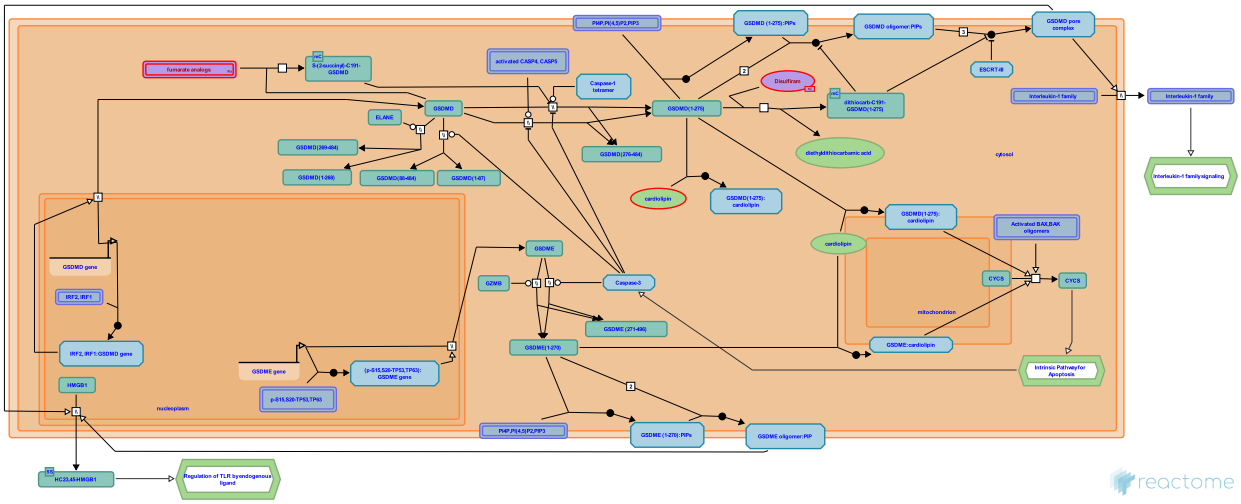
Pyroptosis



D'Eustachio, P., Granucci, F., Jupe, S., Kanneganti, TD., Matthews, L., Pinteaux, E., Ray, KP., Shamovsky, V., Shao, F., Shorser, S., Zanoni, I.

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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.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

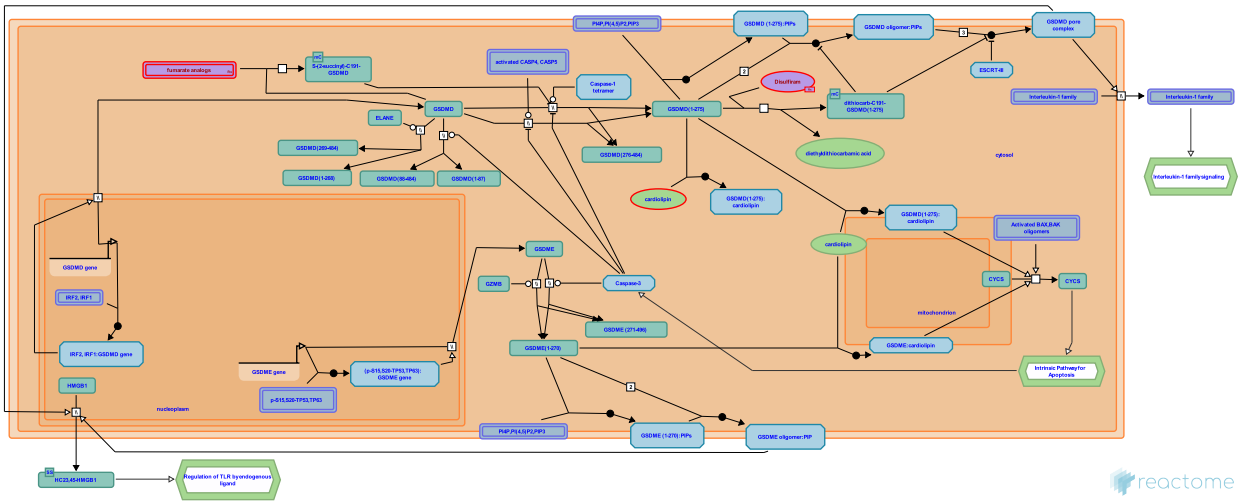
Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

Pyroptosis ↗

Stable identifier: R-HSA-5620971



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-forming activity of GSDMs in the cell membrane facilitates the release of inflammatory molecules such as interleukin (IL)-1β and IL-18 (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 box-1 (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 caspase-3 (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

Ryan, KM., Debatin, KM., Dawson, VL., Altucci, L., Simon, HU., Tsujimoto, Y. et al. (2018). Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ, 25*, 486-541. ↗

Karki, R., Man, SM., Kanneganti, TD. (2017). Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol. Rev., 277*, 61-75. ↗

Zheng, G., Tang, L., Burgering, BM., Lu, C. (2020). Emerging insights on the role of gasdermins in infection and inflammatory diseases. *Clin Transl Immunology, 9*, e1186. ↗

Shi, J., Shao, F., Gao, W. (2017). Pyroptosis: Gasdermin-Mediated Programmed Necrotic Cell Death. *Trends Biochem Sci, 42*, 245-254. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
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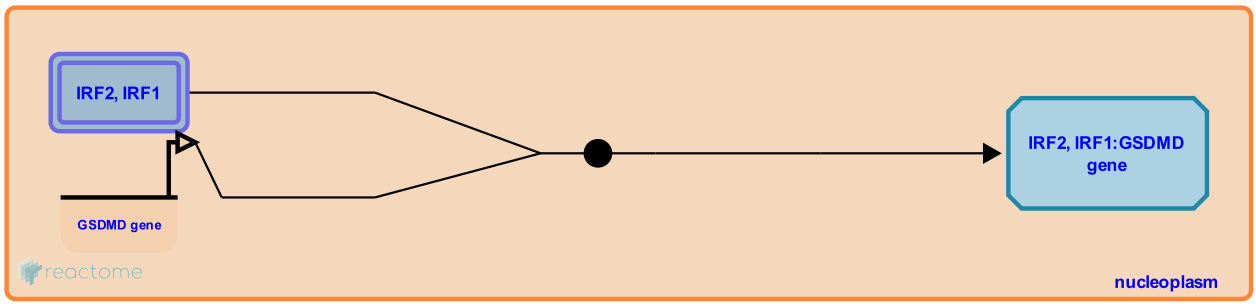
IRF2 or IRF1 binds the GSDMD gene promoter ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710295

Type: binding

Compartments: nucleoplasm



The transcription factor interferon regulatory factor 2 (IRF2) is essential for the transcriptional activation of gasdermin D (GSDMD) in human and mouse cells (Kayagaki N et al. 2019). IRF2 chromatin immunoprecipitation sequencing (ChIP-seq) analysis and genome-wide analysis of IRF2-binding sequences identified a highly conserved IRF2-binding site within the GSDMD promoter. A luciferase reporter assay using human embryonic kidney HEK293 cells revealed that IRF2 drives GSDMD promoter activation by binding to its consensus site. IRF2 deficiency markedly reduced GSDMD abundance in mouse bone marrow-derived macrophages (BDMD) and human EA.hy926 endothelial cells. Further, IRF1 was found to regulate GSDMD gene expression in EA.hy926 cells in the absence of IRF2 (Kayagaki N et al. 2019).

Followed by: GSDMD expression mediated by IRF2, IRF1

Literature references

Modrusan, Z., Bertram, EM., Reja, R., Mirrashidi, KM., Watanabe, C., O'Rourke, K. et al. (2019). IRF2 transcriptionally induces GSDMD expression for pyroptosis. *Sci Signal, 12*. ↗

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2021-02-17	Edited	Shorser, S.
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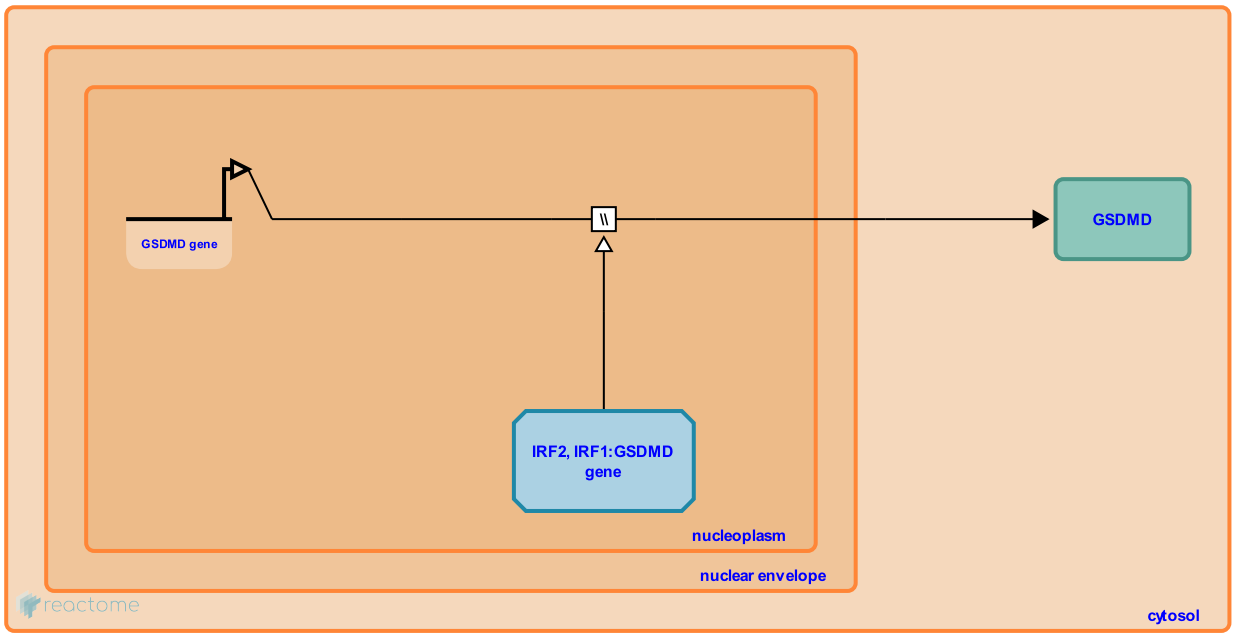
GSDMD expression mediated by IRF2, IRF1 ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710294

Type: omitted

Compartments: nucleoplasm, cytosol



Gasdermin D (GSDMD), a key protein in the activation of pyroptosis, is expressed in a wide range of tissues and immune cells. The transcription factor interferon regulatory factor 2 (IRF2) was found to be essential for the transcriptional activation of GSDMD in human and mouse cells (Kayagaki N et al. 2019).

Preceded by: IRF2 or IRF1 binds the GSDMD gene promoter

Followed by: CASP4, CASP5 cleave GSDMD, ELANE cleaves GSDMD, CASP1 cleaves GSDMD

Literature references

Modrusan, Z., Bertram, EM., Reja, R., Mirrashidi, KM., Watanabe, C., O'Rourke, K. et al. (2019). IRF2 transcriptionally induces GSDMD expression for pyroptosis. *Sci Signal, 12*. ↗

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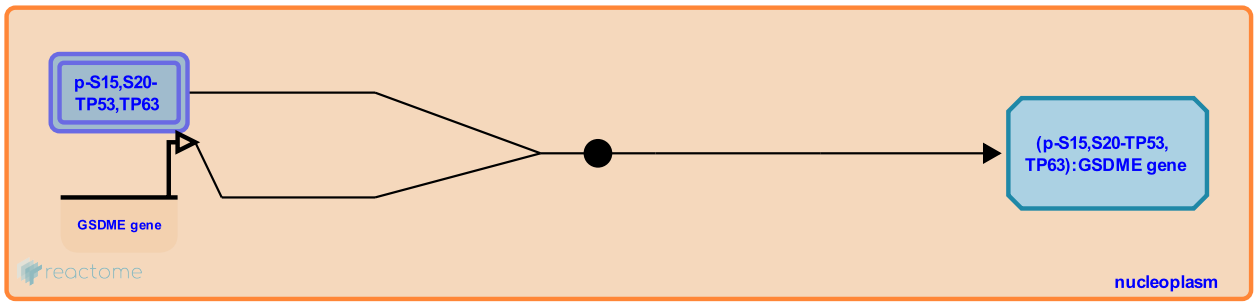
TP53 or TP63 binds the GSDME gene ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710323

Type: binding

Compartments: nucleoplasm



Gasdermin E (GSDME, also known as DFNA5) was identified as a direct target gene of the transcription factor p53 (Masuda Y et al. 2006). Increased expression of GSDME in a human hepatocellular carcinoma cell line (HepG2) treated with H2O2, UV (30 J/m2) or gamma rays (50 Gy) was p53-dependent. A chromatin immunoprecipitation (ChIP) assay using HepG2 identified a potential p53-binding site in intron 1 of the GSDME gene. A luciferase reporter gene assay using human non-small cell lung carcinoma H1299 cells further confirmed that the site is the p53-responsive sequence involved in the recruitment of TP53 (p53) to the GSDME gene. In addition, P63γ and p73β also upregulated GSDME in human breast cancer MCF7 cells treated with demethylating agent 5-aza-2'-deoxycytidine (DAC), suggesting that the GSDME expression can be regulated by multiple members of the p53 family (Fujikane T et al. 2010).

Followed by: GSDME expression mediated by TP53, TP63

Literature references

Arakawa, H., Ohnishi, S., Ichikawa, H., Futamura, M., Ohta, T., Baba, H. et al. (2006). The potential role of DFNA5, a hearing impairment gene, in p53-mediated cellular response to DNA damage. *J Hum Genet, 51*, 652-664. ↗

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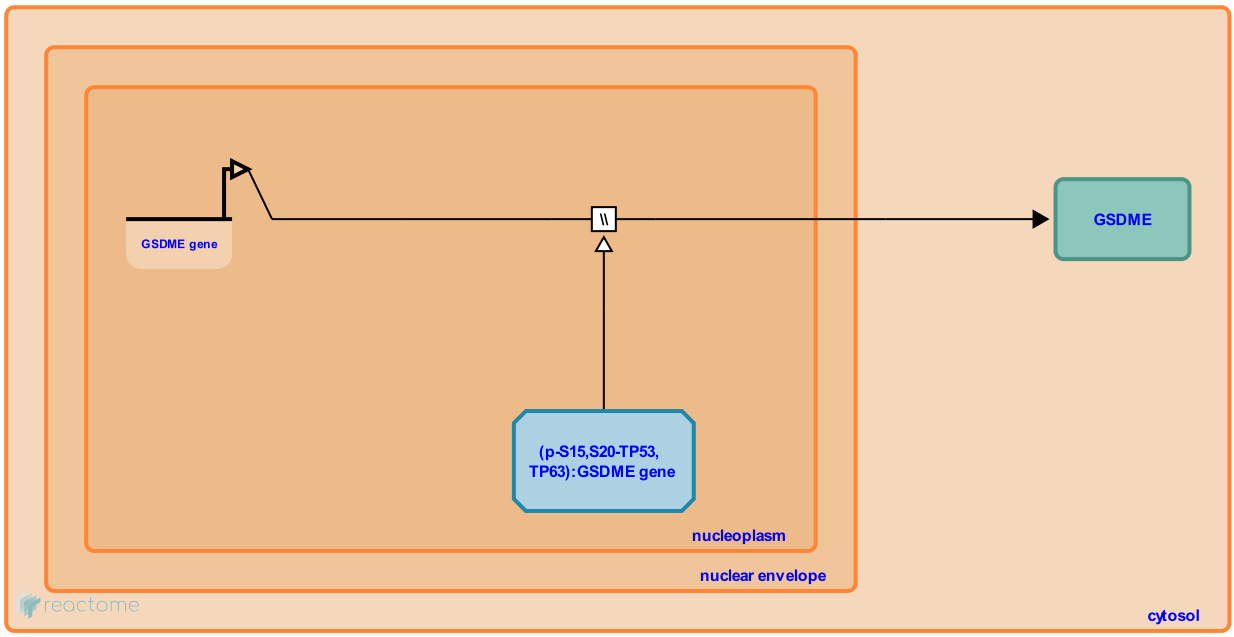
GSDME expression mediated by TP53, TP63 ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710306

Type: omitted

Compartments: nucleoplasm, cytosol



Gasdermin E (GSDME, also known as DFNA5) was identified as a direct target gene of the transcription factor p53 (Masuda Y et al. 2006). Increased expression of GSDME in a human hepatocellular carcinoma cell line (HepG2) treated with H2O2, UV (30 J/m2) or gamma rays (50 Gy) was p53-dependent. TP53 (p53) induced GSDME expression via a specific p53 binding site in intron 1 of the GSDME gene (Masuda Y et al. 2006). In addition, P63γ and p73β also upregulated GSDME in demethylating agent 5-aza-2'-deoxycytidine (DAC)-treated human breast cancer MCF7 cells, suggesting that the GSDME expression can be regulated by multiple members of the p53 family (Fujikane T et al. 2010).

Preceded by: TP53 or TP63 binds the GSDME gene

Followed by: GZMB cleaves GSDME, CASP3 cleaves GSDME

Literature references

Ohe-Toyota, M., Ohmura, T., Toyota, M., Nishikawa, N., Kai, M., Sasaki, Y. et al. (2010). Genomic screening for genes upregulated by demethylation revealed novel targets of epigenetic silencing in breast cancer. *Breast Cancer Res Treat, 122*, 699-710. ↗

Arakawa, H., Ohnishi, S., Ichikawa, H., Futamura, M., Ohta, T., Baba, H. et al. (2006). The potential role of DFNA5, a hearing impairment gene, in p53-mediated cellular response to DNA damage. *J Hum Genet, 51*, 652-664. ↗

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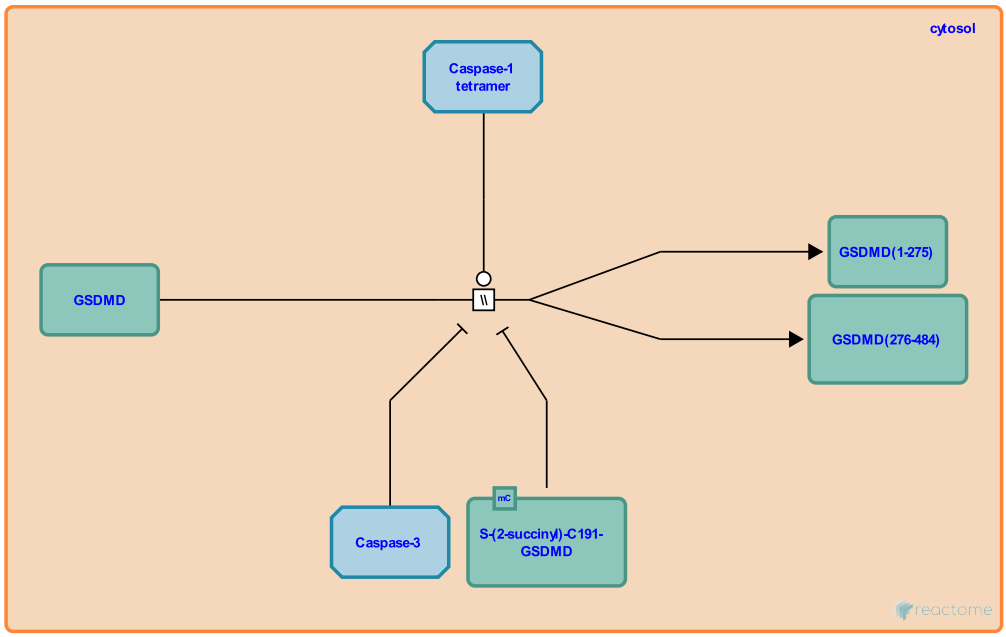
CASP1 cleaves GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647680

Type: omitted

Compartments: cytosol



Inflammatory caspase-1 (CASP1) is activated within the canonical inflammasome, a multiprotein complex assembled in response to sensing of pathogen-derived particles or host-derived danger signals (reviewed in Kelley N et al. 2019; Zheng D et al. 2020). Activated, CASP1 cleaves gasdermin D (GSDMD) within the central linker region generating a 31-kDa N-terminal fragment (GSDMD (1-275)) which has an intrinsic pore-forming activity to execute pyroptosis and a 22-kDa C-terminal fragment (GSDMD (276-484)) which otherwise inhibits cell death through intramolecular domain association (Shi J et al. 2015; Ding J et al. 2016; Sborgi L et al. 2016; Liu Z et al. 2019; Yang J et al. 2018; Kuang S et al. 2017). The expression of GSDMD (1-275) in human embryonic kidney 293 (HEK293) cells induced pyroptosis (Shi J et al. 2015). The catalytic domain of CASP1 was found to directly bind to GSDMD or its cleavage site peptide, FLTD (Yang J et al. 2018). A GSDMD-derived inhibitor, N-acetyl-Phe-Leu-Thr-Asp-chloromethylketone (Ac-FLTD-CMK), inhibited GSDMD cleavage in vitro and suppressed pyroptosis downstream of both canonical and noncanonical inflammasomes. The structure of human CASP1 in complex with Ac-FLTD-CMK revealed extensive enzyme–inhibitor interactions involving both hydrogen bonds and hydrophobic contacts (Yang J et al. 2018). The most critical element determining the specific targeting of GSDMD by CASP1 as well as CASP4/5 and mouse Casp11 is a hydrophobic surface on the C-terminal fragment (GSDMD (276-484)) that is recognized by a short two-stranded β sheet at the dimer interface of the caspases, which leads to an unconventional tetrapeptide (cleavage-site sequence)-independent cleavage of GSDMD (Wang K et al. 2020; Liu Z et al. 2020). In addition, biochemical and structural studies of human GSDMD and mouse GSDMA3 showed the auto-inhibitory conformation of gasdermin domains which is released upon interdomain cleavage by inflammatory caspases, including CASP1 (Shi J et al. 2015; Ding J et al. 2016; Liu Z et al. 2019; Yang J et al. 2018; Kuang S et al. 2017; reviewed in Orning P et al. 2019). Thus, the CASP1-mediated cleavage is thought to release the cytotoxic GSDMD (1-275) from intramolecular autoinhibition by the C-terminal fragment of GSDMD. The N-terminal domain GSDMD (1-275) binds and inserts into lipid membranes where it assembles into pores 10-16 nm in diameter (Ding J et al. 2016; Sborgi L et al. 2016). GSDMD pores facilitate the secretion of active forms of interleukin-1β (IL-1β) and IL-18 from pyroptotic cells (Shi J et al. 2015; He W et al. 2015; Ding J et al. 2016; Evavold CL et al. 2018). "The increasing abundance of membrane pores ultimately leads to membrane rupture and

pyroptosis, releasing the entire cellular contents" - Feng S et al. 2018.

This Reactome event shows CASP1-mediated cleavage of GSDMD at D275.

Preceded by: GSDMD expression mediated by IRF2, IRF1

Followed by: GSDMD (1-275) binds bacterial cardiolipin, GSDMD (1-275) binds PIPs, GSDMD (1-275) binds cardiolipin

Literature references

Chen, Y., Liu, Z., Wang, C., Yang, J., Rathkey, JK., Pinkard, OW. et al. (2018). Mechanism of gasdermin D recognition by inflammatory caspases and their inhibition by a gasdermin D-derived peptide inhibitor. *Proc. Natl. Acad. Sci. U.S.A., 115*, 6792-6797. ↗

Cai, T., Shi, J., Shao, F., Wang, K., Shi, X., Zhao, Y. et al. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature, 526*, 660-5. ↗

Shi, J., Shao, F., Zhao, Y. (2018). Inflammatory Caspases: Activation and Cleavage of Gasdermin-D In Vitro and During Pyroptosis. *Methods Mol. Biol., 1714*, 131-148. ↗

Ding, J., Shao, F., Wang, K., Zeng, M., Li, Z., Shi, X. et al. (2020). Structural Mechanism for GSDMD Targeting by Autoprocessed Caspases in Pyroptosis. *Cell, 180*, 941-955.e20. ↗

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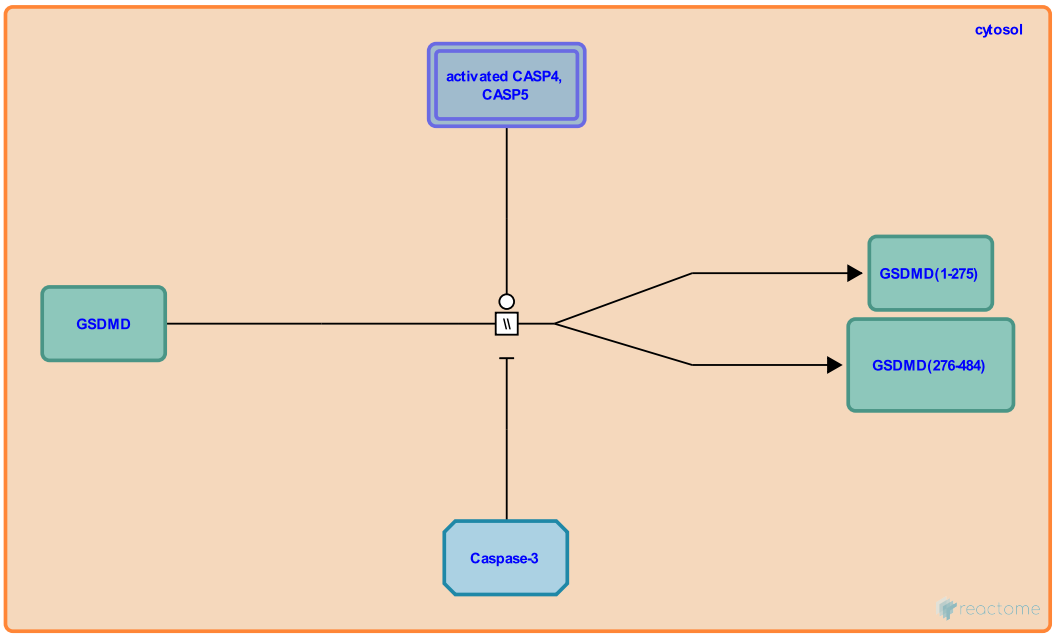
CASP4, CASP5 cleave GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710101

Type: omitted

Compartments: cytosol



Activation of the inflammatory caspase-4 (CASP4) is induced upon sensing of intracellular bacterial lipopolysaccharide (LPS) (Shi J et al. 2014; Kajiwara Y et al. 2014; Casson CN et al. 2015; Vigano E et al. 2015; Lagrange B et al. 2018). Activated CASP4 drives non-canonical inflammasome responses to fight bacterial infections (reviewed in Rathinam VAK et al. 2019; Zamyatina A & Heine H 2020; Downs KP et al. 2020). LPS triggers auto-processing at D289 in the inter-subunit linker of CASP4, generating a CASP4 subunit p10 (290-377) (Wang K et al. 2020). The CASP4 autoprocessing at D289 was required for induction of gasdermin D (GSDMD) cleavage thus promoting pyroptosis in human CASP4-/- epidermoid carcinoma A431 and HeLa cells that stably expressed Flag-tagged CASP4 (Wang K et al. 2020). Human CASP5 is thought to function similarly to CASP4 (Vigano E et al. 2015; Shi J et al. 2015). The protease activity of CASP5 can initiate pyroptosis through processing of GSDMD at D275, which is covered by this Reactome annotation. However, the mechanisms underlying activation and LPS recognition by CASP5 require further study. Further, structural studies suggest that binding of GSDMD to CASP4 allosterically enhanced the catalytic activity of CASP4 by stabilizing the dimeric form of processed CASP4 (2xp10:p20) (Wang K et al. 2020). Once activated, CASP4, CASP5 cleave GSDMD at D275 within the central linker region generating a 31-kDa N-terminal fragment (GSDMD (1-275)) which has an intrinsic pore-forming activity to initiate pyroptosis and a 22-kDa C-terminal fragment (GSDMD (276-484)) which inhibits cell death through intramolecular domain association (Shi J et al. 2015; Ding J et al. 2016; Liu Z et al. 2019; Yang J et al. 2018; Kuang S et al. 2017). The expression of GSDMD (1-275) in human embryonic kidney 293 (HEK293) cells induced pyroptosis, whereas overexpression of the C-terminal fragment of GSDMD (276-484) blocked cell death (Shi J et al. 2015). In addition, biochemical and structural studies of human GSDMD and mouse GSDMA3 showed the auto-inhibitory conformation of gasdermin domains which is released upon interdomain cleavage by inflammatory caspases, including CASP4 (Shi J et al. 2015; Ding J et al. 2016; Liu Z et al. 2019; Yang J et al. 2018; Kuang S et al. 2017). Thus, the CASP4, CASP5-mediated cleavage is thought to release the cytotoxic GSDMD (1-275) from intramolecular autoinhibition mediated by the C-terminal fragment of GSDMD. The N-terminal domain of GSDMD (1-275) binds and inserts into lipid membranes where it assembles into pores 10-16 nm in diameter (Ding J et al. 2016; Sborgi L et al. 2016). GSDMD pores facilitate the secretion

of active forms of interleukin-1β (IL-1β) and IL-18 from pyroptotic cells (Shi J et al. 2015; Ding J et al. 2016; Evavold CL et al. 2018). "The increasing abundance of membrane pores ultimately leads to membrane rupture and pyroptosis, releasing the entire cellular content" - Feng S et al. 2018. The murine homolog of CASP4/5, CASP11, can also cleave GSDMD (Kayagaki N et al. 2015).

This Reactome event shows CASP4/CASP5-mediated cleavage of GSDMD at D275.

Preceded by: GSDMD expression mediated by IRF2, IRF1

Followed by: GSDMD (1-275) binds bacterial cardiolipin, GSDMD (1-275) binds PIPs, GSDMD (1-275) binds cardiolipin

Literature references

Chen, Y., Liu, Z., Wang, C., Yang, J., Rathkey, JK., Pinkard, OW. et al. (2018). Mechanism of gasdermin D recognition by inflammatory caspases and their inhibition by a gasdermin D-derived peptide inhibitor. *Proc. Natl. Acad. Sci. U.S.A., 115*, 6792-6797.

Cai, T., Shi, J., Shao, F., Wang, K., Shi, X., Zhao, Y. et al. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature, 526*, 660-5.

Shi, J., Shao, F., Zhao, Y. (2018). Inflammatory Caspases: Activation and Cleavage of Gasdermin-D In Vitro and During Pyroptosis. *Methods Mol. Biol., 1714*, 131-148.

Ding, J., Shao, F., Wang, K., Zeng, M., Li, Z., Shi, X. et al. (2020). Structural Mechanism for GSDMD Targeting by Autoprocessed Caspases in Pyroptosis. *Cell, 180*, 941-955.e20.

Diamond, CE., Sobota, RM., Balachander, A., Viganò, E., Spreafico, R., Mortellaro, A. (2015). Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nat Commun, 6*, 8761.

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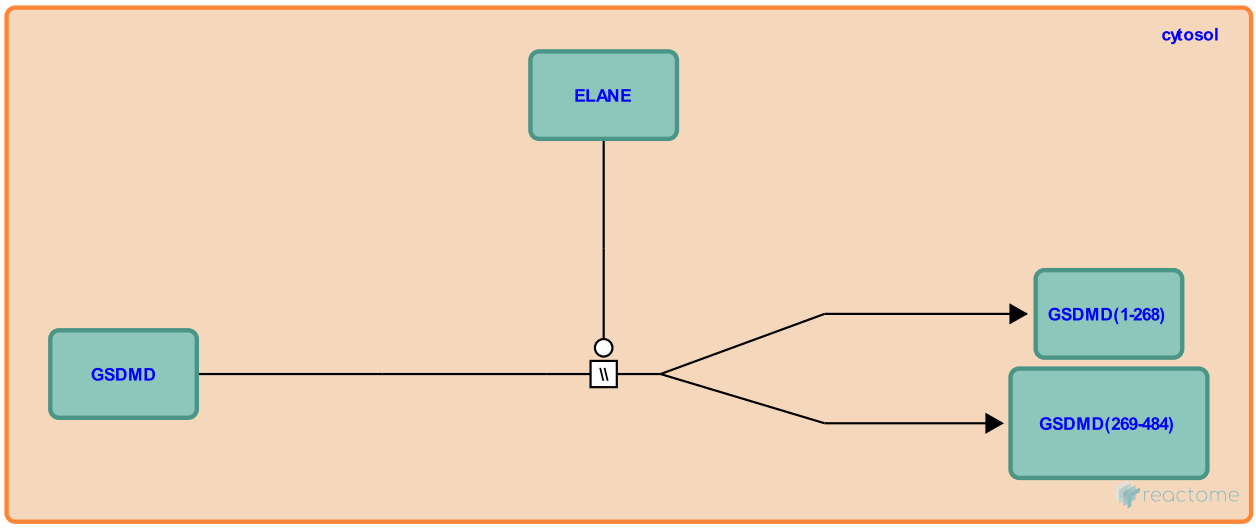
ELANE cleaves GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710106

Type: omitted

Compartments: cytosol



In neutrophils, gasdermin D (GSDMD) is cleaved by the serine protease, neutrophil elastase (NE or ELANE), which is released from the granules to cytosol during formation of neutrophil extracellular traps (NET) (Sollberger G et al. 2018; Kambara H et al. 2018). The process of NET formation and NET release is called NETosis, a programmed neutrophil cell death, which is induced in response to microbial infection and endogenous danger signals (reviewed in Papayannopoulos V 2018; Vorobjeva NV & Chernyak BV 2020). NETosis is characterized by the release of granule components such as ELANE or MPO into the cytosol, as well as chromatin decondensation associated with histone modification (reviewed in Papayannopoulos V 2018; Vorobjeva NV & Chernyak BV 2020). Although NETs may protect the host against microbes, excessive NET formation can contribute to the pathogenesis of immune-related diseases (reviewed in Papayannopoulos V 2018; Mutua V & Gershwin LJ 2020).

The cleavage of GSDMD by human neutrophil lysate was inhibited by ELANE-specific inhibitors in a dose-dependent manner (Kambara H et al. 2018). The identified ELANE cleavage site C268 in human GSDMD is different from the caspase cleavage site D275 and is not conserved between humans and mice (Kambara H et al. 2018). The N-terminal fragment of GSDMD (1-268) formed oligomers and induced lytic cell death upon overexpression in human embryonic kidney HEK293 cells (Kambara H et al. 2018). High-resolution total internal reflection fluorescence (TIRF) microscopy showed that after NET formation GSDMD localized to the plasma membrane in human primary neutrophils (Sollberger G et al. 2018). These data suggest that GSDMD cleavage by ELANE at D268 induces lytic cell death in neutrophils (Sollberger G et al. 2018; Kambara H et al. 2018). In addition, the N-terminal fragment of GSDMD was shown to target azurophilic (primary) granules and autophagosomes in human and murine neutrophils (Karmakar M et al. 2020). In this study the N-terminal fragment of GSDMD facilitated release of ELANE into the cytosol by permeabilization of azurophilic granules and secretion of IL-1β via an autophagy machinery-dependent pathway (Karmakar M et al. 2020).

Preceded by: GSDMD expression mediated by IRF2, IRF1

Followed by: GSDMD (1-275) binds bacterial cardiolipin, GSDMD (1-275) binds PIPs, GSDMD (1-275) binds cardiolipin

Literature references

Xu, Y., Bajrami, B., Han, M., Zhang, X., Zhou, S., Liu, P. et al. (2018). Gasdermin D Exerts Anti-inflammatory Effects by Promoting Neutrophil Death. *Cell Rep, 22*, 2924-2936. ↗

Nussbaumer, P., Herzig, A., Habenberger, P., Eickhoff, J., Klebl, B., Menninger, S. et al. (2018). Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci Immunol, 3*. ↗

Editions

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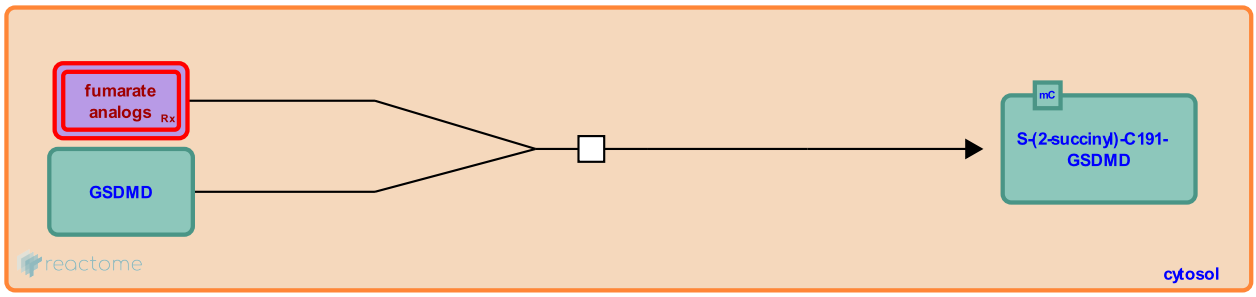
Dimethyl fumarate modifies Cys191 in GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9716258

Type: transition

Compartments: cytosol



During inflammation, the inflammatory caspase-1 (CASP1) can be activated downstream of canonical inflammasome activation in response to sensing of pathogen-derived particles or host-derived danger signals (reviewed in Kelley N et al. 2019; Zheng D et al. 2020). The non-canonical inflammasome assembly is mediated by CASP4, CASP5 in humans and CASP11 in mice upon sensing intracellular bacterial lipopolysaccharide (LPS) (Vigano E et al. 2015; Kayagaki N et al. 2015). Activated inflammatory caspases induce a proinflammatory cell death known as pyroptosis via the proteolytic processing of gasdermin D (GSDMD) (Shi J et al. 2015; Kayagaki N et al. 2015; He W et al. 2015; Ding J et al. 2016; Liu X et al. 2016; Sborgi L et al. EMBO J 2016). Intact GSDMD cannot form pores due to the inhibitory function of its C-terminal domain. Caspase-mediated cleavage of GSDMD releases the C-terminal fragment of GSDMD (276-484) (Shi J et al. 2015), enabling the N-terminal fragment of GSDMD (1-275) to form pores in cellular membranes leading to cytokine release and pyroptosis (Ding J et al. 2016; Liu X et al. 2016; Sborgi L et al. 2016; Mulvihill E et al. 2018). Dimethyl fumarate (DMF, trade name:Tecfidera®) was found to modify Cys191 of GSDMD to form S-(2-succinyl)-cysteine, a process known as succination of proteins (Humphries F et al. 2020). Cys191 in human GSDMD (corresponding to Cys192 in mouse) is thought to be critical for the GSDMD oligomerization and pore formation (reviewed in Pandeya A et al. 2019). DMF inhibited cell death and lactate dehydrogenase (LDH) release in LPS-primed mouse macrophages and human monocyte-like THP-1 cell in response to nigericin. Similarly, two fumarate analogs, diroximel fumarate (trade name: Vumerity®) and tepilamide fumarate, also blocked LPS-nigericin-induced pyroptosis and formation of GSDMD (1-275) (Humphries F et al. 2020). Treatment with DMF is thought to inhibit the interaction of GSDMD with CASP1, cleavage by CASP1 and oligomerization of GSDMD (Humphries F et al. 2020). Moreover, GSDMD-mediated pyroptosis when overactivated can lead to sepsis. Elevated levels of GSDMD were noted in microparticles isolated from plasma of septic patients (Homsy E et al. 2019). In the murine sepsis model, Gsdmd-deficient mice showed significantly improved survival compared to the wild type mice (Kambara H et al. 2018). Treatment with fumarate protected mice from LPS-induced septic shock (Humphries F et al. 2020). In addition, Gsdmd-/- mice are protected from disease in mouse models of familial Mediterranean fever and multiple sclerosis (MS) (Kanneganti A et al. 2018; Li S et al. 2019). Administration of DMF reduced GSDMD-driven responses in these mouse models (Humphries F et al. 2020). Further, DMF reduced levels of both interleukin (IL)-1β and GSDMD (1-275) in peripheral blood mononuclear cells (PBMCs) from patients with MS. The data suggest that DMF blocks GSDMD pore formation and pyroptosis by modifying Cys191 of GSDMD (Humphries F et al. 2020). Endogenous fumarate may also inactivate GSDMD by succinating Cys191 (Humphries F et al. 2020).

This Reactome event shows the covalent modification of GSDMD, namely S-(2-succinyl)-Cys191-GSDMD, formed by a Michael addition reaction between DMF and the reactive thiol group on Cys191 of GSDMD.

Literature references

Dutta, R., Fitzgerald, KA., Thompson, PR., Wilson, R., Humphries, F., Shmuel-Galia, L. et al. (2020). Succination inactivates gasdermin D and blocks pyroptosis. *Science, 369*, 1633-1637. ↗

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2020-11-09	Authored	Shamovsky, V.
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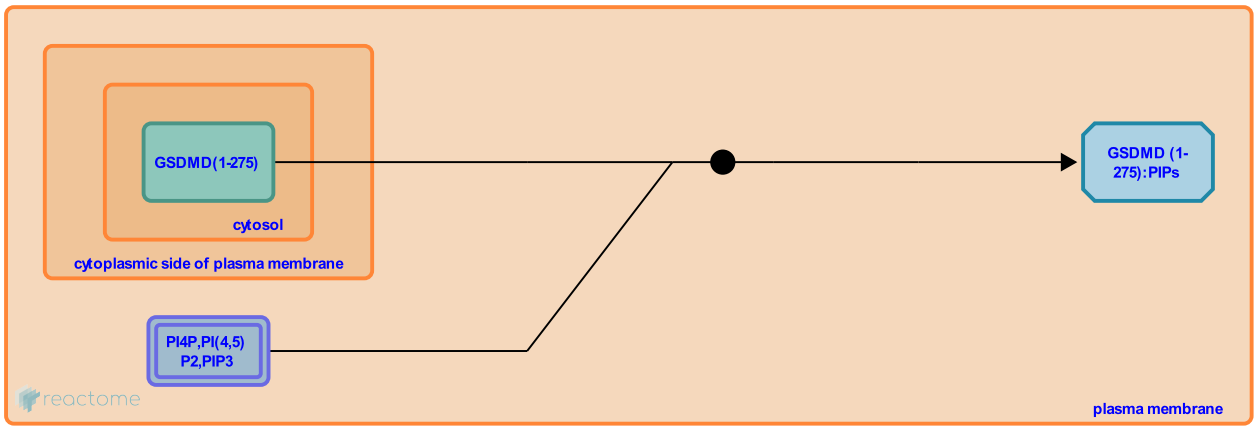
GSDMD (1-275) binds PIPs ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647631

Type: binding

Compartments: plasma membrane, cytosol



Human gasdermin D (GSDMD) is a member of the gasdermin (GSDM) protein family, which is processed by inflammatory caspases and cleaved into N-terminal (GSDMD(1-275)) and C-terminal (GSDMD(276-484)) fragments (Shi et al. 2015). The N-terminal fragment of GSDMD (1-275) by itself caused pyroptosis when expressed ectopically in human embryonic kidney HEK293 cells, whereas the overexpression of the GSDMD C-terminus was found to block pyroptosis (Shi et al, 2015). The N-terminal fragment, GSDMD (1-275), targets and permeabilizes cellular membranes by assembling transmembrane pores (Ding et al, 2016; Liu X et al, 2016; Sborgi et al, 2016; Mulvihill et al. 2018). High-resolution (≤ 2 nm) atomic force microscopy (AFM) showed that the N-terminal fragment of GSDMD inserts into various lipid membranes (Mulvihill E et al. 2018). The lipid composition of the membrane was found to directly influence the ability of GSDMD to permeabilize liposomes (Ding et al, 2016; Liu et al, 2016; Mulvihill et al. 2018). Whereas phosphoinositides (PIPs) facilitated binding of GSDMD (1-275), cholesterol reduced insertion of GSDMD (1-275) and pore formation (Ding et al, 2016; Sborgi et al, 2016; Mulvihill et al. 2018). Once inserted, GSDMD (1-275) assembles arc-, slit-, and ring-shaped oligomers (Ding et al, 2016; Liu et al, 2016; Sborgi et al, 2016; Mulvihill et al. 2018).

Preceded by: ELANE cleaves GSDMD, CASP1 cleaves GSDMD, CASP4, CASP5 cleave GSDMD

Followed by: GSDMD oligomerizes into arc-, slit-shaped structures

Literature references

Pfreundschuh, M., Mari, SA., Sborgi, L., Hiller, S., Müller, DJ., Mulvihill, E. (2018). Mechanism of membrane pore formation by human gasdermin-D. *EMBO J., 37*. ↗

Feng, S., Fox, D., Man, SM. (2018). Mechanisms of Gasdermin Family Members in Inflammasome Signaling and Cell Death. *J. Mol. Biol., 430*, 3068-3080. ↗

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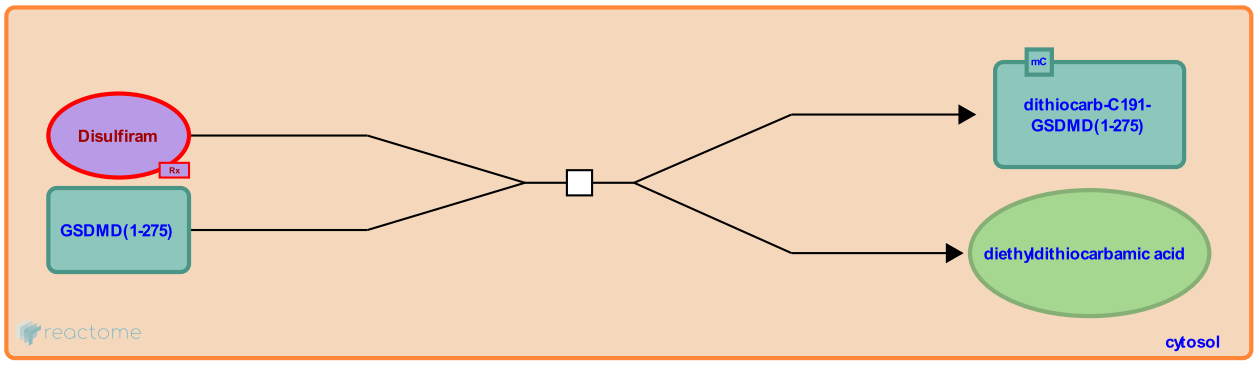
Disulfiram covalently modifies Cys191 in GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9693324

Type: transition

Compartments: cytosol



During inflammation, the inflammatory caspase-1 (CASP1) can be activated downstream of canonical inflammasome activation in response to sensing of pathogen-derived particles or host-derived danger signals (reviewed in Kelley N et al. 2019; Zheng D et al. 2020). The non-canonical inflammasome assembly is mediated by CASP4, CASP5 in humans and CASP11 in mice upon sensing intracellular bacterial lipopolysaccharide (LPS) (Vigano E et al. 2015; Kayagaki N et al. 2015). Activated inflammatory caspases induce a proinflammatory cell death known as pyroptosis via the proteolytic processing of gasdermin D (GSDMD) (Shi J et al. 2015; Kayagaki N et al. 2015; He W et al. 2015; Ding J et al. 2016; Liu X et al. 2016; Sborgi L et al. EMBO J 2016). Intact GSDMD cannot form pores due to the inhibitory function of its C-terminal domain. Caspase-mediated cleavage of GSDMD releases the C-terminal fragment of GSDMD (276-484) (Shi J et al. 2015), enabling the N-terminal fragment of GSDMD (1-275) to form pores in cellular membranes leading to cytokine release and pyroptosis (Ding J et al. 2016; Liu X et al. 2016; Sborgi L et al. 2016; Mulvihill E et al. 2018). Disulfiram, the thiol-reactive drug also known as antabuse, was found to inhibit nigericin-induced NLRP3-mediated pyroptosis and inflammatory cytokine release in LPS-primed human monocytic THP-1 cells (Hu JJ et al. 2020). Similar results were obtained for the non-canonical (caspase-11-dependent) mouse inflammasome pathway induced by LPS electroporation in mouse immortalized bone marrow-derived macrophages (iBMDMs) (Hu JJ et al. 2020). Nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) identified a dithiodiethylcarbamoyl adduct of Cys191 in human GSDMD suggesting that disulfiram covalently modified Cys191 of GSDMD. The importance of Cys191 of GSDMD for disulfiram activity was further confirmed by a site-directed mutational analysis (Hu JJ et al. 2020). In line with these findings, necrosulfonamide (NSA) was identified as a potent inhibitor of pyroptosis by targeting GSDMD at Cys191 (Rathkey JK et al. 2018), and dimethyl fumarate modifies Cys191 to form S-(2-succinyl)-cysteine and block pyroptosis (Humphries F et al. 2020). Cys191 in human GSDMD (corresponding to Cys192 in mouse) is thought to be critical for the GSDMD oligomerization and pore formation (reviewed in Pandeya A et al. 2019). Further, disulfiram allowed cleavage of pro-interleukin 1β (IL-1β) and GSDMD, but abrogated GSDMD pore formation and blocked IL-1β release in human and mouse cells (Hu JJ et al. 2020). Moreover, GSDMD-mediated pyroptosis when overactivated can lead to sepsis. Elevated levels of GSDMD were noted in microparticles isolated from plasma of septic patients (Homsy E et al. 2019). In the murine sepsis model, GSDMD-deficient mice showed significantly improved survival compared to the wild type mice (Kambara H et al. 2018). Disulfiram activity protected mice from LPS-induced septic shock (Hu JJ et al. 2020). The data suggest that disulfiram blocks GSDMD pore formation and pyroptosis by modifying Cys191 of GSDMD and point to the possibility of using disulfiram to counteract human diseases due to excessive inflammation (Hu JJ et al. 2020).

Literature references

Lou, X., Zhang, Y., Wang, J., Luo, X., Zhang, Z., Zhao, J. et al. (2020). FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. *Nat. Immunol., 21*, 736-745. ↗

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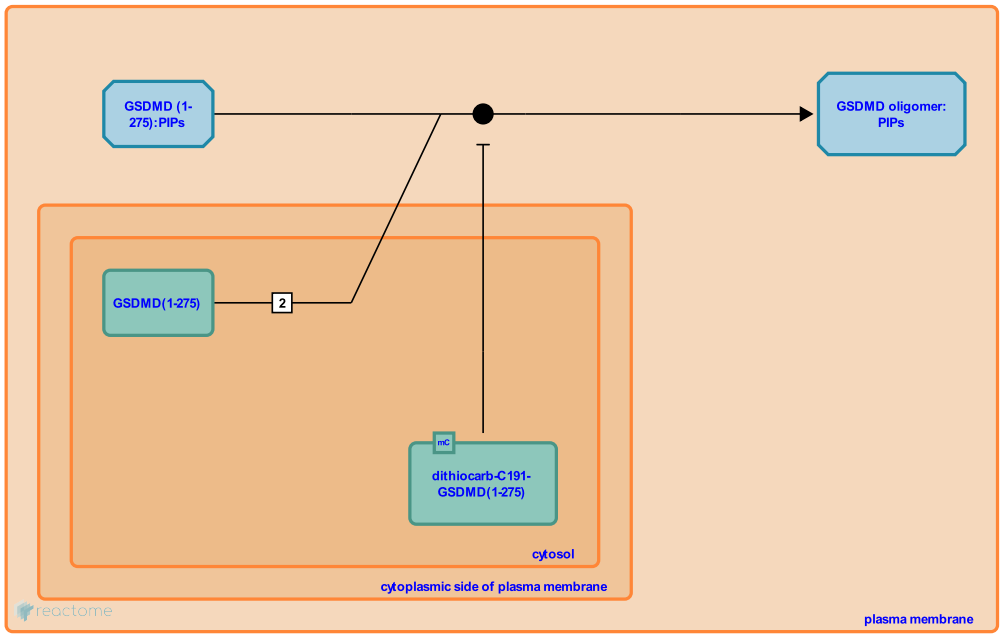
GSDMD oligomerizes into arc-, slit-shaped structures ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647645

Type: binding

Compartments: plasma membrane, cytosol



Gasdermin D (GSDMD) is a member of the gasdermin (GSDM) protein family, which is processed by inflammatory caspases and cleaved into an N-terminal (GSDMD(1-275)) and a C-terminal (GSDMD (276-484)) fragments (Shi J et al, 2015). Once GSDMD is cleaved, the N-terminal fragment of GSDMD (1-275) targets and permeabilizes cellular membranes by assembling transmembrane pores (Ding J et al, 2016; Liu X et al, 2016; Sborgi L et al, 2016). High-resolution (≤ 2 nm) atomic force microscopy (AFM) showed that GSDMD N-terminus inserts into various lipid membranes (Mulvihill E et al. 2018). Once inserted, the N-terminal fragment of GSDMD assembles arc-, slit-, and ring-shaped oligomers, which eventually incorporate additional oligomers and transform into larger thermodynamically stable ring-shaped oligomers (Mulvihill E et al. 2018).

Preceded by: GSDMD (1-275) binds PIPs

Followed by: GSDMD forms ring-shaped oligomers

Literature references

Pfreundschuh, M., Mari, SA., Sborgi, L., Hiller, S., Müller, DJ., Mulvihill, E. (2018). Mechanism of membrane pore formation by human gasdermin-D. *EMBO J., 37*. ↗

Editions

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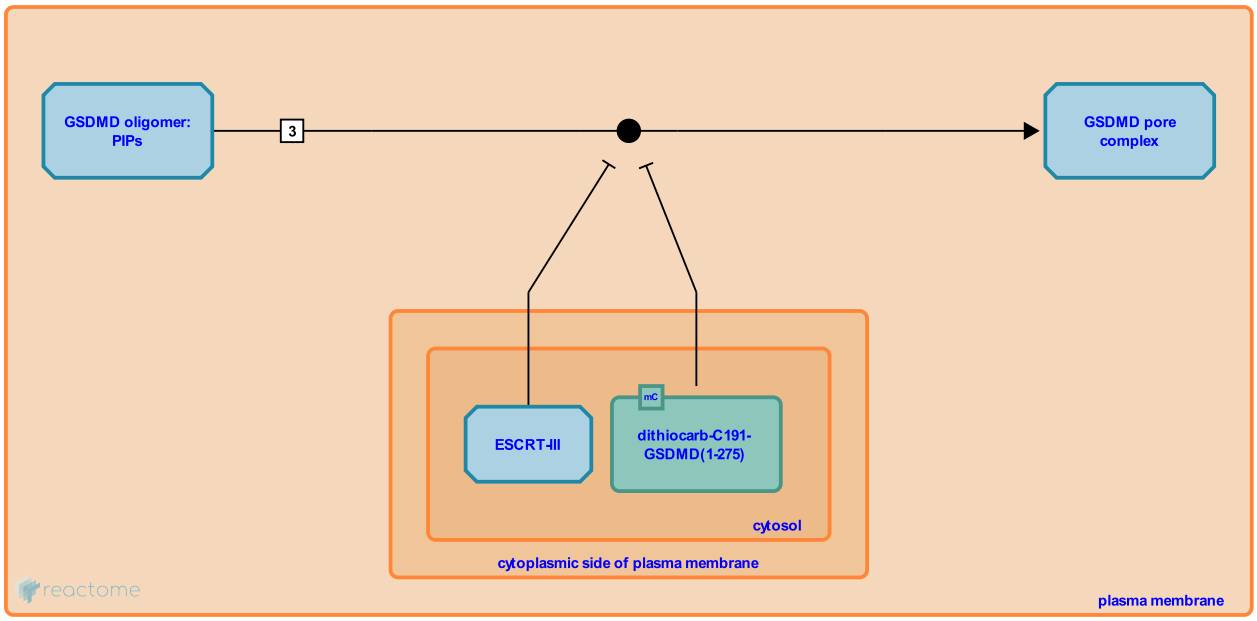
GSDMD forms ring-shaped oligomers ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647619

Type: binding

Compartments: plasma membrane



Gasdermin D (GSDMD) is cleaved by inflammatory caspases into an N-terminal (GSDMD(1-275)) and a C-terminal (GSDMD (276-484)) fragment (Shi et al, 2015). The liposome-based assays indicated that the N-terminal doman of GSDMD (1-275) binds membrane lipids assembling large pores (Ding J et al. 2016; Liu X et al. 2016). High-resolution (≤ 2 nm) atomic force microscopy (AFM) showed that the GSDMD N-terminus inserts into various lipid membranes (Mulvihill E et al. 2018). Once inserted, the N-terminal fragment of GSDMD assembles arc-, slit-, and ring-shaped oligomers, which eventually can incorporate additional oligomers and transform into larger thermodynamically stable ring-shaped oligomers (Mulvihill E et al. 2018). Ca2+ influx through GSDMD pores was shown to recruit the endosomal sorting complexes required for transport (ESCRT) machinery to damaged areas of the plasma membrane, leading to membrane repair. ESCRT-III–dependent membrane repair is thought to negatively regulate cell death and interleukin (IL)-1β, IL18 secretion following inflammasome activation (Rühl S et al. 2018).

Preceded by: GSDMD oligomerizes into arc-, slit-shaped structures

Literature references

Pfreundschuh, M., Mari, SA., Sborgi, L., Hiller, S., Müller, DJ., Mulvihill, E. (2018). Mechanism of membrane pore formation by human gasdermin-D. *EMBO J., 37*. ↗

Broz, P., Heilig, R., Pipercevic, J., Rühl, S., Sborgi, L., Farady, CJ. et al. (2016). GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J., 35*, 1766-78. ↗

Wang, DC., Ding, J., Liu, W., Shi, J., Shao, F., Wang, K. et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature, 535*, 111-6. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
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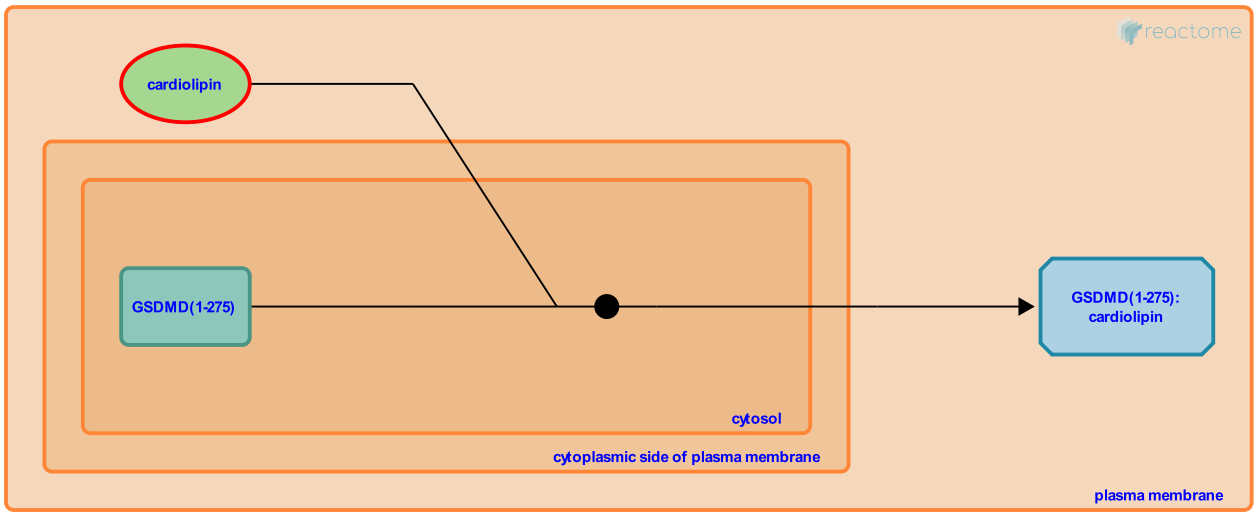
GSDMD (1-275) binds bacterial cardiolipin ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647643

Type: binding

Compartments: cytosol



The N-terminal domain of gasdermin D (GSDMD(1-275), also known as GSDMD-NT) binds to cardiolipin, a lipid found on the bacterial cell membrane, and oligomerizes to form pores on the bacterial cell membrane (Ding J et al. 2016; Liu X et al. 2016). GSDMD(1-275) was reported to damage and lyse Gram-positive and Gram-negative bacteria directly, including Escherichia coli, Staphylococcus aureus and Bacillus megaterium protoplasts (Ding J et al. 2016; Liu X et al. 2016). GSDMD(1-275) also interacts with cardiolipin present in the inner leaflet of the host mitochondrial membrane (Rogers C et al. 2019). However, it is not known how GSDMD can pass the outer membrane to access the inner member if it can disrupt the bacterial and mitochondrial membrane under physiological conditions.

Preceded by: ELANE cleaves GSDMD, CASP1 cleaves GSDMD, CASP4, CASP5 cleave GSDMD

Literature references

Wang, DC., Ding, J., Liu, W., Shi, J., Shao, F., Wang, K. et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature, 535*, 111-6. ↗

Lieberman, J., Wu, H., Zhang, Z., Magupalli, VG., Ruan, J., Pan, Y. et al. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature, 535*, 153-8. ↗

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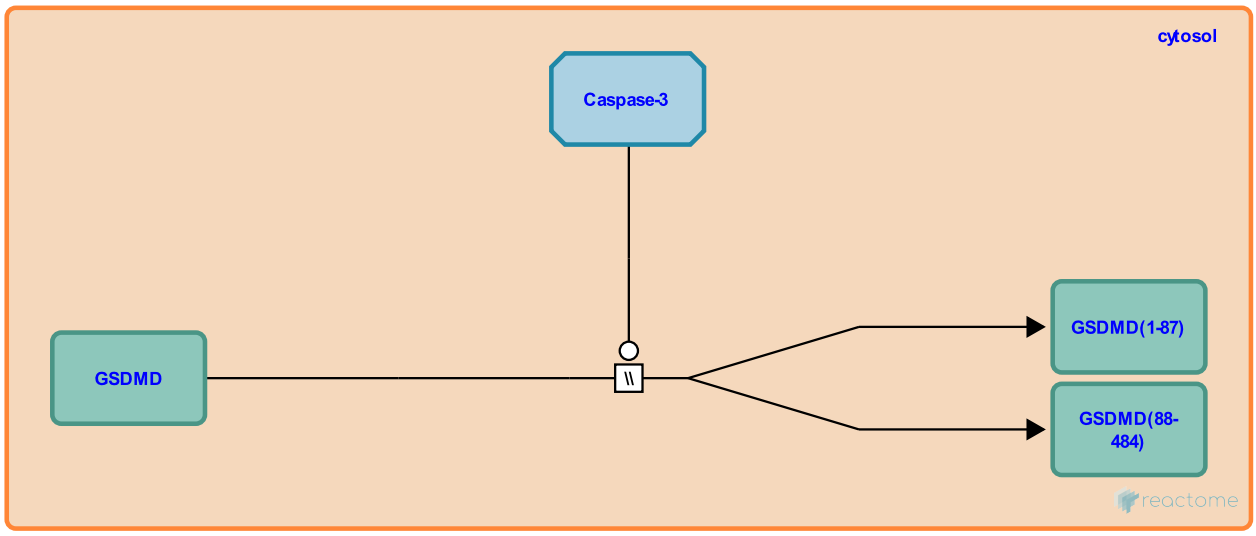
CASP3 cleaves GSDMD ↗

Location: Pyroptosis

Stable identifier: R-HSA-9686088

Type: omitted

Compartments: cytosol



During apoptosis, activated caspase-3 (CASP3) and to a lesser degree CASP7 inactivate gasdermin D (GSDMD) by cleaving GSDMD at position D87 generating p20 (GSDMD(1-87)) and p43 (GSDMD(88-484)) fragments (Taabazuing CY et al. 2017). This cleavage site is evolutionarily conserved and lies within the N-terminal fragment (p30, GSDMD(1-275)) that forms membrane pores. Proteolysis at this site renders GSDMD incapable of activating pyroptosis (Taabazuing CY et al. 2017). This pathway does not seem to play a key role during animal development as wild-type and Gsdmd-deficient mice both develop normally.

Literature references

Bachovchin, DA., Okondo, MC., Taabazuing, CY. (2017). Pyroptosis and Apoptosis Pathways Engage in Bidirectional Crosstalk in Monocytes and Macrophages. *Cell Chem Biol, 24*, 507-514.e4. ↗

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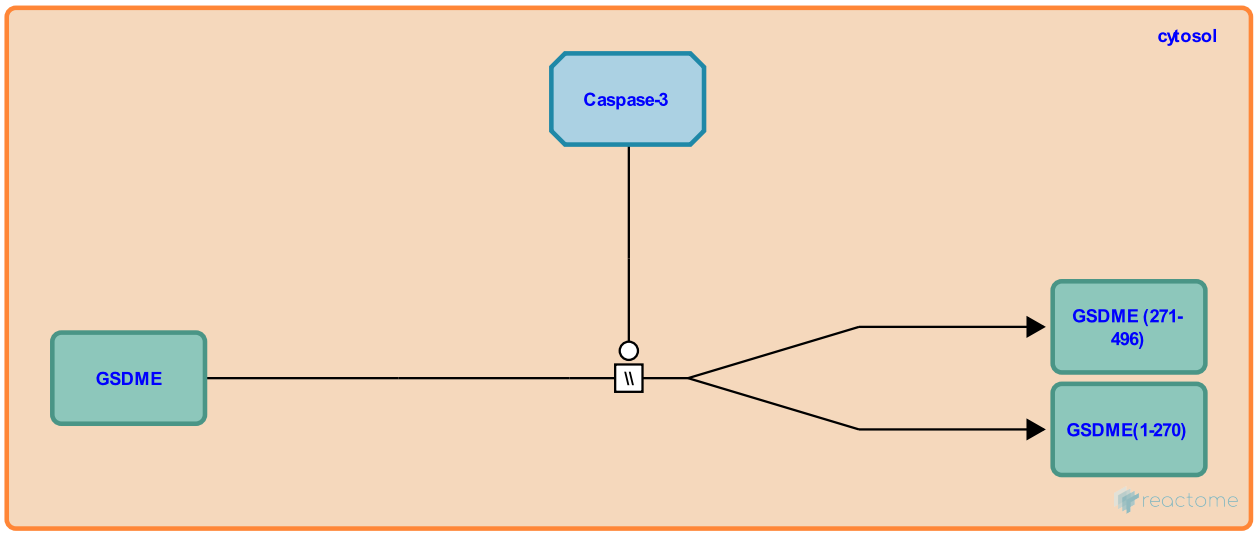
CASP3 cleaves GSDME ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647632

Type: omitted

Compartments: cytosol



Gasdermin E (GSDME, also known as DFNA5) was identified as a substrate of activated caspase-3 (CASP3), the executioner caspase of apoptotic cell death (Wang Y et al. 2017; Rogers C et al. 2017). GSDME can switch TNF- or chemotherapy drug-induced CASP3-mediated apoptosis to secondary necrosis/pyroptosis (Wang Y et al. 2017; Rogers C et al. 2017; Zhang CC et al. 2019; Zhang J et al. 2020; Zhang Z et al. 2020; reviewed in Jiang M et al. 2020). The expression level of GSDME determines the type of cell death (Wang Y et al. 2017). CASP3-mediated cleavage of GSDME drives pyroptosis in GSDME-expressing cells, including normal and certain types of cancer cells, while cells lacking sufficient levels of GSDME undergo apoptosis without progression into pyroptosis or secondary necrosis (Wang Y et al. 2017; reviewed in Jiang M et al. 2020). CASP3 cleaves GSDME in the linker after Asp270, generating the GSDME N-terminal fragment GSDME(1-270) that disrupts cell membranes and induces secondary necrotic/pyroptotic cell death (Wang Y et al. 2017; Rogers C et al. 2017). GSDME D267A and GSDME D270A mutations resisted cleavage in TNF-stimulated HeLa cells and showed no death-switching activity (Wang Y et al. 2017). The processing of GSDME was inhibited by the specific CASP3 inhibitor (zDEVD) in GSDME-expressing human neuroblastoma SH-SY5Y (Zhang J et al. 2020) and colon cancer HT-29 and HCT116 cells (Yu J et al. 2019) upon treatment with a chemotherapeutic drug. Similarly, a specific CASP3 inhibitor and a pan-caspase inhibitor (zVAD) suppressed drug-induced GSDME(1-270) generation and reduced pyroptosis in human lung cancer A549 cells (Zhang CC et al. 2019; Zhang J et al. 2020). In addition, GSDME(1-270) was shown to permeabilize the mitochondrial membrane, releasing cytochrome c and activating the apoptosome in GSDME-expressing human embryonic kidney (HEK293) cells (Rogers C et al. 2019). The release of cytochrome c and CASP3 activation in response to apoptotic stimuli were significantly reduced in GSDME-deficient human T-lymphoblastic (CEM-C7) cells (Rogers C et al. 2019). Moreover, GSDME deficiency accelerated cell growth in human melanoma cell line (MeWo) and in mouse models of melanoma, colon (CT26) and breast (EMT6) tumors (Lage H et al. 2001; Zhang Z et al. 2020). Gsdme-/- mice were protected from chemotherapy-induced tissue damage (Wang Y et al. 2017). These data suggest that GSDME may have cytotoxic effects in tumor cells by triggering pyroptotic cell death. GSDME-induced pyroptosis was shown to suppress tumor growth by increasing anti-tumor functions of tumor-infiltrating NK and CD8+ T killer lymphocytes (Zhang Z et al. 2020). Cancer-related GSDME mutations significantly reduced lactate dehydrogenase (LDH) release, a hallmark of lytic cell death (Zhang Z et al. 2020). The tumor suppressor role of GSDME is further supported by studies showing reduced expression of GSDME due to increased methylation

of the GSDME gene promoter in primary gastric tumors, colorectal adenocarcinomas and breast tumors (Akino K et al. 2006; Kim MS et al. 2008; Yokomizo K et al. 2012; Croes L et al. 2017; Ibrahim J et al. 2019). GSDME-deficient tumors are associated with reduced survival in patients (reviewed in Xia X et al. 2019). Thus, CASP3 can induce pyroptosis and apoptosis in a manner that is dependent on the expression level of GSDME. GSDME was dispensable for the regulation of pyroptosis in human Jurkat T cells and THP-1 monocytes (Tixeira R et al. 2018). In line with this study, GSDME was not required for pyroptosis in mouse Casp1-and Casp11-deficient bone marrow-derived macrophages (BMDMs) treated with flagellin, cytochrome c or Fas ligand (Lee BL et al. 2018). Together, these findings suggest that factors in addition to the ones annotated here play a role in connecting apoptosis and pyroptosis, which has been shown in the context of PANoptosis (Karki R et al. 2021).

This Reactome event shows CASP3-mediated cleavage of GSDME at D270.

Preceded by: GSDME expression mediated by TP53, TP63

Followed by: GSDME (1-270) binds PIPs, GSDME (1-270) binds cardiolipin

Literature references

Cingolani, G., Rogers, C., Mayes, L., Alnemri, D., Alnemri, ES., Fernandes-Alnemri, T. (2017). Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat Commun, 8*, 14128. ↗

Ding, J., Liu, W., Shao, F., Wang, K., Shi, X., Wang, Y. et al. (2017). Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature, 547*, 99-103. ↗

Mok, TMY., Sengupta, S., Li, S., Meza-Sosa, KF., Junqueira, C., Zhang, Y. et al. (2020). Gasdermin E suppresses tumour growth by activating anti-tumour immunity. *Nature, 579*, 415-420. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
2021-02-17	Edited	Shorser, S.
2021-04-22	Reviewed	Shao, F.

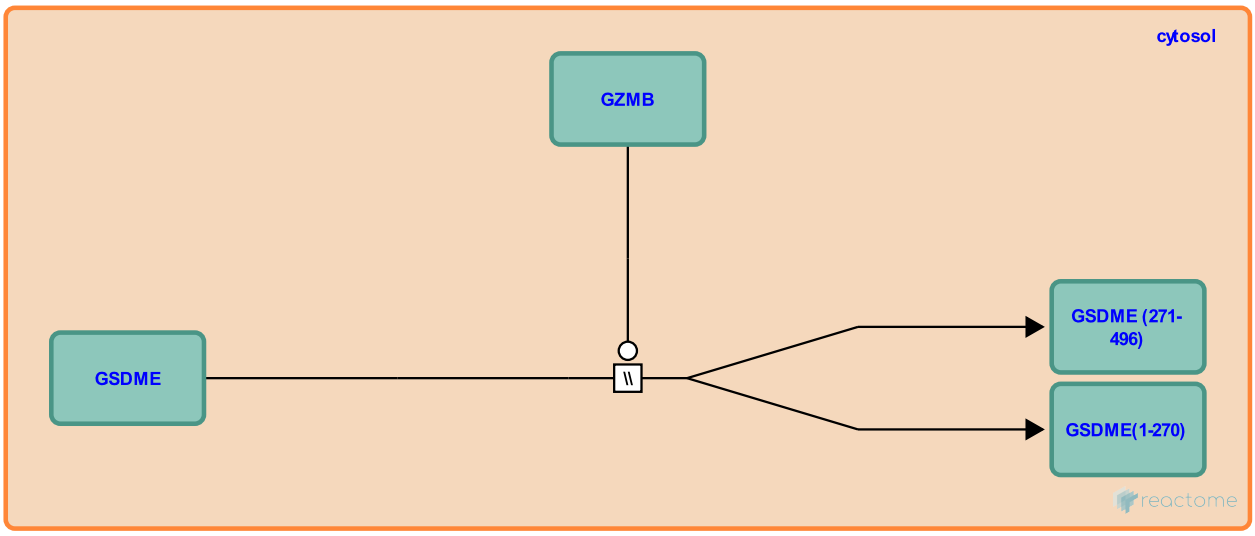
GZMB cleaves GSDME ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710263

Type: omitted

Compartments: cytosol



Granzyme B (GZMB) belongs to a family of serine proteases stored in the cytotoxic granules of natural killer (NK) cells and cytotoxic T lymphocytes. GZMB weakly cleaves recombinant gasdermin E (GSDME) in vitro and in lysates from GSDME-overexpressing human embryonic kidney 293T (HEK293T) cells (Zhang Z et al. 2020). Mutational analysis suggests that GZMB activates GSDME at the same site (D270) as caspase-3 (CASP3). In the presence of perforin (PFN), GZMB cleaved GSDME in human neuroblastoma SH-SY5Y cells inducing pyroptotic cell death. Despite that direct targeting of GSDME by GZMB is not efficient, GSDME can be cleaved by CASP3. Activation of CASP3 by GZMB was also detected in GZMB +PFN -treated SH-Y5Y cells (Zhang Z et al. 2020). In addition, human NK line YT or NK-92 triggered pyroptosis in GSDME-overexpressing HeLa cells in both CASP3-dependent and -independent manners. The data suggest that GZMB released from killer cytotoxic lymphocytes may induce GSDME-dependent lytic cell death in tumor targets via GZMB/CASP3-mediated cleavage of GSDME (Zhang Z et al. 2020). Similar findings were reported for chimeric antigen receptor (CAR) T cells that release a large amount of PFN and GZMB and result in the activation of GSDME in B leukemic cells leading to cell pyroptosis (Liu Y et al. 2020).

Preceded by: GSDME expression mediated by TP53, TP63

Followed by: GSDME (1-270) binds PIPs, GSDME (1-270) binds cardiolipin

Literature references

Mok, TMY., Sengupta, S., Li, S., Meza-Sosa, KF., Junqueira, C., Zhang, Y. et al. (2020). Gasdermin E suppresses tumour growth by activating anti-tumour immunity. *Nature, 579*, 415-420. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
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2021-02-17	Edited	Shorser, S.
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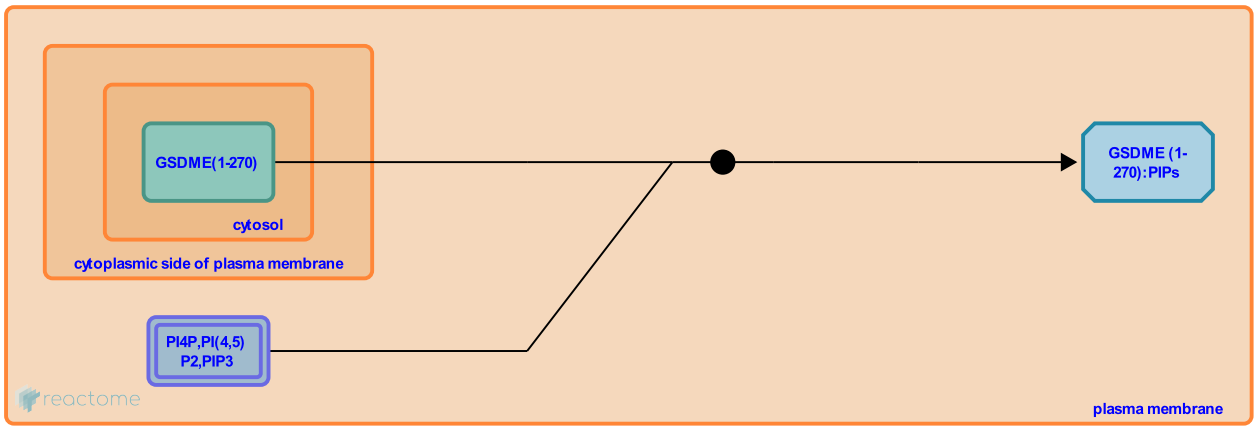
GSDME (1-270) binds PIPs ↗

Location: Pyroptosis

Stable identifier: R-HSA-9647660

Type: binding

Compartments: plasma membrane, cytosol



Gasdermin E (GSDME/DFNA5) cleavage at D270 by caspase-3 (CASP3) or by granzyme B (GZMB) liberates the N-terminal fragment of GSDME (GSDME(1-270)), which moves to the plasma membrane where it strongly binds to inner leaflet lipids such as phosphatidylinositol (4,5)-bisphosphate (Rogers C et al. 2017; Wang Y et al. 2017). Residues 1–56 of GSDME include 19 hydrophobic ones thought to be involved in membrane targeting and penetration (Rogers C et al. 2017). Upon cleavage by CASP3, the liberated N-terminal fragment of GSDME forms pores in the plasma membrane to either drive cells directly into pyroptosis or induce secondary necrosis after apoptosis in cells with low expression level of GSDME (that is insufficient to override the CASP3-mediated apoptotic program) (Rogers C et al. 2017; Wang Y et al. 2017).

Preceded by: GZMB cleaves GSDME, CASP3 cleaves GSDME

Followed by: GSDME oligomerizes

Literature references

Cingolani, G., Rogers, C., Mayes, L., Alnemri, D., Alnemri, ES., Fernandes-Alnemri, T. (2017). Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat Commun, 8*, 14128. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
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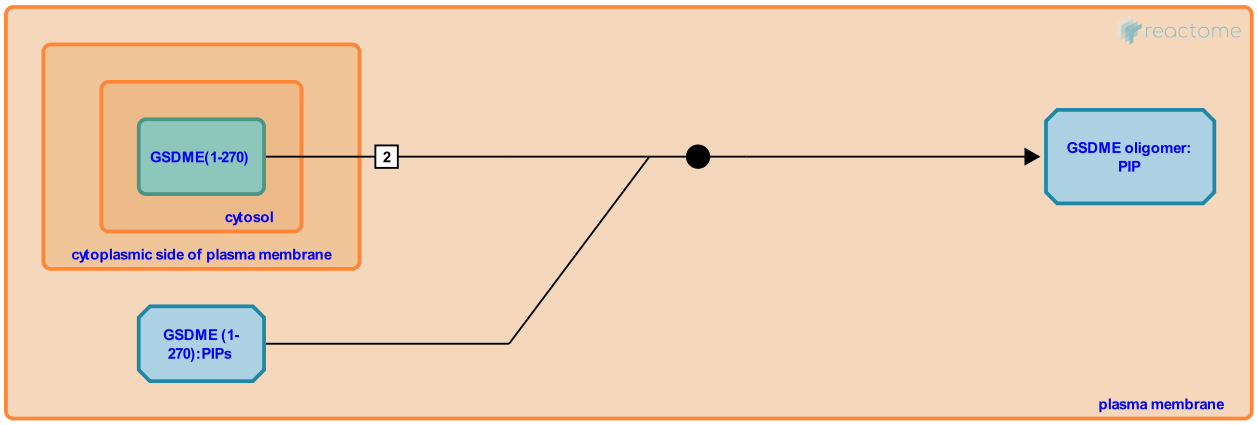
GSDME oligomerizes ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710254

Type: binding

Compartments: plasma membrane, cytosol



The N-terminal fragment of gasdermin E (GSDME(1-270)) produced by cleavage by caspase-3 (CASP3) or granzyme B (GZMB) targets the plasma membrane (Rogers C et al. 2017; Wang Y et al. 2017). In vitro oligomerization assays showed that GSDME(1-270) formed dimers and high-molecular weight oligomers when incubated with purified cell membranes from human embryonic kidney 293 (HEK293T) cells (Rogers C et al. 2019). Structural and biochemical studies suggest that the N-terminal fragments of gasdermins form membrane-spanning pores allowing release of inflammatory molecules such as interleukin (IL)-1β, IL-18, and high-mobility group box 1 (HMGB1) and eventually causing cell rupture (Shi J et al. 2015; Ding J et al. 2016; Liu X et al. 2016; Feng S et al. 2018; Mulvihill E et al. 2018). Phosphorylation of GSDME at T6 may influence its pore-forming activity (Rogers C et al. 2019).

Preceded by: GSDME (1-270) binds PIPs

Literature references

Nardone, A., Aplin, AE., Rogers, C., Erkes, DA., Alnemri, ES., Fernandes-Alnemri, T. (2019). Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. *Nat Commun, 10*, 1689. ↗

Wang, DC., Ding, J., Liu, W., Shi, J., Shao, F., Wang, K. et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature, 535*, 111-6. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
2021-02-17	Edited	Shorser, S.
2021-04-22	Reviewed	Shao, F.

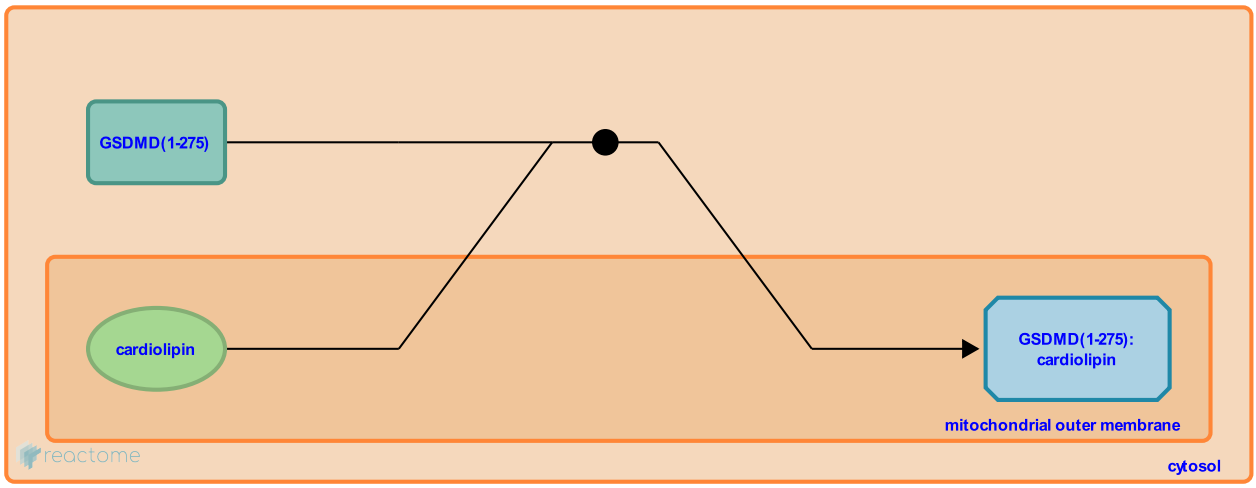
GSDMD (1-275) binds cardiolipin ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710353

Type: binding

Compartments: cytosol, mitochondrial outer membrane



Gasdermin D (GSDMD) is cleaved by inflammatory caspases (CASP) downstream of inflammasome activation (Shi J et al. 2015). The released N-terminal fragment of GSDMD (1-275) targets the plasma membrane to drive pyroptosis. In addition, GSDMD (1-275) can bind to and permeabilize liposomes containing cardiolipin, a phospholipid found on the mitochondrial membrane and bacterial membranes (Ding J et al. 2016; Liu X et al. 2016). Although cardiolipin is primarily located in the inner mitochondrial membrane, the outer mitochondrial membrane also contains around 10-20% cardiolipin and cardiolipin has been shown to translocate in a regulatable manner between the compartments (Liu et al. 2003; reviewed in Dudek J 2017). Further, upon expression in human embryonic kidney 293T (HEK293T) cells, GSDMD (1-275) induces cytochrome c (CYCS) release from the mitochondria leading to the CASP3 activation (Rogers C et al. 2019). In a mouse model of inflammatory lung injury, lipopolysaccharide (LPS) triggered caspase-11-mediated cleavage of mouse GSDMD, which formed pores on the mitochondrial membrane and induced mitochondrial DNA (mtDNA) release into the cytosol of endothelial cells (Huang LS et al. 2020). Moreover, single-cell analysis of pyroptosis dynamics in mouse macrophages revealed that GSDMD disrupts the mitochondrial membrane potential and leads to mitochondrial decay that precedes pyroptotic cell lysis (de Vasconcelos NM et al. 2019). These data suggest that the N-terminal fragment of GSDMD binds mitochondrial cardiolipin and forms pores triggering the release of mitochondrial proteins and DNA, however, the physiological relevance of this event remains to be determined.

This Reactome event describes the GSDMD (1-275) binding to mitochondrial cardiolipin leading to CYCS release.

Preceded by: ELANE cleaves GSDMD, CASP1 cleaves GSDMD, CASP4, CASP5 cleave GSDMD

Literature references

Pfreundschuh, M., Mari, SA., Sborgi, L., Hiller, S., Müller, DJ., Mulvihill, E. (2018). Mechanism of membrane pore formation by human gasdermin-D. *EMBO J., 37*. ↗

Nardone, A., Aplin, AE., Rogers, C., Erkes, DA., Alnemri, ES., Fernandes-Alnemri, T. (2019). Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. *Nat Commun, 10*, 1689. ↗

Wang, DC., Ding, J., Liu, W., Shi, J., Shao, F., Wang, K. et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature, 535*, 111-6. ↗

Lieberman, J., Wu, H., Zhang, Z., Magupalli, VG., Ruan, J., Pan, Y. et al. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature, 535*, 153-8. ↗

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2020-11-09	Authored	Shamovsky, V.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
2021-02-17	Edited	Shorser, S.
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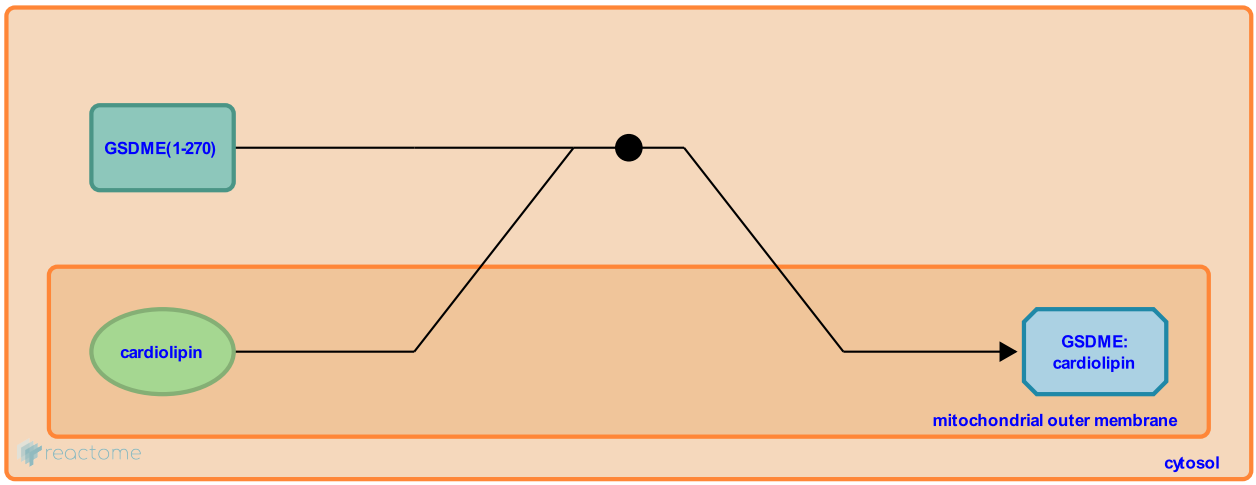
GSDME (1-270) binds cardiolipin ↗

Location: Pyroptosis

Stable identifier: R-HSA-9710354

Type: binding

Compartments: cytosol, mitochondrial outer membrane



Gasdermin E (GSDME) is cleaved by caspase 3 (CASP3) at D270 in response to apoptotic stimuli (Rogers C et al. 2017; Wang Y et al. 2017). The released N-terminal fragment of GSDME (1-270) targets the plasma membrane to drive pyroptosis in GSDME-expressing cells (Wang Y et al. 2017). In addition, the N-terminal fragment of mouse GSDME binds to cardiolipin liposomes causing severe leakage (Wang Y et al. 2017). Although cardiolipin is primarily located in the inner mitochondrial membrane, the outer mitochondrial membrane also contains around 10-20% cardiolipin and cardiolipin translocates in a regulatable manner between the compartments (Liu J et al. 2003; reviewed in Dudek J 2017). Confocal microscopy and biochemical analysis revealed that tagged-GSDME (1-270) localized to mitochondria and triggered release of proapoptotic proteins such as cytochrome c (CYCS) upon ectopic expression in human HeLa cells or human embryonic kidney 293T (HEK293T) cells (Rogers C et al. 2019). Endogenous GSDME (1-270) also localized to the mitochondrial fraction during apoptosis in TNFα plus actinomycin D (TNFα/actD)-treated human lymphoid CEM-C7 cells. Apoptotic stimuli-triggered cleavage of GSDME (1-270) induced CYCS release and ROS production in CEM-C7 cells (Rogers C et al. 2019). These data suggest that the N-terminal fragment of GSDME (1-270) can permeabilize the mitochondria in response to apoptotic stimuli (Rogers C et al. 2019), however, the physiological relevance of this event remains to be determined.

This Reactome event describes the GSDME (1-275) binding to mitochondrial cardiolipin leading to CYCS release from the mitochondria.

Preceded by: GZMB cleaves GSDME, CASP3 cleaves GSDME

Literature references

Pfreundschuh, M., Mari, SA., Sborgi, L., Hiller, S., Müller, DJ., Mulvihill, E. (2018). Mechanism of membrane pore formation by human gasdermin-D. *EMBO J., 37*. ↗

Nardone, A., Aplin, AE., Rogers, C., Erkes, DA., Alnemri, ES., Fernandes-Alnemri, T. (2019). Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. *Nat Commun, 10*, 1689. ↗

Wang, DC., Ding, J., Liu, W., Shi, J., Shao, F., Wang, K. et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature, 535*, 111-6. ↗

Editions

2020-11-09	Authored	Shamovsky, V.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
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2021-04-22	Reviewed	Shao, F.

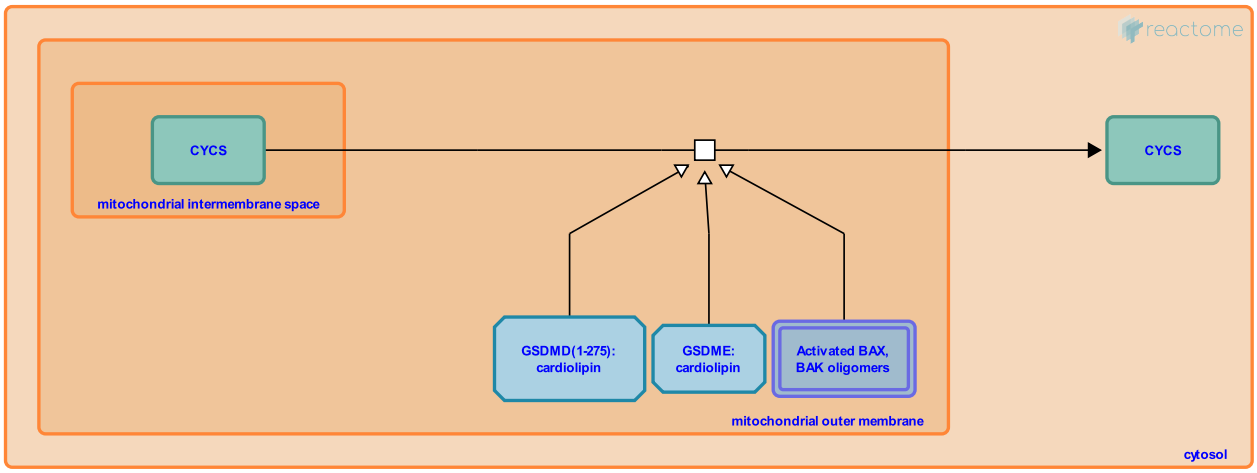
Release of Cytochrome c from mitochondria ↗

Location: Pyroptosis

Stable identifier: R-HSA-114284

Type: transition

Compartments: mitochondrial outer membrane



Permeabilization of the outer mitochondrial membrane by pro-apoptotic BCL2 family proteins, such as BAK and BAX, allows cytochrome c eflux from the mitochondrial intermembrane space into the cytosol (Arnoult et al. 2003).

Literature references

Song, Z., Wu, M., Yao, X. (2003). Direct interaction between survivin and Smac/DIABLO is essential for the anti-apoptotic activity of survivin during taxol-induced apoptosis. *J Biol Chem, 278*, 23130-40. ↗

Arnoult, D., Karbowski, M., Gaume, B., Cecconi, F., Sharpe, JC., Youle, RJ. (2003). Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. *EMBO J, 22*, 4385-99. ↗

Editions

2018-09-25	Reviewed	Matthews, L.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
2021-04-22	Reviewed	Shao, F.

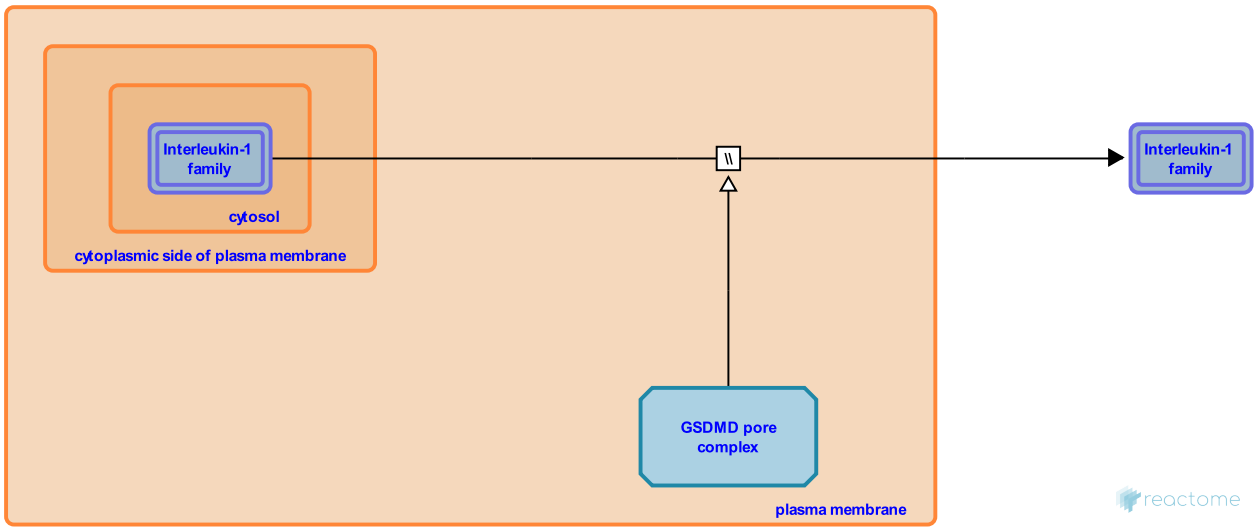
Interleukin-1 family are secreted ↗

Location: Pyroptosis

Stable identifier: R-HSA-449058

Type: omitted

Compartments: plasma membrane



Interleukin-1β (IL-1β) lacks signal sequences for compartmentation within the Golgi and classical secretory vesicles, so release of the mature form to extracellular compartments requires nonclassical mechanisms of secretion which are poorly understood (Eder C 2009; Piccioli P & Rubartelli A 2013). Several secretory pathways were proposed involving secretory lysosomes, exosomes, microvesicles, and autophagic vesicles, possibly through a mechanism similar to chaperone-mediated autophagy (CMA) (Andrei C et al. 2004; Ward JR et al. 2010; MacKenzie A et al. 2001; Gudipaty L et al. 2003; Qu Y et al. 2007; Iula L et al. 2018: reviewed by Eder C 2009; Piccioli P & Rubartelli A 2013; Claude-Taupin A et al. 2018). Further, the route of IL-1β secretion was found to be dependent on the type and strength of the inflammatory stimuli (Semino C et al. 2018; Sitia R & Rubartelli A 2018). Thus, in primary human monocytes small trauma or low pathogen load (LPS) activated a pathway involving secretory lysosomes that allows slow release of IL-1β, followed by apoptotic cell death that switches off the inflammatory response (Semino C et al. 2018). Differently, a stronger stimulus (LRZ) resulted in gasdermin D (GSDMD) cleavage with generation of the N-terminal domain that assembles in N-rings with formation of pores through which IL-1β can be externalized: this pathway of secretion is followed by pyroptosis, with membrane ruptures through which DAMPs can leave cells, further amplifying the inflammatory response (Semino C et al. 2018). Caspase-8 and FADD are required for NLRP3 inflammasome activation and IL-1β release (Gurung P et al. 2014, 2016).

Literature references

Franchi, L., Nunez, G., Qu, Y., Dubyak, GR. (2007). Nonclassical IL-1 beta secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. *J Immunol , 179*, 1913-25. ↗

Editions

2010-05-17	Authored	Ray, KP.
2010-08-06	Edited	Jupe, S.
2010-09-06	Reviewed	Pinteaux, E.
2021-02-17	Reviewed	D'Eustachio, P., Kanneganti, TD.
2021-04-22	Reviewed	Shao, F.

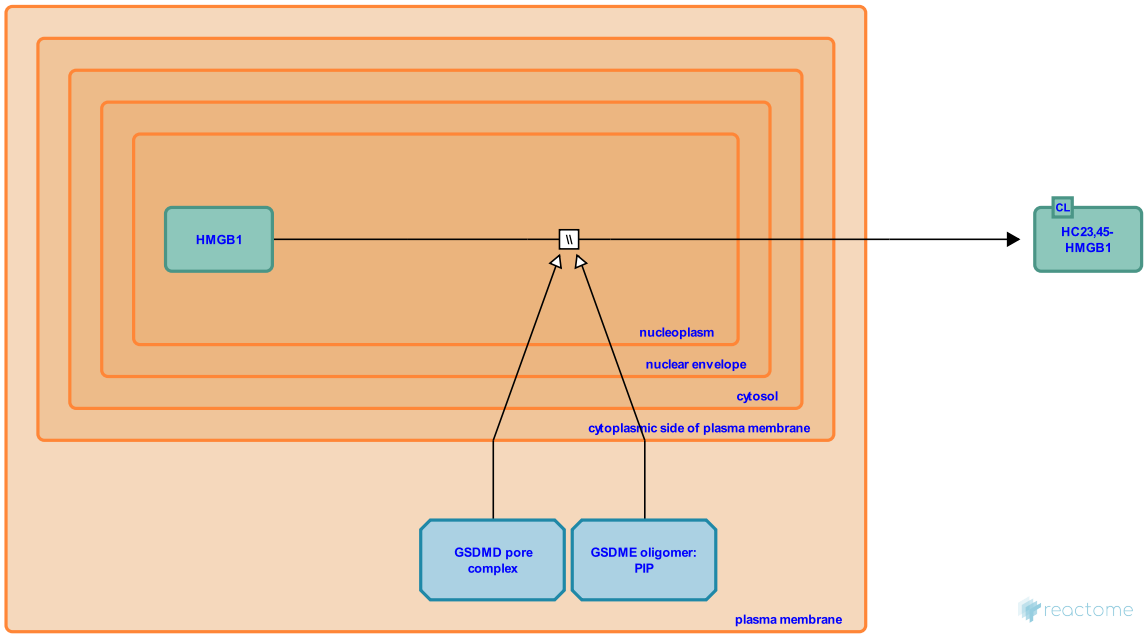
HMGB1 release from cells ↗

Location: Pyroptosis

Stable identifier: R-HSA-6805981

Type: omitted

Compartments: nucleoplasm, extracellular region



High mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that under normal conditions binds and bends DNA and facilitates gene transcription. In response to infection or injury, HMGB1 is actively secreted by innate immune cells and/or released passively by necrotic or damaged cells to function as an alarmin (Andersson U et al. 2000; Scaffidi P et al. 2002; Bonaldi T et al. 2003; Chen G et al. 2004; Lamkanfi M et al. 2010; Beyer C et al. 2012; Yang H et al. 2013). Earlier studies reported that HMGB1 did not diffuse out of cells undergoing apoptosis as HMGB1 was found to be tightly associated with the chromatin in apoptotic cells, even when the cell membrane was permeabilized artificially with detergents (Scaffidi P et al. 2002). This finding is in agreement with the general observation that apoptosis does not promote inflammation. However, further work showed that cells that undergo apoptosis do release HMGB1 (Bell CW et al. 2006; Yamada Y et al. 2011; Spencer DM et al. 2014). In human apoptotic cells (acute myeloid leukemia H60, HeLa, Jurkat T lymphocyte, pancreatic carcinoma PANC1 cell lines) HMGB1 was found to translocate into membrane-bound vesicles which are generated and released by cells during apoptosis (Spencer DM et al. 2014; Schiller M et al 2013). Outside the cell, HMGB1 can serve as an alarmin to activate innate immune responses including chemotaxis and cytokine release in both normal and aberrant immunity (Andersson U et al. 2000; Zetterström CK et al. 2002; Voll RE et al. 2008; Harris HE et al. 2012; Diener KR et al. 2013; Yang H et al. 2013).

Literature references

Andersson, U., Antoine, DJ., Tracey, KJ., Yang, H. (2013). The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. *J. Leukoc. Biol., 93*, 865-73. ↗

Bell, CW., Pisetsky, DS., Jiang, W., Reich, CF. (2006). The extracellular release of HMGB1 during apoptotic cell death. *Am. J. Physiol., Cell Physiol., 291*, C1318-25. ↗

Bianchi, ME., Misteli, T., Scaffidi, P. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature, 418*, 191-5. ↗

Giessl, A., Pisetsky, DS., Distler, JH., Schett, G., Stearns, NA., Beyer, C. (2012). The extracellular release of DNA and HMGB1 from Jurkat T cells during in vitro necrotic cell death. *Innate Immun, 18*, 727-37. ↗

Editions

2015-09-12	Reviewed	D'Eustachio, P.
2015-09-12	Authored	Shamovsky, V.
2016-05-10	Edited	Shamovsky, V.
2016-05-12	Reviewed	Zanoni, I., Granucci, F.
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2021-04-22	Reviewed	Shao, F.

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