Lecture 1

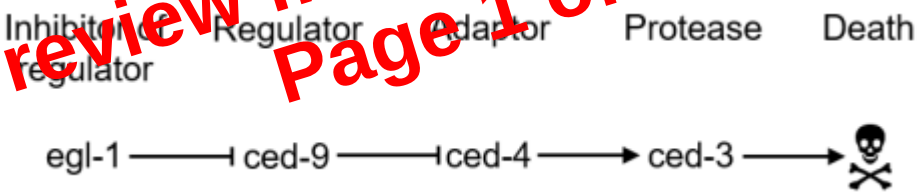
Introduction to Apoptosis

- Apoptosis, concept of which was established in **1972**, is associated with **fragmentation of genomic DNA**. In addition, apoptosis is morphologically characterised by the **cytoplasmic condensation**, **nuclear pyknosis, chromatin condensation**, cell rounding, **membrane blebbing** and **formation of membrane-bound apoptotic bodies** that are rapidly phagocytosed and digested by macrophages or neighbouring cells without activating immune response.

- ***Characteristics of apoptosis and necrosis***

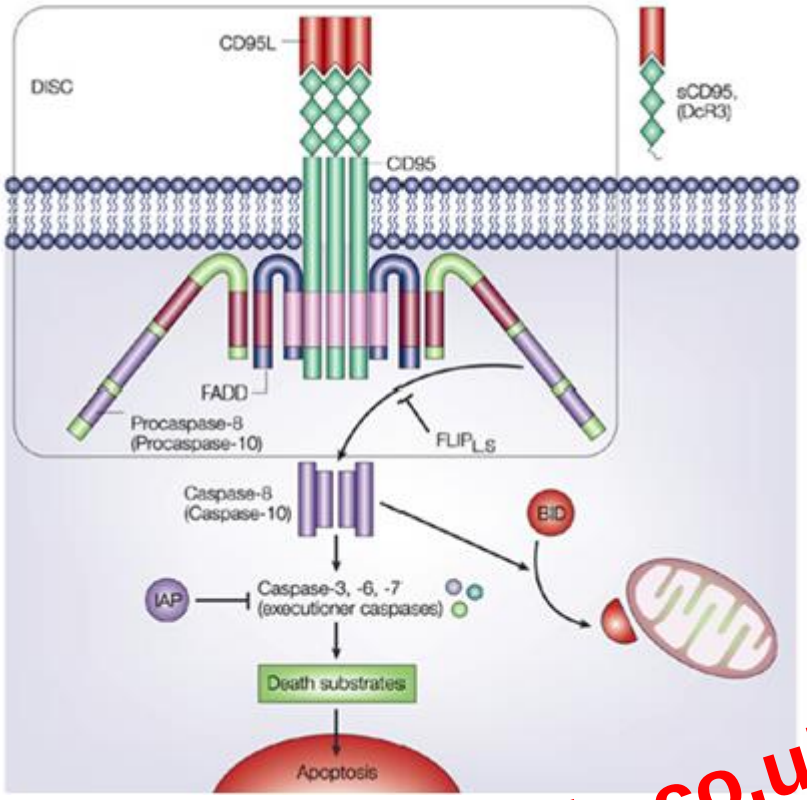
Apoptosis (Death by design)	**Necrosis (Death by accident)**
Affects single cells	Affects groups of neighbouring cells
Initiated by a signal transduction process (physiological)	Initiated by direct cell damage (pathological)
An active process: requires macromolecule synthesis	Passive: does not require macromolecule synthesis
Cells shrink	Cells swell
Membrane blebs (buds off) but integrity is maintained	Membrane integrity lost
Increased mitochondrial membrane permeability	Organelle swelling and lysosomal leakage
Chromatin condenses and genomic DNA fragments into a 200 bp ladder	No condensation of chromatin and random degradation of genomic DNA
Formation of apoptotic body	No formation of apoptotic body
Apoptotic bodies ingested by neighbouring cells	Lysed cells ingested by macrophages
Does not cause inflammation	Causes significant inflammation

  - Caenorhabditis elegans (C. elegans) are/were used for very early days of apoptotic research.
  - Why do we use them? Because they are small, fast growing, culture them on agar plates, they are tough as they can even survive space shuttle disaster.
  - ***Order of the C.elegans programmed cell death machinery:-***



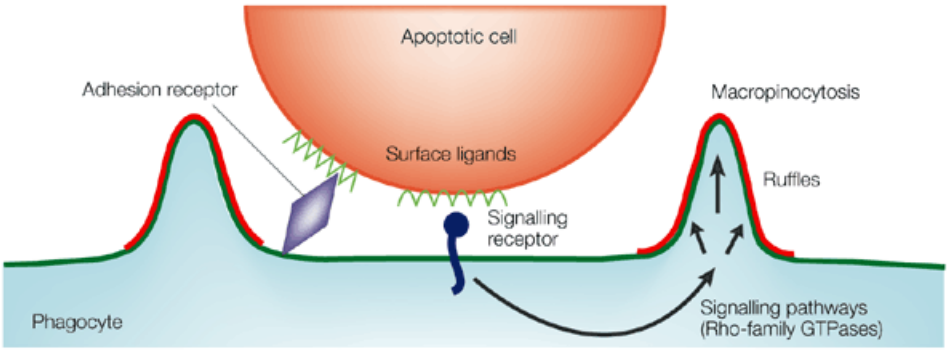
    - Order remains the same whatever kind of system it may be.
    - The outcome of apoptosis is death that is trying to be achieved
    - Part of that is chopping part of the cells up which is done my proteases, in the case of C. elegans called ced-3
    - To make the proteases activated, we need an adaptor protein in C.elegans being ced-4.
    - Because we don't want the proteases to be activated too quickly, we need a regulatory process wherein, ced-9 inhibits the activity of ced-4 else, ced-4 would activate ced-3 all the time giving unwanted cell death. Thus, putting a block on the system.
    - The block also needs to be able to be released. So every regulator has an inhibitor. So, egl-1 inhibits ced-9 activity. Once this is taken out of the picture, ced-4 can be activated, which goes on to activate ced-3 to give cell death.
    - Mammalian apoptosis follows the same pathway but with many more components (Inhibitors, Regulator, Adaptor, Protease, Death).
    - Thus, even though C. elegans and humans are separated by years of evolution, the rules and proteins of how they work are very similar.

  - ***Mouse model of Apaf-1 (-/-) knockout***
    - Apaf1 is the homolog of ced-4 (adaptor protein which activates the caspases)
    - There are a lot of events during development that fail due to apaf1 knockout.



- Threshold exists, usually requiring three ligands to attach in order for this to work
- CD95 and TRAILR1/TRAILR2 DISCs consist of oligomerized, probably trimerized, receptors the DD-containing adaptor molecule FADD, two isoforms of procaspase-8, procaspase-10 and the cellular FLICE inhibitory protein (FLIP$_{L,S}$)
- Interactions between the molecules at the DISC are based on homotypic contacts.
- DD of the receptor interacts with the DD of FADD whereas the death effector domain (DED) of FADD interacts with the N-terminal tandem of DEDS of procaspase-8, procasepase-10 and FLIP$_{L/S}$
- Activation of procasepase-8 is believed to follow an 'induced proximity' model, in which high local concentrations of procaspase-8 at the DISC lead to its autoproteolytic activation, a multi-step cleavage process resulting in formation of a caspase-8 heterotetramer containing two large subunits (p18) and two small subunits (p10).
- This is then released into the cytosol to propagate the apoptotic signal.
- Procaspase-10 is also activated at the DISC, forming an active heterotetramer however, whether caspase-10 can trigger cell death in absence of caspase-8 in response to CD95 or TRAILR1/R2 stimulation is controversial.
- FLIP$_L$ and FLIP$_S$ inhibit activation of procaspase-8 at the DISC by blocking its processing.
- There is increasing evidence that FLIP$_L$ also facilitates the cleavage of procaspase-8 at the DISC by forming FLIP$_L$-procaspase-8 heterodimers.
- Two types of CD95 signaling have been established.
- Type I cells are characterized by high levels of DISC formation and increased amounts of active caspase-8.
- Activated caspase-8 directly leads to the activation of downstream effector caspases.
- In type II cells, there are lower levels of CD95 DISC formation and thus, lower levels of active capase-8.
- In this case, signalling requires an additional amplification loop that involves the cleavage by caspase-8 of the Bcl-2 family protein Bid to generated truncated Bid and subsequent tBid-mediated release of cytochrome C from the mitochondria.

- Activation of signalling receptors causes the tickle by lots of PS molecules which bind to the receptor with low avidity but activate enough of the signalling receptors to discriminate a dying cell.
- Signalling activates remodelling of engulfing cell via Rho-GTPases, meaning ROCK1 signalling is probably involved.



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- Stages of Efferocytosis