Regulated Necrosis

Chan, FK., D'Eustachio, P., Gillespie, ME., Kanneganti, TD., Murphy, JM., Shamovsky, V., Shao, F., Shorser, S.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

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https://reactome.org
**Introduction**

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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**Literature references**


Reactome database release: 82

This document contains 3 pathways (see Table of Contents)
Regulated Necrosis

Stable identifier: R-HSA-5218859

Necrosis has traditionally been considered as a passive, unregulated cell death. However, accumulating evidence suggests that necrosis, like apoptosis, can be executed by genetically controlled and highly regulated cellular process that is morphologically characterized by a loss of cell membrane integrity, intracellular organelles and/or the entire cell swelling (oncosis) (Rello S et al. 2005; Galluzzi L et al. 2007; Berghe TV et al. 2014; Ros U et al. 2020). The morphological hallmarks of the necrotic death have been associated with different forms of programmed cell death including (but not limited to) parthanatos, necroptosis, glutamate-induced oxytosis, ferroptosis, inflammasome-mediated necrosis etc. Each of them can be triggered under certain pathophysiological conditions. For example UV, ROS or alkylating agents may induce poly(ADP-ribose) polymerase 1 (PARP1) hyperactivation (parthanatos), while tumor necrosis factor (TNF) or toll like receptor ligands (LPS and dsRNA) can trigger necrosome-mediated necroptosis. The initiation events, e.g., PARP1 hyperactivation, necrosome formation, activation of NADPH oxidases, in turn trigger one or several common intracellular signals such as NAD+ and ATP-depletion, enhanced Ca2+ influx, dysregulation of the redox status, increased production of reactive oxygen species (ROS) and the activity of phospholipases. These signals affect cellular organelles and membranes leading to osmotic swelling, massive energy depletion, lipid peroxidation and the loss of lysosomal membrane integrity. Different mechanisms of permeabilization have emerged depending on the cell death form. Pore formation by gasdermins (GSDMs) is a hallmark of pyroptosis, while mixed lineage kinase domain-like (MLKL) protein facilitates membrane permeabilization in necroptosis, and phospholipid peroxidation leads to membrane damage in ferroptosis. This diverse repertoire of mechanisms leading to membrane permeabilization contributes to define the specific inflammatory and immunological outcome of each type of regulated necrosis. Regulated or programmed necrosis eventually leads to cell lysis and release of cytoplasmic content into the extracellular region that is often associated with a tissue damage resulting in an intense inflammatory response.

The Reactome module describes necroptosis and pyroptosis.
Literature references


Editions

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RIPK1-mediated regulated necrosis

Location: Regulated Necrosis

Stable identifier: R-HSA-5213460

Receptor-interacting serine/threonine-kinase protein 1 (RIPK1) and RIPK3-dependent necrosis is called necroptosis or programmed necrosis. The kinase activities of RIPK1 and RIPK3 are essential for the necroptotic cell death in human, mouse cell lines and genetic mice models (Cho YS et al. 2009; He S et al. 2009, 2011; Zhang DW et al. 2009; McQuade T et al. 2013; Newton et al. 2014). The initiation of necroptosis can be stimulated by the same death ligands that activate extrinsic apoptotic signaling pathway, such as tumor necrosis factor (TNF) alpha, Fas ligand (FasL), and TRAIL (TNF-related apoptosis-inducing ligand) or toll like receptors 3 and 4 ligands (Holler N et al. 2000; He S et al. 2009; Feoktistova M et al. 2011; Voigt S et al. 2014). In contrast to apoptosis, necroptosis represents a form of cell death that is optimally induced when caspases are inhibited (Holler N et al. 2000; Hopkins-Donaldson S et al. 2000; Sawai H 2014). Specific inhibitors of caspase-independent necrosis, necrostatins, have recently been identified (Degterev A et al. 2005, 2008). Necrostatins have been shown to inhibit the kinase activity of RIPK1 (Degterev A et al. 2008). Importantly, cell death of apoptotic morphology can be shifted to a necrotic phenotype when caspase 8 activity is compromised, otherwise active caspase 8 blocks necroptosis by the proteolytic cleavage of RIPK1 and RIPK3 (Kalai M et al. 2002; Degterev A et al. 2008; Lin Y et al. 1999; Feng S et al. 2007). When caspase activity is inhibited under certain pathophysiological conditions or by pharmacological agents, deubiquitinated RIPK1 is engaged in physical and functional interactions with the cognate kinase RIPK3 leading to formation of necosome, a necroptosis-inducing complex consisting of RIPK1 and RIPK3 (Sawai H 2013; Moquin DM et al. 2013; Kalai M et al. 2002; Cho YS et al. 2009, He S et al. 2009, Zhang DW et al. 2009). Within the necosome RIPK1 and RIPK3 bind to each other through their RIP homotypic interaction motif (RHIM) domains. The RHIMs can facilitate RIPK1:RIPK3 oligomerization, allowing them to form amyloid-like fibrillar structures (Li J et al. 2012; Mompean M et al. 2018). RIPK3 in turn interacts with mixed lineage kinase domain-like protein (MLKL) (Sun L et al. 2012; Zhao J et al. 2012; Murphy JM et al. 2013; Chen W et al. 2013). The precise mechanism of MLKL activation by RIPK3 is incompletely understood and may vary across species (Davies KA et al. 2020). Mouse MLKL activation relies on transient engagement of RIPK3 to facilitate phosphorylation of the pseudokinase domain (Murphy JM et al. 2013; Petrie EJ et al. 2019a), while it appears that stable recruitment of human MLKL by necrosomal RIPK3 is an additional crucial step in human MLKL activation (Davies KA et al. 2018; Pet-
RIPK3-mediated phosphorylation is thought to initiate MLKL oligomerization, membrane translocation and membrane disruption (Sun L et al. 2012; Wang H et al. 2014; Petrie EJ et al. 2020; Samson AL et al. 2020). Studies in human cell lines suggest that upon induction of necroptosis MLKL shifts to the plasma membrane and membranous organelles such as mitochondria, lysosome, endosome and ER (Wang H et al. 2014), but it is trafficking via a Golgi-microtubule-actin-dependent mechanism that facilitates plasma membrane translocation, where membrane disruption causes death (Samson AL et al. 2020). The mechanisms of necroptosis regulation and execution downstream of MLKL remain elusive. The precise oligomeric form of MLKL that mediates plasma membrane disruption has been highly debated (Cai Z et al. 2014; Chen X et al. 2014; Dondelinger Y et al. 2014; Wang H et al. 2014; Petrie EJ et al. 2017, 2018; Samson AL et al. 2020). However, microscopy data revealed that MLKL assembles into higher molecular weight species upon cytoplasmic necrosomes within human cells, and upon phosphorylation by RIPK3, MLKL is trafficked to the plasma membrane (Samson AL et al. 2020). At the plasma membrane, phospho-MLKL forms heterogeneous higher order assemblies, which are thought to permeabilize cells, leading to release of DAMPs to invoke inflammatory responses. MLKL also exerts non-necroptotic functions such as regulation of endosomal trafficking or MLKL-induced activation of the NLRP3 inflammasome (Yoon S et al. 2017; Shlomovitz I et al. 2020; Yoon S et al. 2022). While RIPK1, RIPK3 and MLKL are the core signaling components in the necroptosis pathway, many additional molecules have been proposed to positively and negatively tune the signaling pathway. Currently, this picture is evolving rapidly as new modulators continue to be discovered.

The Reactome module describes MLKL-mediated necrototic events on the plasma membrane.

**Literature references**


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Pyroptosis is a form of lytic inflammatory programmed cell death that is triggered by microbial infection or pathological stimuli, such as stroke or cancer (reviewed in Shi J et al. 2017; Man SM et al. 2017; Tang D et al. 2019; Zheng Z & Li G 2020). The process of pyroptosis protects the host from microbial infection but can also lead to pathological inflammation if overactivated. The morphologic characteristics of pyroptosis include cell swelling, rupture of the cell membrane and release of intracellular contents into the extracellular environment. Pyroptosis is also characterized by chromatin condensation, however this is not the key or universal feature of pyroptosis (reviewed in Man SM et al. 2017; Tang D et al. 2019). Pyroptosis is executed by proteins of the gasdermin family, which mediate formation of membrane pores (Liu X et al. 2016; Ding J et al. 2016; Mulvihill E et al. 2018; Broz P et al. 2020). Pyroptosis can be defined as gasdermin-mediated programmed necrotic cell death (Shi J et al. 2017; Galluzzi L et al. 2018). The gasdermin (GSDM) superfamily includes GSDMA, GSDMB, GSDMC, GSDMD, GSDME (or DFNA5) and PJVK (DFNB59) (Kovacs SB & Miao EA 2018). Each protein contains an N-terminal domain with intrinsic necrotic pore-forming activity and a C-terminal domain reported to inhibit cell death through intramolecular domain association (Liu X et al. 2016; Ding J et al. 2016; Liu Z et al. 2018, 2019; Kuang S et al. 2017). Proteolytic cleavage in the linker connecting the N and C-terminal domains of gasdermins releases the C-terminus, allowing the gasdermin N-terminus to translocate to the cell membrane and oligomerize to form pores (Shi J et al. 2015; Ding J et al. 2016; Sborgi L et al. 2016; Feng S et al. 2018; Yang J et al. 2018; Mulvihill E et al. 2018). Although PJVK (DFNB59) is included to the gasdermin family, it is not known whether PJVK is cleaved and whether the full length or the N-terminal portion of PJVK is responsible for forming membrane pores. The N-terminal fragments of GSDMs strongly bind to phosphatidylinositol phosphates and weakly to phosphatidylserine, found on the inner leaflet of the plasma membrane (Liu X et al. 2016; Ding J et al. 2016; Mulvihill E et al. 2018). Gasdermins are also able to target cardiolipin, which is often found in mitochondrial membranes and membranes of bacteria (Liu X et al. 2016; Rogers C et al. 2019). The size of the GSDMD pore is estimated to be 10–20 nm (Ding J et al. 2016; Sborgi L et al. 2016). The pore-formation activity of GSDMs in the cell membrane facilitates the release of inflammatory molecules such as interleukin (IL)1β and IL18 (mainly in GSDMD-mediated pyroptosis), and eventually leads to cytolysis in mammalian cells, releasing additional proinflammatory cellular contents including danger signals such as high mobility group box1 (HMGB1) (Shi J et al. 2015; He W et al. 2015; Evavold CL et al. 2017; Semino C et al. 2018; Volchuk A et al. 2020). Pyroptosis can occur in immune cells such as macrophages, monocytes and dendritic cells and non-immune cell types such as intestinal epithelial cells, trophoblasts and hepatocytes (Taabazuing CY et al. 2017; Li H et al. 2019; Jia C et al. 2019). GSDME
can be cleaved by caspase3 (CASP3) to induce pyroptosis downstream of the “apoptotic” machinery (Wang Y et al. 2017; Rogers C et al. 2017), whereas GSDMD is cleaved by inflammatory CASP1, CASP4 and CASP5 in humans, and CASP1, CASP11 in mice to induce pyroptosis associated with inflammasome activation (Shi J et al. 2015; Kayagaki N et al. 2015). CASP3 cleavage of GSDMD results in its inactivation (Taabazuing et al. 2017). In mouse macrophages, CASP8 can also cleave GSDMD and cause pyroptosis when TAK1 is inhibited (Malireddi R et al. 2018; Orning P et al. 2018; Sarhan J et al. 2018), and TAK1 inhibition also leads to GSDME cleavage (Sarhan J et al. 2018). Furthermore, activated CASP8 can drive inflammasome-independent cleavage of both pro-IL-1β and GSDMD downstream of the extrinsic cell death receptor signaling pathway switching apoptotic signaling to GSDMD-dependent pyroptotic-like cell death (Donado CA et al. 2020). The cleavage and activation of GSDMD in neutrophils is mediated by neutrophil elastase (NE or ELANE), which is released from azurophil granules into the cytosol during neutrophil extracellular trap (NET) formation (Kambara H et al. 2018). Further, granzyme A (GZMA) released from cytotoxic T lymphocytes and natural killer (NK) cells specifically target GSDMB for interdomain cleavage to activate GSDMB-dependent pyroptosis in target tumor cells (Zhou Z et al. 2020). Similarly, granzyme B (GZMB) released from cytotoxic T lymphocytes and natural killer (NK) cells, can induce GSDME-dependent lytic cell death in tumor targets via the CASP3-mediated cleavage of GSDME (Zhang Z et al. 2020).

This Reactome module describes pyroptotic activities of GSDMD and GSDME. While the N-terminal domains of mammalian GSDMA, GSDMB, and GSDMC also have the ability to form pores (Feng S et al. 2018; Ruan J et al. 2018), their functions in the induction of pyroptosis, secretion of proinflammatory cytokines or in bactericidal activity in host remain to be studied and are not covered by this Reactome module.

**Literature references**


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