IPSSC + CMBO

STUDENTS' CONFERENCE

Jožef Stefan International Postgraduate School and Young Researchers' Day CMBO 19 and 20 April

Die hard - use a detergent!

Lysosomal membrane permeabilization - a cellular suicide strategy

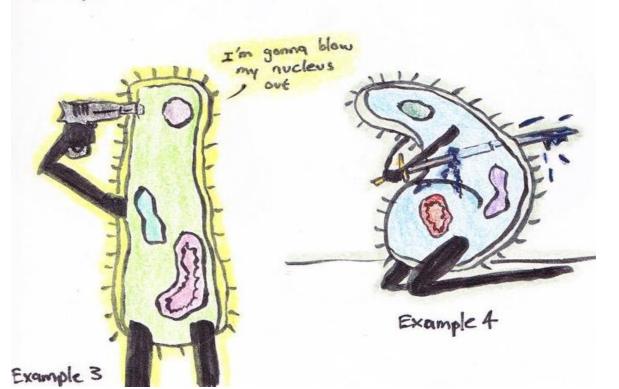
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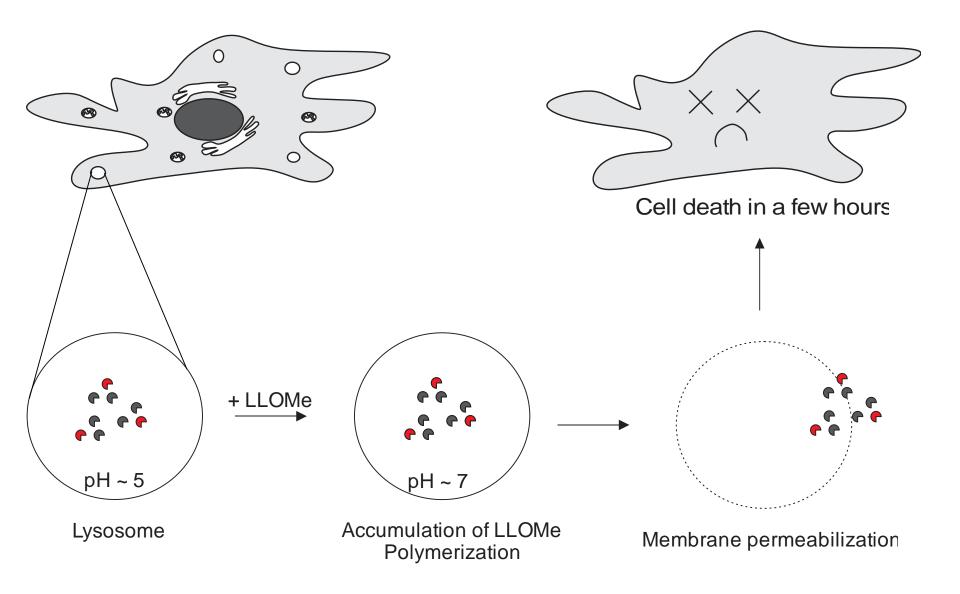
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What is a cell?

- the basic structural, functional and biological unit of all known organisms
- constantly works to stay alive
- and like any living thing it dies







Acknowledgement

- Boris Turk (mentor)
- Barbara Sobotič
- Miha Butinar
- other co-workers from B1 department

Cathepsin C is critical for the release of other cathepsins from lysosomes in LLOMe-triggered apoptosis

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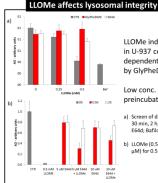
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BACKGROUND

Lysosomotropic detergents remain captured in acidic vesicles and can induce lysosomal membrane permeabilization (LMP). LMP is a point-of-no-return, followed by cell death, usually mediated by cathepsins. L-Leu-Leu-methyl ester (LLOMe) is a synthetic lysosomotropic detergent, which showed extremely good results in preventing the graph-versus-host disease in vivo by successfully eliminating immune cells of the donor. The mechanism of compound action is dependent on the transferase activity of cathepsin C, which leads to the formation of LeuLeu oligomers that in turn act as detergent, leading to lysosomal leakage, release of cathepsins into the cytosol and triggering of apoptotic cell death. However, the exact mechanism of action and interconnection between different cathepsins were never clarified.

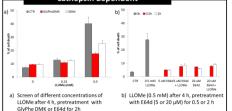


LLOMe induced lysosomal disruption in U-937 cells is concentration dependent and it can be prevented by GlyPheDMK.

Low conc. of E64d or shorter preincubation is less efficient

- Screen of different concentrations of LLOMe after
 30 min, 2 h; pretreatment with GlyPheDMK or
 E64d: Bafilomycin (30 nM) as positive control
- b) LLOMe (0.5 mM), pretreatment E64d (5 or 20 μM) for 0.5 or 2 h

LLOMe induced cell death is cysteine cathepsin dependent



LLOMe induced cell death in U-937 cells is concentration dependent and it can be prevented by GlyPheDMK or E64d.

LLOMe induced leakage of cathepsins can be prevented by GlyPheDIMK a) 1.4 Get 1.1 Get 2.1 Get 2.1 Get 3.1 Actin Diamont Stut b) Cat 5 Cat 5 Civ LLOMe GlyPheDIMK LLOMe a) and b) Pretreatment with GlyPheDIMK for 2 h, LLOMe (0.5 mM) for 15 min c vt - cytosolic lysates, tot - total lysates

cat C is essential for LMP and leakage of cathepsins in U-937 cells

contribution to apoptosis is the largest due to their high concentrations in the cells

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CONCLUSIONS in – 15 minutes



- a) LLOMe causes the increase in lysosomal pH, LMP, followed by cathepsins' release and apoptosis.
- b) GlyPheDMK efficiently prevents lysosomal disruption and apoptosis
 c) E64d prevents apoptosis, but only partially lysosomal pH increase.
- d) Cathepsin C is required for LMP, other released cathepsins trigger cell death → confirmed by cat B and cat L single or double KO.