Necroptosis, one type of regulated necrosis

Classification of cellular death modalities and biomedical applications

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INTRODUCTION

The ability to undergo programmed cell death (an active cellular process that culminates in cell death) is a built-in latent capacity in virtually all cells of multicellular organisms. Cell death is important for embryonic development, maintenance of tissue homeostasis, establishment of immune self-tolerance, killing by immune effector cells, and regulation of cell viability by hormones and growth factors. Abnormalities of the cell death program contribute to a number of diseases, including cancer, Alzheimer's disease, and acquired immune deficiency syndrome (AIDS).

The aim of this project is to explain and clarify what types of cellular death exist nowadays, their actual classification, focusing on regulated necrosis and its main types. Finally, it introduces the newest applications, coming from the knowledge of necroptosis cellular death pathway, for some biomedical therapeutic approaches (cancer and virus infections).

MOLECULAR CLASSIFICATION OF CELL DEATH MODALITIES

Apoptosis

Programmed cell death based on caspase activation (extrinsic) or MOMP (intrinsic).

- ✓ Loss of microvilli and intracellular junctions
- ✓ Loss of plasma membrane asymmetry
- ✓ Phosphatidylserine out of the cell
- ✓ Chromatine hypercondensation
- ✓ Apoptotic bodies
- ✓ Cell shrink
- ✓ No inflammation

Necrosis

Collapse of cell physiology due to a result of ATP depletion. Accidental cell death triggered by structural or chemical insult.

- ✓ Burst of cellular membranes
- ✓ Cell swelling
- ✓ Local inflammation

Cellular death

Autophagy

Mitotic catastrophe

Cornification

Entosis

Ferroptosis

Netosis

Anoikis

Regulated necrosis

Programmed cell death based on ligand-receptor interactions with characteristics of both necrosis and apoptosis, which are diverse and so it has been done a regulated necrosis classification.

Parthanatos

Independent of caspases and it has two principal molecules: PARP and AIF (apoptosis inducing factor)

- ✓ Fragmentation and condensation of chromatin
- ✓ Loss of mitochondrial membrane potential

Regulated necrosis

Necroptosis

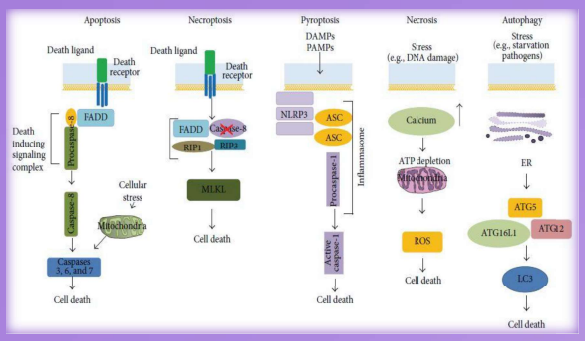
Pyroptosis

Dependent on casp-1 and casp-7, induction of pyroptosis forms the inflamasome and creates a pore on the plasmatic membrane.

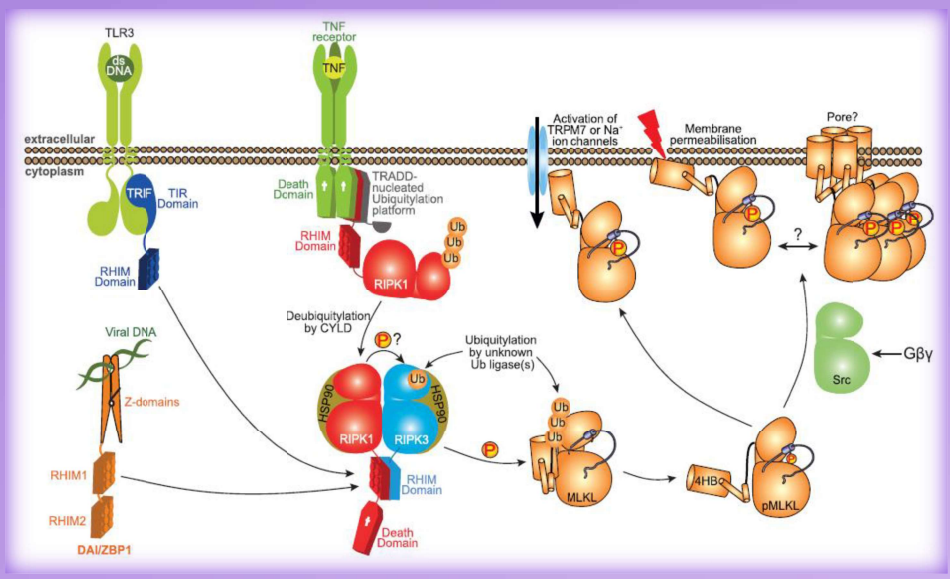
- ✓ Osmotic cell lysis
- ✓ Secretion of interleukins

There are several ways to induce necroptosis. The most common, a stimuli binds to **TNFR1**, which trimerize and forms complex I. **TRADD** binds to complex I and recruits RIPK1. **RIPK1** translocates to form Complex II with FADD, Caspase8 and cFLIP. Under caspase-inhibitory conditions, **CYLD** deubiquitiles RIPK1 and **RIPK3** is recruited in the Complex II forming the necrosome. RIPK1 and RIPK3 phosphorylate themselves and **MLKL** pseudokinase, which oligomerizes by its RHIM domains and translocates to the plasma membrane where it creates **pores** and causes massive entrance of Ca and Na ions.

- ✓ Early loss of plasma membrane integrity
- ✓ Organelle swelling and breakdown
- ✓ Leakage of intracellular contents



Molecular cascades of apoptosis, necroptosis, pyroptosis, necrosis and autophagy (Negroni, Cucchiara, & Stronati, 2015)



Molecular pathways of Necroptosis (Murphy & Vince, 2015)

NECROPTOSIS, AN ALTERNATIVE TO APOPTOSIS IN CANCER AND VIRUS INFECTIONS

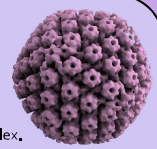
BIOMARKERS AND DETECTION

- **Mesurement of RIPK1, RIPK3 and MLKL expression**
- **Phosphorilation status of RIPK1, RIPK3 and MLKL** (RIPK3 on S227, MLKL on Thr357 and Ser358)
- **Detection of necrosome formation** (RIPK1-RIPK3 or RIPK3-MLKL interactions)
- **Oligomerization of MLKL**
- **Transgenic animal models** (RIPK1, RIPK3 and MLKL knock-out models)

VIRAL INFECTIONS

Necroptosis as a defense mechanism against viral infection

RIPK3 interacts with DAI (intracellular sensor of viral DNA) via RHIM domains



Reference: Thomas Splettstoesser from https://commons.wikimedia.org/wiki/File:HSV-1-EM.png

- ➢ Cytomegalovirus (MCMV)

vIRA MCMV protein: viral inhibitor of RIPK activation, disrupting RIPK3/RIPK1 complex.

- ➢ Herpes Simplex Virus (HSV)

ICP6 *(HSV-1)* and ICP10 *(HSV-2)*: viral proteins which inhibit apoptosis through Casp8, are able to interact with the necrosome enhancing RIPK3/MLKL-necroptosis in mouse cells, but inhibiting it in human cells in HSV-1 case.

CANCER

Alterations of necroptosis in cancer cells

- **Downregulation or mutations** in SMAC, RIPK1, RIPK3, MLKL and/or CYLD.
- **Relevance of molecular genetics**: some single nucleotide polymorphisms (SNPs) in RIPK3 gene are associated with susceptibility for colorectal cancer.
- **Overexpression of IAPs**: pro-survival proteins that promote NF-κB and block caspase activity

Necroptosis and metastasis

- **High level of Reactive Oxigen Species (ROS)**: necroptosis induces a high level of ROS which restricts cancer metastasis.

Reference: https://en.wikipedia.org/wiki/Lung_cancer#/media/File:Squamous_carcinoma_lung_3_cytology.jpg

Necroptosis and inflammation

- **Necroptosis, a potent inducer of immune response** through the release of DAMPs into the tissue. DAMP recrui inflammatory cells to aid tissue repairing.
- **Activation of immunity**: specially it triggers the activation of natural killer cells, dendritic cells which will activate CD8+ lymphocytes.
  - CD8+ T cells activation is RIPK1-signalling dependent
  - RIPK3 is critical for NK cells by regulating cytokine expression

Anticancer therapies

- Necrostatin-1	- cFLIP	- Shikonin
- Necrosulfonamide	- Homoharringtonine	- SMM of Smac
- 5-Fluorouracil	- Cisplatin	- Decitabine
- Etoposide	- Obatoclax	- Taxol
- Campthotecin	- Staurosporine	- Curcumin

CONCLUSIONS

- Environment conditions and inducers of cell death pathways are stimuli that dictate which cell death program will happen. This is possible because cell death modalities share a lot of molecular pathways.
- Necroptosis is an interesting programmed cell death type which is useful for the cell as an alternative way to apoptosis, the main programmed cell death program of the cell, when it is inhibited.
- Necroptosis can be induced by so many compounds and there are several molecular pathways to finally end with the activation of the necrosome and MLKL activation that leads to cell death. To see its possibilities, more research is needed, taking an exclusive effort to improve diseases treatment.
- In future therapies for cancer, personalized phamacotherapeutic strategies based on detection and expression of cancer-type-specific cell death regulators may become an essential option to sensitize tumor cells to anticancer agents, used alone or most likely as a combination of target-specific compounds, taking them together with standard chemotherapeutic agents.

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