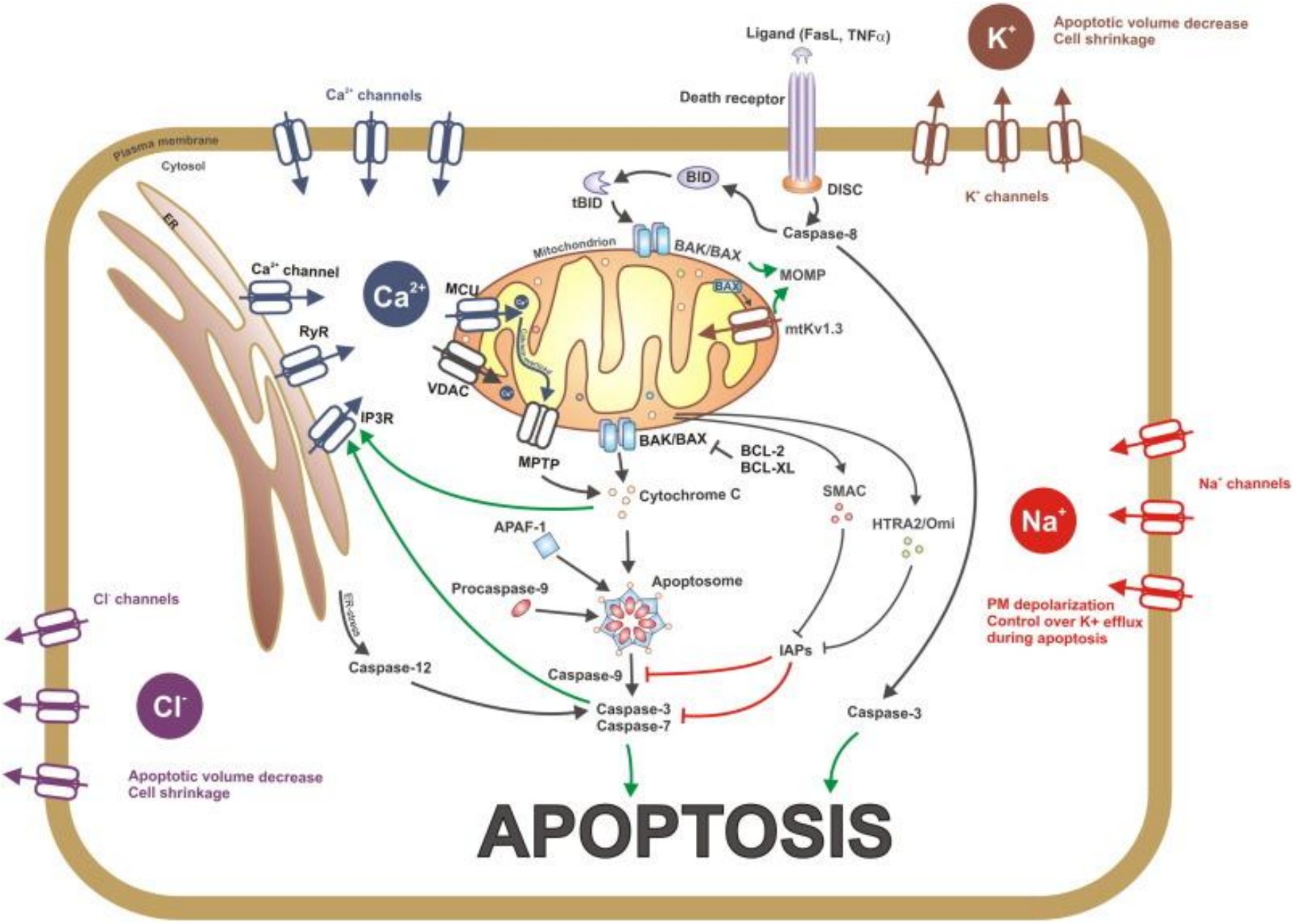
- Interplay between ion channels and apoptosis dysfunctions have been implicated in cancer initiation and progression as well as chemotherapy resistance.

- Various cytoplasmic and extracellular ion concentrations as well as ion channels in the regulation of apoptosis in cancer have been reported, however their contribution varies depending on cancer types.

- Major types of ions/ion channels involved in apoptosis regulation are:

  - Calcium and calcium-permeable channels
  - Potassium and potassium channels
  - Sodium and sodium channels
  - Chloride and chloride channels

# Ions and Ion channels also regulate Apoptosis (Cont'd)




Biochimica et Biophysica Acta (BBA)-Biomembranes, 1848, 2015, 2532-46.

# Caspase-independent Cell death (CICD)

Cell death may occur in a programmed fashion but in complete absence and independent of caspase activation. Major categories are:

**Paraptosis:**

A type of programmed cell death characterized by dilation of the endoplasmic reticulum and/or mitochondria. Involves cytoplasmic vacuolation, mitochondrial swelling in the absence of caspase activation or typical nuclear changes.

**Mitotic catastrophe:**

Occurs as a default pathway after mitotic failure caused by defective cell cycle checkpoints, development of aneuploid cells, and eventual necrosis-like death and centrosome aberration. Most commonly found in tumor cells with impaired p53 function exposed to various cytotoxic and genotoxic agents.

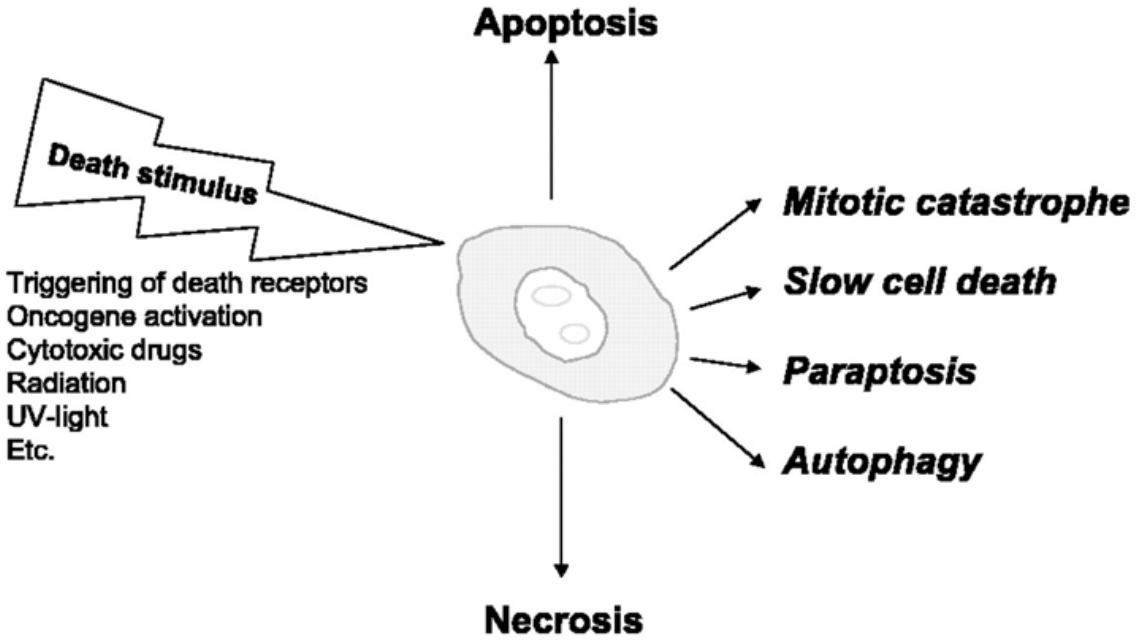
# Caspase-independent Cell death (CICD) (Cont'd)

**Slow cell death:**

It was proposed by Blagosklonny (*Leukemia 2000;14:1502–8.*) to describe the delayed type of programmed cell death that occurs if caspases are inhibited or absent.

**Autophagy (Type II cell death):**

It is characterized by sequestration of cytoplasm or organelles in autophagic vesicles and their subsequent degradation by the cell's own lysosomal system.

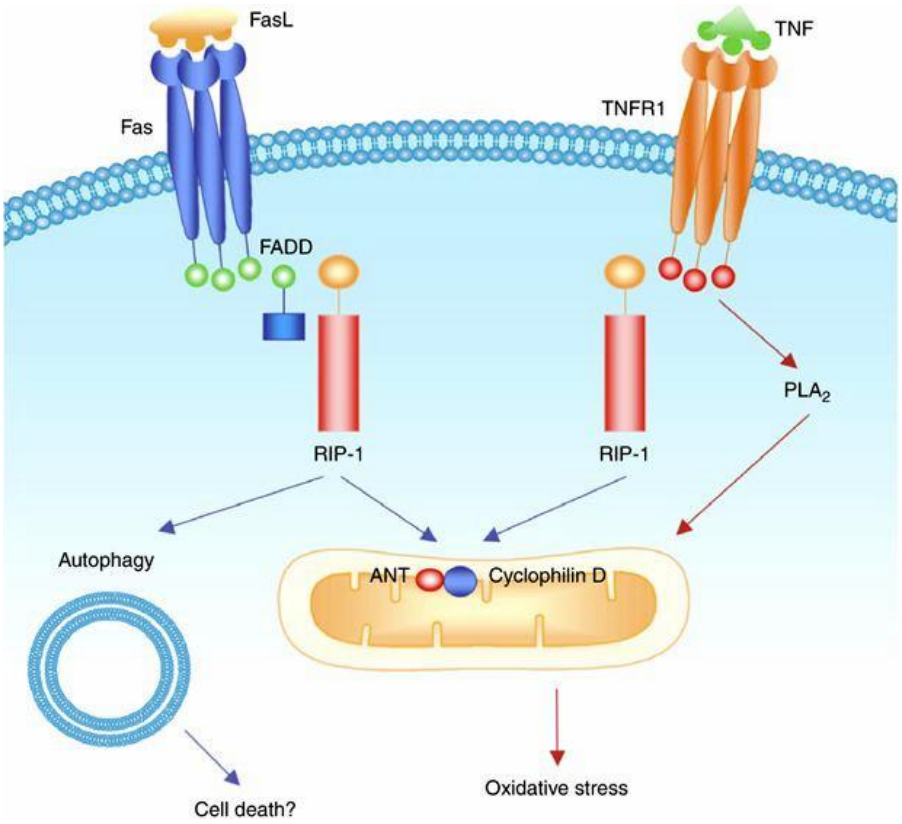



Clin Cancer Res 2005;11:3155-3162

# Mechanisms of CICD

- **Death receptor-induced necroptosis:**

In the absence of caspase activity, death receptor activation leads to necroptosis through the upregulation of Phospholipase $A_2$ that, cause increased oxidative stress. Necroptosis can also be triggered by the activation of Receptor-interacting protein-1 kinase that directly affects mitochondrial function or autophagy.

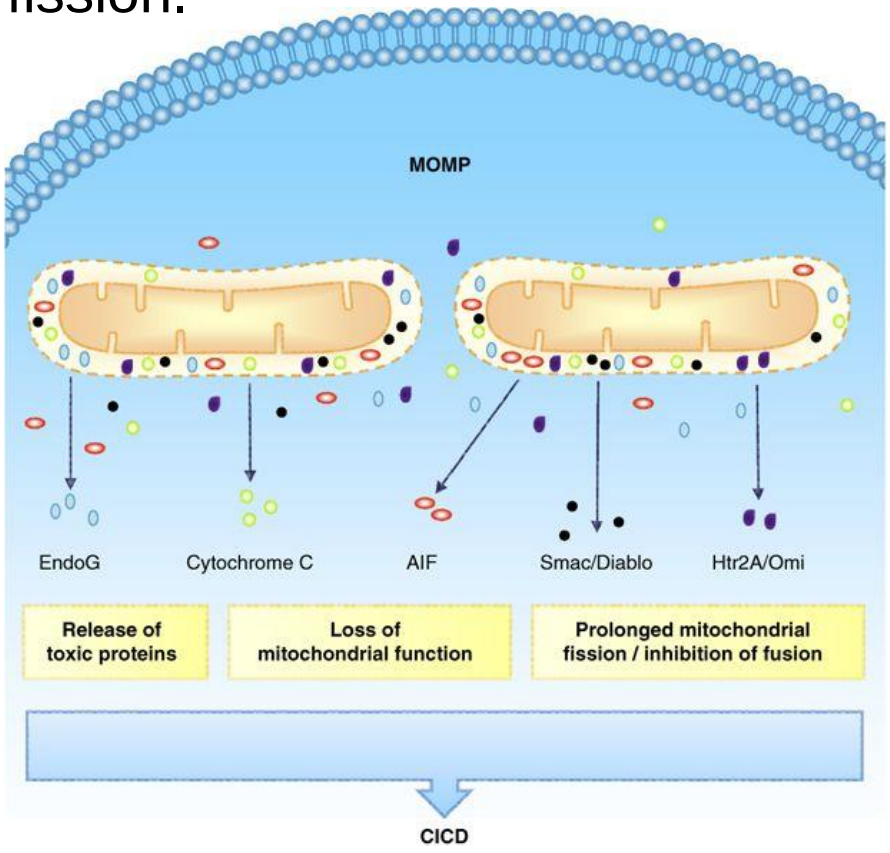



Oncogene, 27, 6452–6461(2008)

# Mechanisms of CICD (Cont'd)

- **MOMP-induced CICD:**

In the absence of caspase activity, MOMP-inducing stimuli trigger cell death cause release of mitochondrial intermembrane space proteins eventually leading to loss of mitochondrial function or disruption of mitochondrial morphology via activation of mitochondrial fission.




Oncogene, 27, 6452–6461(2008)

# Apoptosis *vs* CICD

| | Apoptosis | Caspase-independent cell death (CICD) |
|---|---|---|
| **Nucleus** | Cleavage of caspase targets<br>DNA laddering<br>Nuclear fragmentation<br>Chromatin condensation | Partial chromatin condensation<br>Chromatin marginalization around the nuclear membrane<br>No DNA laddering<br>Nuclear shrinkage but no fragmentation |
| **Mitochondrion** | Outer-membrane permeabilization<br>Cleavage of caspase targets<br>Rapid loss of membrane potential<br>Activation of fission/inhibition of fusion | Outer-membrane permeabilization<br>Swollen cristae<br>Gradual loss of membrane potential<br>Activation of fission/inhibition of fusion |
| **Cytoplasm** | Cleavage of caspase targets<br>Increased [$Ca^{2+}$]<br>Protein crosslinking<br>Condensation | Abundant autophagosomes<br>Ribosome aggregation<br>Vacuolated |
| **Plasma membrane** | Cleavage of caspase targets<br>Loss of membrane-phospholipid asymmetry<br>Annexin V-positive<br>Membrane blebbing | Ragged membrane<br>Annexin V-negative |
| **Cell** | Loss of proliferation<br>Detachment from matrix<br>Phagocytosis of dying cell | Loss of proliferation<br>Often remain attached to matrix<br>Removal through unknown means |
| **Kinetics** | Variable up to point of MOMP<br>Rapid execution following MOMP | Variable up to point of MOMP<br>Slow execution following MOMP |

*Adapted from, Oncogene, 27, 6452–6461(2008)*

# Apoptosis-evasion mechanisms

Acquired resistance to apoptosis is the common feature of most and perhaps all types of cancer. Various ways of evading apoptosis are as follows:

**A) Evasion of the Death receptor Pathway:**

- Reduced expression of death signals.
- Downregulation or epigenetic silencing (due to CpG-island hypermethylation of gene promoters) of death receptors (van Noesel et al., 2002; Petak et al., 2003; Debatin et al., 2003).

**B) Evasion of the Mitochondrial Pathway:**

- **Disrupted balance of Bcl-2 Family Proteins:** Increase in anti-apoptotic molecules and decrease in pro-apoptotic molecules.
- **Reduced form of cytochrome c:** The oxidized form, not the reduced form, of cytochrome c induces caspase activation via the apoptosome **(***The Journal of Biological Chemistry*, 282, 31124–31130, 2007.**)**
- **Increased expression of "Inhibitor of Apoptosis" (IAP) proteins.**

# Apoptosis-evasion mechanisms (Cont'd)

**C) At the post-mitochondrial level:**

At the post-mitochondrial level, translation of XIAP and cIAP1 is sustained via an IRES-dependent mechanism even under cellular stress conditions. For example, reduced expression level or activity of apoptotic protease activating factor-1 (Apaf-1) due to promoter hypermethylation or loss of heterozygosity at chromosome 12q22-23, leads to impaired assembly of a functional apoptosome (*Nature*, 409, 207–211, 2001; *Oncogene*, 22, 451–455, 2003).

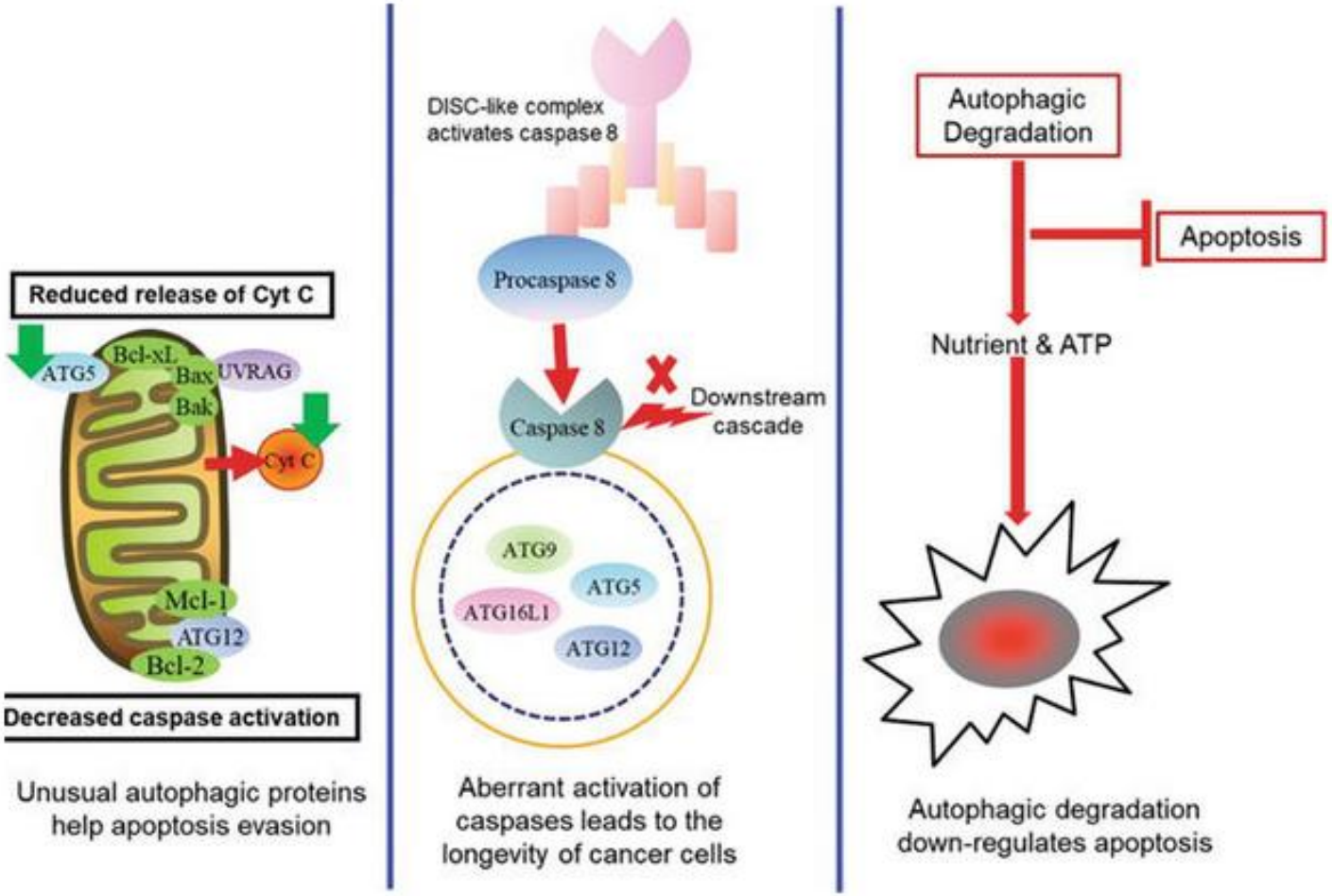
**D) Downregulation of tumor suppressor genes:**

Inactivation, elimination, abnormal expression, and mutation of the p53 gene is the most common defect in human cancer (Oda et al., 2000; Yu et al., 2001).

# Apoptosis-evasion mechanisms (Cont'd)

## E) Abnormal apoptosis-autophagy cross-talk:

In the tumor microenvironment, autophagy supplies nutrients to cancer cells and promotes tumor growth.




*https://www.intechopen.com/books/current-understanding-of-apoptosis-programmed-cell-death/role-of-apoptosis-in-cancer-resistance-to-chemotherapy*

# Thank You!