APOPTOSIS:
SOME GENERAL NOTIONS

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Definition

ΑΠΟΠΤΩΣΙΣ: from the Greek: « the fall », as for leaves in Autumn, by opposition to necrosis = accidental death.

Synonymous: programmed cell death, cell suicide.

Survival = active pathway

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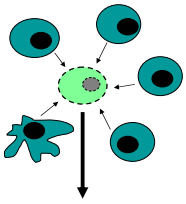
Apoptosis = default pathway

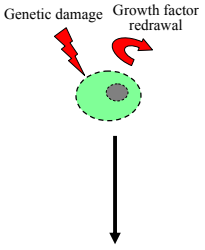
« SOCIAL CONTROL »



APOPTOSIS

- Cell numbers
- Geographic localization
- Tissue composition

« INTRINSIC CONTROL »



APOPTOSIS

Elimination of altered cells

Apoptosis and physiology

- **DEVELOPMENTAL LIFE:**
  - Morphogenesis (hand…)
  - Sexual differentiation
  - Nervous system establishment
  - Immune system establishment

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- **ADULT LIFE**:
  - Tissue homeostasis
  - Elimination of damaged or abnormal cells
  - Defense against infections
  - Maintenance of self-tolerance

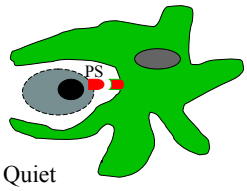
Cellular changes during apoptosis

- Cell shrinkage
- Plasma membrane blebbing with partial maintenance of impermeability
- Phospholipid externalization => ingestion by neighboring competent cells.
- Mitochondria outer membrane permeabilization
- Nuclear chromatin condensation and fragmentation
- Genomic DNA fragmentation
- Cytoskeletal modification
- Segmentation into apoptotic bodies

Cellular changes during necrosis

- Due to toxic stimuli such as hyperthermia, metabolic poisons, direct cell trauma =>
- Cell swelling
- Plasma cell rupture
- Cell content swelling

Apoptosis



Quiet
but not silent death

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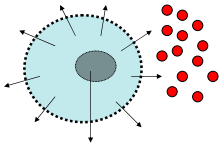
Immune response
Immune tolerance…

Necrosis



Inflammation

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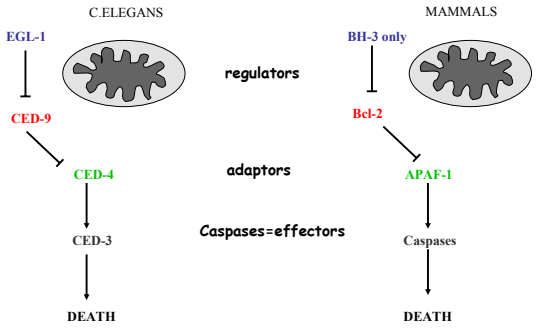
Tissue destruction

Molecular aspects of apoptosis

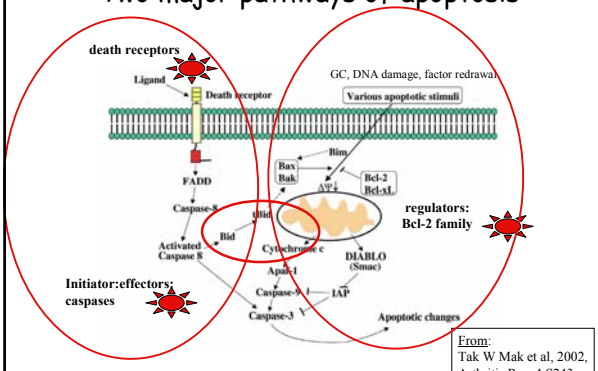
Apoptosis: a highly conserved mechanism

- C. *elegans* => 3 families of proteins involved in different steps of apoptosis
  - regulators
  - adaptors/initiators
  - effectors
- Highly conserved from nematodes to Mammals

The conservation of cell death machinery



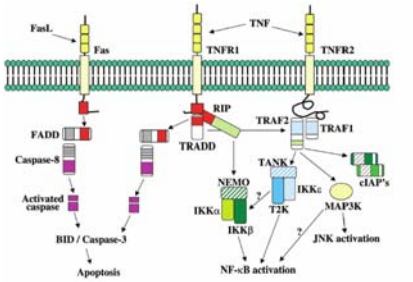
Two major pathways of apoptosis



From:
Tak W Mak et al, 2002,
Arthritis Res, 4:S243.

- The death-receptors
- The Bcl-2 family proteins
- The caspases

The death receptors



- Contain Death Domain (DD)
- Homodimers interaction upon ligand interaction
- Required adaptor molecules

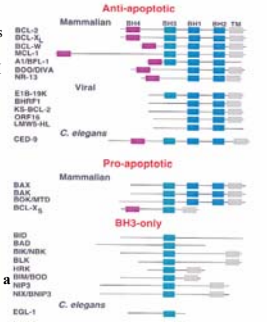
From:
Tak W Mak et al, 2002, Arthritis Res, 4:S243.

- The death-receptors
- The Bcl-2 family proteins
- The caspases

The regulator proteins of mitochondria-dependent apoptosis = the Bcl-2 family

- Mitochondria proteins.
- 4 α-helical segments named Bcl-2 homologous (BH) domains.
- 3 sub-families according to their content in BH domains.
  - **BH1-4**: anti-apoptotic proteins
    Bcl-2, Bcl-x_L, Mcl-1, A1.
  - **BH1-3**: pro-apoptotic proteins
    Bax, Bak, Bok
  - **BH3** only: pro-apoptotic proteins
    Bad, Bid, Bik, Bim, Noxa, Puma

- **The tightly regulated balance between anti-apoptotic and pro-apoptotic molecules within a cell will determine its sensitivity to apoptosis.**
- Interconnection between molecules? Mechanisms (ratio? Post-translational events?)



From: A. Gros et al, Genes & Dev., 1999, 13: 1899.

BH1-4: anti-apoptotic protein

- Bcl-2, Bcl-x_L, Mcl-1, A1.
- C terminal part in the outer mitochondria membrane
- Interact with pro-apoptotic protein through BH domains.
- Bcl-$2^{-/-}$ mice => lymphopenia
- Bcl-$xL^{-/-}$ mice => no CNS development, death at day E13

BH1-3: pro-apoptotic protein

- Bax, Bak, Bok.
- Involved in intrinsic death pathway, caspase-dependent or independent pathway.
- Make hole in the mitochondria membrane => control the integrity of mitochondria and the release of many pro-apoptotic molecules into plasma.
- Inhibited by Bcl-x_L

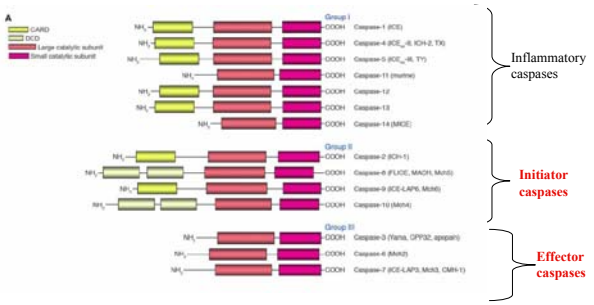
BH3 only: pro-apoptotic protein

- Bad, Bid, Bik, Bim, Noxa, Puma.
- BH3 only → BH1-3
- BH3 only → BH1-4

- Bim ⊣ All BH1-4
- Bad ⊣ Bcl-2, Bcl-x_L
- Bix ⊣ Bcl-x_L, A1
- Noxa ⊣ Mcl-1, A1

- The death-receptors
- The Bcl-2 family proteins
- The caspases

The caspases



- Cystein aspartate-specific proteases
- Caspases are zymogens (pro-enzymes).
- Activated by cleavage at the specific Asp-X bonds.
- 3 groups according their function in the cell

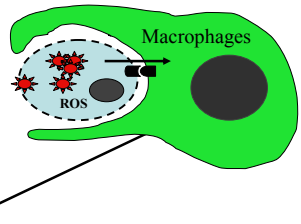
From: IN Lavrik et al, JCI, 2005, 115:2665.

Why, when and how a cell will die?

- **Very complex mechanism**, **highly regulated**, involving **several interconnecting pathways**.
- **The sensitivity of a cell to die and the way it will die depends on:**
  - its present and past interactions with other cells
  - its differentiation stage
  - the integrity of its internal components
  - the balance between anti- and pro-apoptotic molecules

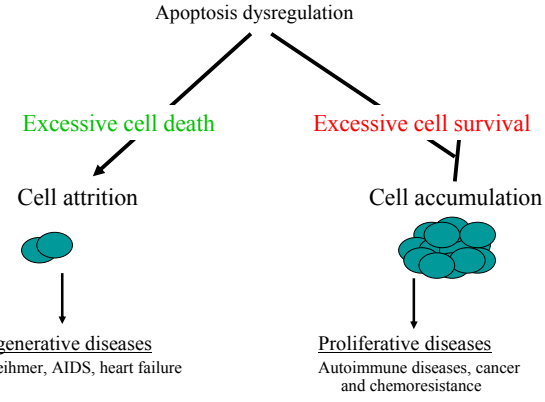
Apoptosis and pathology

INFLAMMATION: on the importance to be eaten



- Anti-inflammatory molecules
- Factors for growth of the dying cell type (homeostasis)

Defect in apoptotic cell clearance may be involved in chronic inflammatory diseases, auto-immune diseases.



Genetic disorders and apoptosis

Mutations in molecules involved in apoptosis pathways =>
- cancers
- auto-immune diseases
- neurodegenerative disorders

Apoptosis dysregulation would contribute to about a half of all the major medical illness for which adequate therapy or prevention is currently lacking.

Neurodegenerative diseases

Example: involvement of **caspase-9** in Alzeihmer disease

Caspase-9 cleavage of amyeloid precursor protein may induce neuronal death.

Activated caspase-9 and caspase-cleaved APP in patient brains, not in control brains.

Cancer

- **Due to**:
  - self-sufficiency in growth signals
  - insensitivity to growth-inhibitory signals
  - **apoptosis resistance**
- **Different molecules**, at different stages of apoptotic pathway, involved:
  - **p53**: half of known cancers have mutations in p53
    P53 => increase Bax, Bid, Puma, Apaf-1, caspase-9
  - increase in **Bcl-2** (CLL, AML, MM, ALL=translocation)
  - increase in **survivin** (lung, colon, pancreas, prostate, breast)
- Transfer of malignancy by engulfment:
  Possible transfer of oncogenes through engulfment of apoptotic bodies from cancer cells => propagation of genetic instability.

Subversion of cell death by viruses

• Many viruses have evolved mechanisms that **repress premature death** in the cells they require for their persistence and/or replication.

Viral protein	Function
Polyomavirus SV40 large protein Papilloma virus E6 Adenovirus EKB-55K HHV8 LANA proteins	P53 suppressors
Adenovirus E1B-19K African Swine fever virus LMW5-HL EBV BHRF-1 HHV8 ORF16	Bcl-2 homologues
Baculovirus p35, IAP Cowpox CrmA Vaccinia SPI-2 Herpes virus v-FLIP	Caspase inhibitors

• Other viruses have evolved mechanisms that **induces premature death** of immune cells by inducing death-R on their surface in order to escape the immune response (HIV nef, CMV).

Autoimmune diseases

Lack of tolerance
Lymphoproliferation

Apoptosis and treatments

Therapeutic targets for cell death inhibition:
D. Green & G. Kroemer, JCI, 2005, 115:2610.

Targets	Drug principles	Indications
p53	Amifostine (Ethyol) Small molecule	Reduction of renal toxicity during chemotherapy, of parotid gland during radiotherapy.
Caspases	Caspase inhibitors Small molecules	HCV, acute alcoholic hepatitis, RA, acute myocardial infarction
Death-R and ligands (TNF-α)	Neutralizing Abs,	RA, psoriasis, Crohn disease
PARP (caspase substrate)	Small molecules (nicotine amide)	Ischemia/reperfusion damage

Apoptosis and treatments

Therapeutic targets for cell death induction:

D. Green & G. Kroemer, JCI, 2005, 115:2610.

Targets	Drug principles	Indications
Bcl-2	Anti-sense oligonucleotides	CLL, MM, NSCLC.
p53	Adenovirus (Advexin)	Head and Neck cancer, breast, lung, colorectal, ovarian cancers
Death-R and ligands (TNF-α)	Recombinant receptor	melanoma
Kinase inhibitors (survival signaling) Ex: HER-1 / HER-2	Small molecules	NSCLC, ovarian, breast

Apoptosis and natural products

• L. Lopez et al, ***Cupressus lusitanica*** **(Cupressaceae) leaf extract induces apoptosis in cancer cells**, 2002, J of Ethnopharmacology, 80:115.

• S. Ming Yuen Lee et al, ***Paeoniae Radix*****, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway**, 2002, Life Sciences, 71:2267.

• Sheng-Teng Huang et al, ***Phyllanthus urinaria*** **triggers the apoptosis and Bcl-2 down-regulation in Lewis lung carcinoma cells**, 2003, Life Sciences, 72:1705.

• Ju-Hyund Woo et al, **Molecular mechanisms of curcumin-induced cytotoxicity: induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-x_L and IAP, the release of cytochrome *c* and inhibition of Akt.**, 2003, Carcinogenesis, 24:1199.