

Review Article

# Actors of necroptosis scenario in cell's scene

Sara Nikseresht<sup>1\*</sup>, Fariba Khodagholi<sup>1</sup>, Abolhassan Ahmadiani<sup>1,2</sup>

1. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. NeuroBiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

## Abstract

Necroptosis, as a novel concept, has been recently introduced in scientific literature. Much of our knowledge about necroptosis comes from ligation of tumor necrosis factor- $\alpha$  to its receptor, TNF receptor 1. Receptor-interacting protein kinase 1, receptor-interacting protein kinase 3 and its substrate, the pseudokinase mixed lineage kinase domain-like protein, have been comprehensively studied as influential components of this process. Emerging pioneering evidence suggests that many molecules, organelles and mechanisms are involved in necroptosis pathway. The aim of this review is presentation of molecular mechanisms of necroptosis in three phases including initiation, regulation and execution of necroptosis. Moreover, this review will summarize unprecedented insights into the contribution of various organelles and cell compartments such as mitochondria, endoplasmic reticulum, nucleus, lysosomes and Golgi apparatus in necroptosis pathway.

## Keywords:

Cell;

Necroptosis;

Organelles

**Received:** 14 Dec 2016

**Accepted:** 8 Mar 2017

## \*Correspondence to:

S. Nikseresht

**Tel:** +98-2122429769

**Fax:** +98-2122432047

## Email:

sara.nikseresht@sbmu.ac.ir

## 1. Background

In the middle of 19<sup>th</sup> century when the first concept of cell death was introduced, nobody knew that molecular science field would be changed by this theory. Approximately 100 years later, the paradigm of regulated cell death was described. After a while, an unknown regulated cell death was observed in animal cells, and named apoptosis. For many decades, scientists and researchers considered necrosis an accidental and uncontrolled cell death which placed in a specific category against apoptosis (Vandenabeele et al., 2010). In the 1990s, the piece of evidence confirmed noticeable role of receptor interacting protein kinase-1 (RIP1) and RIP3 in cell death (Hsu et al., 1996; Sun et al., 1999). Moreover, scientists revealed that RIP1 mediates caspase independent cell death (Holler et al., 2000). In 2003, the term 'programmed necrosis' was introduced in scientific literature (Chan et al., 2003). Two years

later, a group of researchers in Harvard University discovered a new form of nonapoptotic cell death inhibited by necrostatin-1 (Nec-1); thereby calling this nonapoptotic cell death 'necroptosis' (Degterev et al., 2005).

There are two main types of necrosis, regulated and unregulated. The morphological characteristics of cells under regulated/unregulated necrosis are identical, including rounding of the cell, increasing cell volume (oncosis), swelling of organelles, lysosomal membrane permeabilization, plasma membrane discontinuity and permeability, mild chromatin condensation and intact nuclei (Proskuryakov et al., 2003; Dunai et al., 2011). Several types of regulated necrosis are emerging quickly and necroptosis is the well-defined form of them (Table 1).

Many articles provide us with information about pivotal role of cell compartments including mitochondria (Zhang et al., 2009; Maeda and Fadeel, 2014; Shulga and Pastorino, 2016), endoplasmic reticulum (Rizzi et al., 2014; Saveljeva et al., 2015),

**Table1:** Various types of regulated necrosis

Regulated Necrosis	Regulatory factors	Execution factors
<b>Necroptosis</b> <sup>*</sup>	RIP1 RIP3	MLKL, Na <sup>+</sup> and Ca <sup>2+</sup> channels and H <sub>2</sub> O pore formation
<b>Parthanatos</b> <sup>&amp;</sup>	PARP1	PAR synthesis, NAD <sup>+</sup> and ATP depletion
<b>Ferroptosis</b> <sup>#</sup>	GPX4	ROS and Fe <sup>2+</sup>
<b>Pyroptosis</b> <sup>†</sup>	caspase-1	inflammation
<b>MPT-mediated regulated necrosis</b> <sup>*</sup>	CypD	Ca <sup>2+</sup>

RIP, receptor-interacting serine/threonine-protein kinase; PARP1, poly (ADP ribose) polymerase 1; GPX4, glutathione peroxidase 4; CypD, Cyclophilin D; MLKL, mixed lineage kinase like; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; ATP, adenosine 3-phosphate; ROS, reactive oxygen species;

<sup>\*</sup> Necroptosis is an identified form of regulated necrosis, Na<sup>+</sup> and Ca<sup>2+</sup> influx and changes in osmotic pressure are indications of cells under necroptosis (Jouan-Lanhouet et al., 2014).

<sup>&</sup> DNA damage causes PARP-1 overactivation; this enzyme is originally characterized by its role in DNA-repair mechanisms, which leads to poly (ADP-ribose) polymer synthesis and accumulation. PARP-1 overactivation diminishes cellular NAD<sup>+</sup> and ATP that eventually results in necrotic cell death (Fatokun et al., 2014).

<sup>#</sup> This iron-dependent cell death recognized by loss of activity of the lipid repair enzyme, GPX4, and subsequent accumulation of lipid peroxidation products and ROS (Xie et al., 2016).

<sup>†</sup> Pyroptosis occurs after caspase-1 activation which results in the maturation of pro-inflammatory cytokines like IL-1 $\beta$  and ultimate cell lysis (Aki et al., 2015).

• CYPD is the only established constituent of the permeability transition pore complex, and is related to mitochondrial permeability transition (MPT)-mediated regulated necrosis (Pasparakis and Vandenabeele, 2015).

nucleus (Kaku et al., 2015) and *etc.* in necroptosis pathway. Here, in an attempt to integrate the evidence, we have classified the role of cell compartments in necroptosis pathway following a brief review of important molecular mechanisms of necroptosis.

## 2. Molecular mechanisms of necroptosis

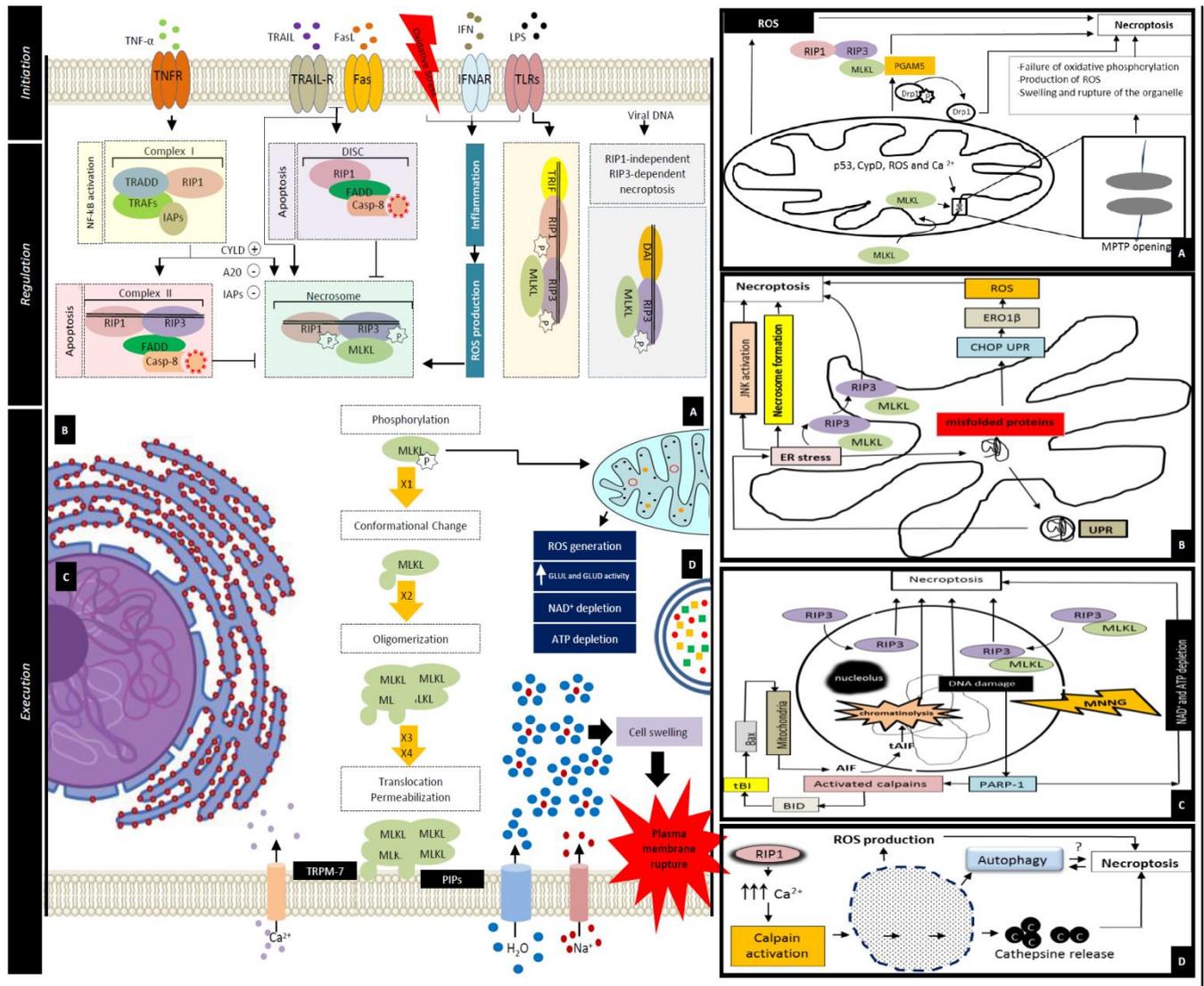
### 2.1 Necroptosis initiation

Regarding history of necroptosis, death receptors (DRs) play the key role in necroptosis recognition story. Among death ligands, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pleiotropic cytokine that induces the expression of some genes and orchestrates inflammatory responses through TNF receptor 1 (TNFR1) (Mc Guire et al., 2011) (Fig. 1). In addition to TNF- $\alpha$ , necroptosis can be triggered by other death ligands like Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL). TNF- $\alpha$  signaling leads to death-inducing complexes while FasL and TRAIL binding to their receptors results in death-

inducing signalling complex (DISC) formation. However, in both conditions, some key molecules determine cell's destiny to apoptosis or necroptosis (Pasparakis and Vandenabeele, 2015). Lack of caspase-8, as a vital molecule in death-inducing complexes and DISC, sensitizes Jurkat T cells to necroptosis induced by TNF, FasL and TRAIL (Holler et al., 2000). In another category of necroptosis inducers, Toll-like receptors (TLRs) trigger necroptosis via pathogen-associated molecular patterns like lipopolysaccharide (Li et al., 2016). Increased level of cytokines following activated TLRs and their downstream events as necroptosis initiators are drawing more attention recently. Other stimuli including (but are not restricted to) T cell receptor stimulation (Ch'en et al., 2008), interferons through IFN- $\alpha$  receptor type I (IFNAR) (McComb et al., 2014) and oxidative stress (Chtourou et al., 2015; Hanus et al., 2015; Zhang et al., 2016) could also induce necroptosis pathway.

### 2.2 Necroptosis regulation

In 1996, an article in *Immunity* journal reported that TNFR1 recruits RIP1 in the TNF signaling cascades



**Fig.1.** Molecular mechanism of necroptosis; initiation, regulation and execution. Necroptosis/programmed necrosis is induced by many factors ranging from ligands of death receptors to oxidative stress. A couple of kinases are main regulators of necroptosis; RIPK1 (receptor interacting protein kinase-1) and RIPK3. They exert their roles by connection to other molecules, and their phosphorylation/dephosphorylation could influence cell destination. MLKL (mixed-lineage kinase domain-like) and its downstream events are momentous executioners of necroptosis pathway which lead to plasma membrane rupture and ultimately cell death. In addition to molecules and events role in necroptosis pathway, role of mitochondria (A), endoplasmic reticulum (B), nucleus (C) and lysosome (D) in this pathway are considerable important. TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ), TNFR1 (TNF receptor 1), TRAIL (TNF-related apoptosis-inducing ligand), FasL (Fas ligand), DISC (death-inducing signalling complex), TLRs (Toll-like receptors), LPS (lipopolysaccharide), IFNAR (interferon alpha receptor) FADD (Fas-associated death domain), TRADD (TNFR1-associated death domain protein), DAI (DNA-dependent activator of interferon regulatory factors), TRIF (Toll/IL-1 receptor (TIR) domain-containing adaptor protein inducing interferon ( $\beta$ )), IAP (inhibitor of apoptosis), CYLD (cylindromatosis), GLUL (glutamate-ammonia ligase), GLUD (glutamate dehydrogenase), TRPM7 (transient receptor potential melastatin related 7), PIPs (phosphatidylinositol phosphates), ROS (reactive oxygen species), ATP (adenosine triphosphate), MPTP (mitochondrial permeability transition pore), CypD (cyclophilin D), C1QBP (Component 1, q Subcomponent Binding Protein), Drp1 (Dynamin-related protein 1), UPR (unfolded protein response), CHOP (C/EBP-homologous protein), ERO1 $\beta$  (endoplasmic reticulum oxidoreductase 1 beta), MNNG (1-methyl-3-nitro-1-nitroso-guanidine), tAIF (truncated AIF)

which resulted in a scientific discovery; RIP1 is the primary molecule to regulate necroptosis (Hsu et al., 1996). About three years later, researchers took an important step towards characterization of RIP3 and

revealed that this molecule binds RIP, and their interaction is recruited by the TNFR1 signaling complex (Sun et al., 1999). RIP3 contains an N-terminal kinase domain and a C-terminal RIP

homotypic interaction motif (RHIM). RHIM on RIP3 and RIP1 mediates a large amyloid-like structure and necroptosis occurs by virtue of this communication (Fig. 1) (Sun et al., 2002; Li et al., 2012; Chan et al., 2015). Various necroptosis regulators might be involved when necroptosis pathway is induced by specific molecules. In death-induced complexes and DISC formation some molecules such as caspase-8, Fas-associated death domain (FADD) and TNFR1-associated death domain protein have determinative roles. Activation and inactivation of these molecules might change the ways, in which cells decide to suicide. Caspase-8 cleavage inhibits RIP1 and RIP3 phosphorylation, and under this condition apoptosis will be executed while these molecules mediate necroptosis when caspase-8 is blocked (Zhou and Yuan, 2014). Caspase-8 and FADD deficiency in mouse embryonic fibroblasts (MEFs) leads to progressive RIP1–RIP3 necrosome formation via both type I ( $\alpha/\beta$ ) and type II ( $\gamma$ ) interferons (IFNs) (Thapa et al., 2013). In RIP1 independent necroptosis, a particular type of necroptosis, viral double-stranded DNA is recognized by DNA-dependent activator of interferon regulatory factors (DAI). DAI through its RHIM domain interacts with RIPK3, then induces the formation of the necrosome and triggers RIP1-independent RIP3-dependent necroptosis (Upton et al., 2012). Another important molecule in necroptosis regulation is TRIF (Toll/IL-1 receptor (TIR) domain-containing adaptor protein inducing IFN- $\beta$ ), which contains a RHIM-domain, allowing communication with RIPK1 and RIPK3 and then necrosome formation (Kaiser et al., 2013; Pasparakis and Vandenabeele, 2015). Although RIP1 and RIP3 kinase activity contribute to regulation of necroptosis, deubiquitination and ubiquitination of these proteins could also control this pathway. RIP1 deubiquitination by cylindromatosis promotes necroptosis while cellular inhibitor of apoptosis 1 & 2 directly ubiquitinate RIP1 and it has been demonstrated that ubiquitinated RIP1 associates with the cell survival (Bertrand et al., 2008; Welz et al., 2011). On the other hand, a negative regulator like A20 blocks RIP3 ubiquitination and rescues cells from necroptosis (Gurung et al., 2015).

### 2. 3 Necroptosis execution

In the effort to look for novel molecules involved in necroptosis execution, mixed-lineage kinase domain-

like (MLKL) pseudokinase was recognized as a substrate of RIP3. This pseudokinase is an essential component of the necroptosis cell death pathway. Phosphorylation of RIPK3 on serine 227, leads to MLKL phosphorylation on threonine 357 and serine 358 which is the critical step for necroptosis execution. MLKL contains a C-terminal pseudokinase domain that suppresses the executioner function of the N-terminal. Phosphorylation of the N-terminal domain results in conformational change. Conformational change leads to MLKL oligomerization (Tri/Tetra), membrane localization and ultimately membrane permeabilization which induces cell death. Some crucial regulators considered as proteins X1–X4 control MLKL activation; X1, conformational change, X2, oligomerization, X3, membrane translocation and X4, permeabilization (Fig. 1) (Sun et al., 2012; Tanzer et al., 2016). The oligomerization of MLKL disrupts the plasma membrane through the transient receptor potential melastatin related 7 (TRPM7)-mediated calcium influx. TRPM was known as a non-voltage-sensitive cation channel. MLKL-mediated  $\text{Ca}^{2+}$  influx triggers plasma membrane damage (Cai et al., 2014). Besides  $\text{Ca}^{2+}$  influx, intracellular sodium concentration increases during necroptosis. Sodium augmentation would disturb the osmotic homeostasis and facilitate the osmosis mediated rupture of the membrane. MLKL complex automatically or through other membrane proteins enhances sodium influx. Furthermore, some amino acids on the surface of N-terminal enable MLKL to connect to phosphatidylinositol phosphates (PIPs) and MLKL connection to PIPs acts to form pores (Fig. 1). The augmented intracellular sodium concentration amplifies osmotic pressure, leading to water influx by assembled pores. These events eventually lead to cell swelling and plasma membrane rupture. This process has been recently recognized as a crucial step for necroptosis execution (Chen et al., 2014b; Dondelinger et al., 2014). Moreover, it has been shown that generation of reactive oxygen species (ROS) is one of the most effective downstream events of MLKL activation during necroptosis (Zhao et al., 2012). In addition to oncosis and ROS formation, alteration of adenosine triphosphate (ATP) level is the other common downstream event that necrotic cells share. RIP1–RIP3 necroptosome triggers glycolysis and glutaminolysis in downstream

which enhances bioenergetics and decreases ATP levels by mitochondria (Vandenabeele et al., 2010). Glycolysis is dependent on  $\text{NAD}^+$ , and  $\text{NAD}^+$  reduction makes cell consume ATP to replete the  $\text{NAD}^+$  level, and this situation leads to energy failure and cell suicide. On the other hand, some mitochondrial related enzymes like glutamate-ammonia ligase (GLUL) and glutamate dehydrogenase (GLUD) have vital functions for the use of glutamate or glutamine as substrates for ATP production. GLUL catalyzes glutamate to produce glutamine transferred into the mitochondria and converted to glutamate to function as an energy substrate. Then, GLUD converts glutamate to  $\alpha$ -ketoglutarate. So, increased activity of these enzymes exacerbates ATP depletion, leading to impaired cellular viability (Devalaraja-Narashimha and Padanilam, 2009; Nikseresht et al., 2015).

Taken together, initiation, regulation and execution of necroptosis are crucial steps to regulate/manipulate programmed necrosis. In Figure 1, some possible molecular events that happen in a necroptotic cell have been shown. In this schematic figure, role of some cellular organelles is also noticeable. Their roles will be described in five categories: mitochondria, endoplasmic reticulum, nucleus, lysosome and Golgi apparatus.

### 3. Mitochondria

Mitochondria are multifunction organelles, which are responsible for aerobic respiration. Disruption of mitochondrial function is the occasion of cell death. Mitochondria are crucial modulators of apoptosis through release of pro- and anti-apoptotic factors in the intrinsic pathway of apoptosis (Jain et al., 2013). Some evidence suggest that mitochondria are not involved in necroptosis pathway, and this organelle may be dispensable for this type of regulated necrosis; yet, various studies have implicated mitochondrial dysfunction as a key event in necroptosis (Marshall and Baines, 2014). The communication of RIP3 with MLKL makes RIP1/RIP3/MLKL complex translocate to the mitochondrial membrane (Chen et al., 2013). Countless molecules and events have been proposed to encompass in necroptosis pathway, and these mediators exert mitochondrial effects on cell fate under various stresses. Here, we point up some important molecules and events related to

mitochondria and their roles in necroptosis.

#### 3.1 ROS

ROS may in fact be primarily known potent molecule to trigger necroptosis. It has been shown that alterations of oxidative status lead to ROS generation by damaged mitochondria (Shindo et al., 2013). Stimulation of ROS formation in T-47D cell's mitochondria causes necroptosis cell death (Shahsavari et al., 2015). Mitochondrial dysfunction contributes to necroptotic execution through excessive ROS production (Tait et al., 2014). Based on a recent study, in some cell lines including L929 and RAW 264.7, mitochondrial ROS but not cytosolic ROS is an essential factor in  $\text{TNF}\alpha$ -induced cell death (Ardestani et al., 2013). Moreover, mitochondrial ROS generation as a result of severe endoplasmic reticulum stress is associated with necroptosis induction (Ma et al., 2016). Some researchers described RIPK1 activation, mitochondrial dysfunction and eventually ROS accumulation as a chain of events following necroptosis initiators administration (Thapa et al., 2011; Ye et al., 2012). It has been shown that mitochondrial complex I is the source of ROS generation in response to neoalbacinol. Neoalbacinol is a necroptosis initiator in some cancer cell lines. This molecule has been verified as an activator of ROS and RIPK3 but not RIPK1 (Yu et al., 2015). However, some studies assume that ROS is not an obligatory effector for necroptosis. They point out the role of ROS as a cell type specific mediator in necroptosis pathway (He et al., 2009; Moquin and Chan, 2010; Wu et al., 2015). In an article published in 2015, ROS inhibition was proposed as a trigger of necroptosis in TRAIL-induced necroptosis in human pancreatic cancer cells (Zhang et al., 2015). Irrespective of ROS source and cell types, ROS is a potent molecule in necroptosis initiation, regulation and execution.

#### 3.2 MPTP

Mitochondrial permeability transition pore (MPTP) is a potential mitochondrial mediator of necroptosis and may present a link between ROS generation and disruption of ATP production. The MPTP is a large, nonspecific channel that leads to a loss of the mitochondrial transmembrane potential and mitochondrial depolarization. Moreover, opening of the MPTP is associated with failure of oxidative

phosphorylation, production of ROS, and swelling and rupture of the organelle (Halestrap, 2009; Baines, 2010). ROS and  $\text{Ca}^{2+}$  increase the possibility of MPTP opening, while adenine nucleotides including ADP and ATP inhibit pore formation. Decrease of ATP level by itself is a possible mechanism to open MPTP and influences mitochondrial transmembrane potential. ATP synthase physically interacts with cyclophilin D (CypD). CypD is a protein which is present in the mitochondrial matrix and regulates MPTP (Giorgio et al., 2009; Elrod and Molkenin, 2013). Responding to various insults, including oxidative stress and  $\text{Ca}^{2+}$  overload, CypD facilitates mitochondrial permeability transition (Kroemer et al., 2007). Mice with declined CypD displayed reduction in infarct size after acute ischemia and reperfusion; this may address CypD as a remarkable molecule in cell death (Schinzel et al., 2005). In addition to CypD, p53 as a principal stress sensor is a key mediator of pore opening by oxidative stress. This protein interacts with CypD and mitochondrial p53-CypD axis induces necrosis cell death in mouse and human cells (Vaseva et al., 2012). Component 1, q subcomponent binding protein (C1QBP) is another related molecule to control MPTP opening. Knockdown of C1QBP sensitizes mitochondria to MPTP opening while overexpression of this molecule attenuates ROS-induction of the MPTP and cellular necrosis. So, C1QBP belongs to MPTP negative regulators category (McGee and Baines, 2011). It is clear that RIP1, RIP3 and MLKL axis is the fundamental effector of necroptotic cell death but it is unknown how they might connect to the MPTP. Based on evidence, necroptosis stimuli obviously amplify MLKL protein level in the mitochondria. Necroptosis also brings about a reduction in the myeloid cell leukemia 1 (Mcl-1). Mcl-1 is an anti-apoptotic Bcl-2 family member that causes mitochondrial dysfunction when removed. MLKL translocation to the mitochondria, matrix Mcl-1 depletion and MPTP opening lead to mitochondrial dysfunction. These are probably the main events in cell death through necroptosis (Karch et al., 2015). It has been shown that inhibition of MPTP could partially attenuate necroptotic related markers (Fakharnia et al., 2017). The executive roles of mitochondrial influential factors including MPTP and CypD in necroptosis have also been questioned and were described in distinct pathways (Ch'en et al.,

2011).

### 3.3 PGAM5

The mitochondrial phosphatase PGAM5 governs cellular oxidative stress through binding to the kelch ECH associating protein 1-nuclear factor-E2-related factor 2 (Keap1-Nrf2) complex (Lo and Hannink, 2008). This phosphatase indirectly promotes Bcl-XL degradation and sensitizes cells to apoptosis (Lo and Hannink, 2006). PGAM5 also promotes mitophagy, a cellular process that eradicates damaged mitochondria (Chen et al., 2014a). PGAM5 provides two splice variants, PGAM5L (long form) and PGAM5S (short form). Similar to apoptosis, necroptosis can also be initiated by two types of signals, extrinsic and intrinsic'. RIP1, RIP3, and MLKL are responsible for the extrinsic pathway while isoforms of PGAM5 function in the intrinsic necroptosis pathway. The role of this 32 KD mitochondrial membrane protein in necroptosis may be confusing. It was first introduced as an anchor of RIP1-RIP3-MLKL complex on mitochondria and a downstream of RIP1/RIP3 to mediate necroptosis (Wang et al., 2012; Belizario et al., 2015). However, a recent study indicates that PGAM5 functions independent of RIPK3 to promote inflammasome activation. They elucidate that PGAM5 is not a necessary element for necroptosis while it has a critical role in processing of pro-IL-1 $\beta$  and inflammation (Moriwaki et al., 2016). On the other hand, necroptotic stimulation enhances RIP1/RIP3 complex on the mitochondria during genetic depletion of PGAM5; it could demonstrate that PGAM5 has insufficient effect on RIP1/RIP3 function. In addition, the cytoprotective mechanisms of PGAM5 against necroptosis have been proved. PGAM5 may in fact be a necroptosis protective factor, both in mice and MEFs. PGAM5 inhibits necroptosis through PINK1-mediated mitophagy (Lu et al., 2016). Mitophagy is a necessary process to preserve intracellular mitochondria homeostasis. This process also decreases augmented ROS and diminishes necrotic cell death (Kubli and Gustafsson, 2012).

### 3.4 Drp1

Mitochondrial morphology is controlled by a balance of fusion and fission. Mitochondrial fission is regulated by Dynamin-related protein 1 (Drp1). Drp1 is primarily a cytoplasmic protein, and following

activation forms ring-like multimers and translocates to the mitochondria (Smirnova et al., 2001; Otera and Mihara, 2011). It has been suggested that the phosphorylation of Drp1 at Ser637 is an important regulatory modification (Chang and Blackstone, 2007). Since mitochondrial fission has been implicated in cell death, cell destination could be influenced by Drp1 activity (Yu et al., 2008; Dubois et al., 2016; Oettinghaus et al., 2016). Although it is indefinable how mitochondrial fission promotes necroptosis, some findings show that following RIP3 phosphorylation PGAM5 activates Drp1 through dephosphorylation of the inhibitory Ser637 site of Drp1. In other words, PGAM5 dephosphorylates and triggers Drp1's GTPase activity that facilitates mitochondrial fission and consequent necroptosis (Kanamaru et al., 2012; Wang et al., 2012). TNF- $\alpha$  stimulation and ATP depletion enhances the protein expression level of Drp1 significantly, while necroptosis inhibition or Drp1-knockdown rescues cells from damage. This evidence indicates that necroptosis inhibition may protect cell, most likely through a mechanism dependent on Drp1 (Zhang et al., 2013). Although Drp1 has been described as a required molecule in necroptosis pathway, some other findings demonstrate that dephosphorylation of Drp1 by PGAM5 and activation of Drp1 is not obligatory for caspase-independent cell death. Indeed, necroptosis caused by RIP3 requires MLKL but not Drp1 (Moujalled et al., 2014).

All the studies described above rely on mitochondria as a pleotropic player in execution of necroptosis pathway (Fig. 1A). However, they have been challenged by other findings which suggest mitochondria may in fact be dispensable for necroptosis (Marshall and Baines, 2014).

#### 4. Endoplasmic reticulum

From many decades ago, it was understood that the endoplasmic reticulum (ER) is responsible for synthesis, folding and maturation of proteins. Disturbance of ER homeostasis is capable of accumulating misfolded proteins and results in a stress response known as unfolded protein response (UPR) which orchestrates the recovery of ER functions. UPR signaling has two different aspects; this process is involved in either promoting cellular survival or inducing cell death. Indeed, mild stress activates the pro-survival adaptation response

module. However, in severe and prolonged ER stress; the UPR is in short supply to renovate homeostasis, and pro-death responses are activated (Clarke et al., 2012; Hoozemans and Scheper, 2012). ER stress is associated with abundant pathophysiological conditions, including ischemia and neurodegenerative diseases (Szegezdi et al., 2006). UPR and ER stress involvement in the extrinsic pathway of apoptosis have been rarely reported; however, it has been suggested that overexpression of ER stress markers results in Bax protein translocation to the mitochondria, and then permeabilization of the outer mitochondrial membrane and ultimately execution of the intrinsic pathway of apoptosis (Oyadomari and Mori, 2004; Deniaud et al., 2008; Hetz, 2012). Apoptosis is not the only regulated cell death that occurs under ER stress. The role of ER stress to trigger necroptosis has been recently questioned. It is becoming increasingly clear that ER has a specific role in necroptosis pathway and it has been demonstrated that ER stress is capable of initiating both regulated cell death modalities, apoptosis and necroptosis. It has been approved that ER stress participates in necroptosis through activation of the RIPK1–RIPK3–MLKL pathway (Iurlaro and Munoz-Pinedo, 2015). However, the detail of ER stress involvement in induction of necroptosis remains as an open question.

#### 4.1 ER stress and necroptosis

Early swelling and vacuolization of the intracellular lumens of the endoplasmic reticulum and mitochondria as programmed necrosis characteristics were established in electron microscopy studies (Pasupuleti et al., 2013). ER stress affects mitochondrial metabolism and triggers cell death through necroptosis. Activation of the pronecrotic C/EBP-homologous protein (CHOP)-UPR pathway leads to expression of endoplasmic reticulum oxidoreductase 1beta (ERO1 $\beta$ ), an enzyme to produce ROS during UPR stress. Overexpression of ERO1 $\beta$  results in ROS formation by ER. Increased ROS from two different sources, ER and mitochondria, brings about DNA damage and finally necroptosis (Coustry et al., 2012). On the other hand, when MLKL is phosphorylated by RIPK3, necrosome formation has been found to translocate to mitochondrially associated endoplasmic reticulum

membranes (Chen et al., 2013). It is worth mentioning that whether or not translocation is required for necroptosis remains unknown. In an electron microscopic study, it has been reported that in an injured spinal cord microglia, MLKL immunoreactivity is detectable remarkably on ER. Similar to MLKL, many RIP3 immunoreactivities are also observed on ER. These data endorse possible contribution of ER in the necroptosis of microglia/macrophages following spinal cord injury. Moreover, ER stress suppression by an ER stress inhibitor like 4-phenylbutyrate (4-PBA) significantly diminishes augmentation of necroptosis markers, RIP3 and MLKL (Fan et al., 2015).

#### 4.2 Necroptosis inhibition and ER stress

It has been shown that ER stress is able to induce necroptosis. All compounds which induce ER stress can also initiate necroptosis pathway, demonstrating a direct link between ER stress and necroptosis. Supporting to this, it has been shown that Nec-1 administration protects cells from ER stress cytotoxicity. In addition, MLKL functions as an effector of ER stress-induced necroptosis (Saveljeva et al., 2015). It has been established that, based on context, necroptosis pathway may be activated following c-Jun N-terminal kinase (JNK) activation. ER stress stimulates JNK activation and consequently necroptotic neuronal cell death. ER stress inhibitors including 4-PBA and tangeretin and necroptosis inhibitors like Nec-1 as well as RIPK1 siRNA successfully attenuate phospho-JNK. So, it could be deduced that both ER stress induction and RIP1-RIP3 complex activation are main steps toward activation of JNK signaling (Oshima et al., 2016). In another study, in cells treated by pravastatin, an ER stress suppressor, various ER stress markers such as glucose-regulated protein 78 (GRP78), activating transcription factor (ATF)-6 and CHOP were reduced. Interestingly, gene expression of RIPK1 and RIPK3 were also down-regulated. Moreover, when necroptosis was repressed by Nec-1, the protein levels of the ER stress markers were also attenuated (Zhao et al., 2016). In a recent study, it has been demonstrated that cell toxicity induced by gefitinib, an autophagy inducer, collaborates with augmentation of ER stress-related genes including GRP78 and CHOP. However, inhibition of necroptosis in presence of Nec-1 could partially but significantly

decline cell toxicity (Mukai et al., 2016). Regarding these reports, a crosstalk between ER stress and necroptosis might be acceptable.

Although ER stress and its role in necroptosis have been reviewed in some articles (Fig. 1B), there is evidence against role of ER in necroptosis. For instance, ATF4 as a transcription factor regulates multiple genes for homeostasis maintenance under ER stress condition. ATF4 mediates two different forms of cell death, apoptosis and necrosis. Intriguingly, necrosis induced by ATF4 is not similar to necroptosis (Leon-Annicchiarico et al., 2015).

### 5. Nucleus

The nucleus is the first organelle to be discovered many years ago. Nuclei remain intact and have been detected throughout necroptosis pathway (Pasparakis and Vandenabeele, 2015). Independent role of nucleus in regulation and execution of necroptosis is not to be expected, however, the pathway could be affected by this organelle (Fig. 1C). In a recent study, it has been reported that following ischemic/reperfusion injury, nuclear translocation of RIP3 takes place throughout necroptosis while RIP1 is only detectable in the cytoplasm. These data suggest that the formation of RIP1-RIP3 complex could be ignored for RIP3 function in the nucleus. Interestingly, Nec-1 could attenuate the upregulation and nuclear translocation of RIP3 in CA1 neurons (Yin et al., 2015). In another assessment, subsequent to MLKL phosphorylation, its location in cells at different times was evaluated; the results indicate that MLKL translocation to the nucleus with RIPK1 and RIPK3 occurs prior to cell death. In fact, stimulation of necroptosis causes nuclear translocation of MLKL. Mutant MLKL blocks translocation of RIPK1 and RIPK3, indicating that the translocation of these two protein kinases are influenced by activated MLKL. It is noticeable that MLKL after oligomerization has two different destinations for translocation, plasma membrane and nucleus, both supporting necroptosis pathway. Nevertheless, the certain role of oligomerized MLKL in the nucleus is not clear (Yoon et al., 2016).

#### 5.1 AIF mediated necroptosis

Another occasion in which nuclei play crucial role to promote necroptosis is apoptosis-inducing factor (AIF) mediated necroptosis. AIF is a pleotropic

protein that plays a vital role in mitochondrial respiration machinery. On the other hand, this protein is particularly recognized for caspase-independent necroptosis induction. AIF is cleaved and released from mitochondria to the cytosol. In programmed cell death, it is mainly known for translocating from the cytosol to the nucleus, where it induces chromatinolysis. Intriguingly, necroptosis and AIF have been associated in neuronal excitotoxicity and AIF release could be controlled by RIP1 inhibition (Delavallee et al., 2011; Baritaud et al., 2012). DNA damage is another trigger for necroptosis. DNA-alkylating agents like 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) are well known to induce necroptosis as a consequence of DNA damage. In addition to RIP1 and RIP3, the PARP-1 pathway is stimulated by MNNG induced-programmed necrosis, however, in TNF-induced necroptosis PARP-1 is not a key mediator and suppression of the PARP-1 pathway has no effect on this process. Both Nec-1 administration and genetic suppression of RIP1 prevent MNNG-induced necroptosis (Baritaud et al., 2012; Cabon et al., 2012; Sosna et al., 2014). In MNNG-induced caspase-independent necroptosis, MNNG breaks DNA double-strand. The DNA damage provokes PARP-1 activation which results in the release of the necroptotic effector truncated AIF (tAIF) from the mitochondria to the cytosol; tAIF makes a connection with CypA to generate a DNA-degrading complex that stimulates AIF-mediated necroptosis as a consequence of chromatinolysis (Baritaud et al., 2012). In another study, it has been shown that following PARP-1 activation, MNNG-induced DNA damage depletes  $\text{NAD}^+$  and ATP and activates calpains in target cells as well. Calpains cleave BID that is a link between calpains and Bax. Smaller form of BID, tBID is capable of activating Bax. Activated Bax facilitates release of tAIF from damaged mitochondria to the cytosol and then nucleus. So, despite pivotal role of Bax in apoptosis, this protein exerts a potent influence over necroptosis pathway. The anti-apoptotic protein, Bcl2, prevents Bax and disturbs AIF release (Cabon et al., 2012).

## 6. Lysosomes

Lysosomes have been associated with unregulated/regulated cell death. In a lethal damage, lysosomal rupture could be considered as a noteworthy early event. Release of high

concentrations of lysosomal hydrolytic enzymes into the cytosol result in unregulated necrosis, while partial and sequential permeabilization cause programmed cell death (Bursch, 2001; Guicciardi et al., 2004). Apoptotic cascade can initiate by a wide variety of events ranging from lysosomal proteases leakage (Vancompernelle et al., 1998) to the mitochondrial release of cytochrome c (Roberg, 2001). Although enhanced generation of oxidants, mitochondrial dysfunction and ATP depletion are main events during necroptosis, activation of cysteine protease calpains and cathepsins, and then lysosomal rupture have been established as major cascades of this process (Golstein and Kroemer, 2007). To date, various factors that are capable of bringing about the lysosomal permeabilization were identified, but their contribution to necroptosis depends on two main issues; first, the way the cell death is induced, second, the cell type. Most likely, the well-known factor of the lysosomal damage, ROS, affects lysosomal membrane integrity. Some experimental results also suggest that antioxidants are able to reduce cell death through stabilizing lysosomal membrane (Persson et al., 2003; Guicciardi et al., 2004). The calpain-mediated lysosomal rupture has been suggested in programmed necrosis. This has been confirmed by various experimental paradigms ranging from *C. elegans* to humans (Yamashima and Oikawa, 2009). Calpains and cathepsins have been accepted to be involved in regulation of programmed necrosis. It has been clearly shown that neuronal programmed necrosis might occur partly by the release of cathepsin-B as a consequence of lysosomal rupture (Wang et al., 2011). Intriguingly, specific inhibitor of necroptosis, Nec-1, prevents the release of cathepsin-B from lysosomes after ischemic reperfusion injury (Yin et al., 2015). On the other hand, it has been shown that administration of Tag7, an innate immunity protein, forms a stable cytotoxic complex with the heat shock protein 70. This complex initiates programmed cell death by interacting with the TNFR1. Following TNFR1 stimulation and then RIP1 kinase activation, augmented intracellular  $\text{Ca}^{2+}$  activates calpain in downstream. Activated calpain results in the lysosomal membrane permeabilization and cathepsins release. These two events lead to mitochondrial damage and ROS accumulation, and ultimately progression of necroptosis (Yashin et al.,

2016).

In addition to lysosomes' responsibility for the release of proteolytic enzymes by virtue of lysosomal membrane rupture, autophagy as a self-digesting mechanism has been implicated in providing another pivotal process for lysosomes. Autophagy is a double-edged sword, and its role as a type of cell death or a pathway to protect cells is a contentious area. Autophagy is distinguishable by the formation of autophagosomes which delivers damaged organelles or cellular components to lysosomes (Shen and Codogno, 2012). Myriad reports demonstrate autophagy is both initiator (Dey et al., 2016; Liu et al., 2016), and inhibitor (Ye et al., 2013) of necroptosis; moreover, some documents show no association between these two processes (Osborn et al., 2010; Button et al., 2016). Hence, finding a clear relationship between autophagy and necroptosis is positively problematic (Fig. 1D).

## 7. Golgi apparatus

The vital role of the Golgi apparatus-complex in the transport and processing of proteins that are produced by the rough endoplasmic reticulum has been studied many years ago (Marsh and Howell, 2002). Although fragmentation of the Golgi apparatus could be a physiological event, it resembles some pathological reactions. The Golgi apparatus is fragmented or dispersed in a number of human degenerative diseases. For instance, in cell and animal models of Alzheimer's disease, following enhancement of amyloid beta processing from the amyloid precursor protein, Golgi fragmentation appears before cell death. Golgi fragmentation is also observed several months before the onset of paralysis (Gonatas et al., 2006; Ceglia et al., 2015). Golgi complex would be supposed as a sensor of stress signals in various platforms (Hicks and Machamer, 2005). In addition to alterations of Golgi complex in response to stress signals, involvement of the Golgi complex in apoptosis pathway or necrosis was reported. It has been represented that apoptotic death receptors are augmented in the Golgi complex before transportation to the plasma membrane, indicating that the Golgi complex is one of the main players in apoptotic signalling. It appears that Golgi complex experiences distinctive changes during apoptosis and necrosis (Nozawa et al., 2002). To confirm the role of this organelle in necroptosis, it has

been revealed that cadmium administration induces cell death; and prior to detection of any cellular/morphological modifications the early appearance is Golgi disintegration. In this context, Nec-1 can partially alleviate destructive effects of cadmium (Krumshabel et al., 2010). To date, it is not clear why and how Golgi complex can influence necroptosis pathway.

## Conclusion

Although necroptotic cells share a recognized signaling pathway which occurs in cytosol, role of other cell's organelles is remarkable in this pathway. Based on many reports, mitochondria, endoplasmic reticulum, nucleus, lysosome and Golgi apparatus highly contribute to programmed necrosis. Indeed, these organelles are actors of necroptosis scenario in cell's scene.

## Acknowledgments

The authors gratefully acknowledge Dr. Mohsen Nategh and Dr. Farinoosh Fakharnia for the useful suggestions and help given by them to prepare this review article.

## Conflict of interest

The authors have no conflict of interest to declare.

## References

- Aki T, Funakoshi T, Uemura K. Regulated necrosis and its implications in toxicology. *Toxicology* 2015; 333: 118-26.
- Ardestani S, Deskins DL, Young PP. Membrane tnfr- $\alpha$ -activated programmed necrosis is mediated by ceramide-induced reactive oxygen species. *J Mol Signal* 2013; 8: 12.
- Baines CP. The cardiac mitochondrion: Nexus of stress. *Annu Rev Physiol* 2010; 72: 61-80.
- Baritaud M, Cabon L, Delavallee L, Galan-Malo P, Gilles ME, Brunelle-Navas MN, et al. Aif-mediated caspase-independent necroptosis requires atm and DNA-pk-induced histone h2ax ser139 phosphorylation. *Cell Death Dis* 2012; 3: e390.
- Belizario J, Vieira-Cordeiro L, Enns S. Necroptotic Cell Death Signaling and Execution Pathway: Lessons from Knockout Mice. *Mediators Inflamm* 2015; 2015: 128076.
- Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreaux A, Durkin J, et al. Ciap1 and ciap2 facilitate cancer cell survival by functioning as e3 ligases that promote rip1 ubiquitination. *Mol Cell* 2008; 30: 689-700.

- Bursch W. The autophagosomal-lysosomal compartment in programmed cell death. *Cell Death Differ* 2001; 8: 569-81.
- Button RW, Vincent JH, Strang CJ, Luo S. Dual pi-3 kinase/mtor inhibition impairs autophagy flux and induces cell death independent of apoptosis and necroptosis. *Oncotarget* 2016; 7: 5157-75.
- Cabon L, Galan-Malo P, Bouharrou A, Delavallee L, Brunelle-Navas MN, Lorenzo HK, et al. Bid regulates aif-mediated caspase-independent necroptosis by promoting bax activation. *Cell Death Differ* 2012; 19: 245-56.
- Cai Z, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, et al. Plasma membrane translocation of trimerized mlkl protein is required for tnf-induced necroptosis. *Nat Cell Biol* 2014; 16: 55-65.
- Ceglia I, Reitz C, Gresack J, Ahn JH, Bustos V, Bleck M, et al. App intracellular domain-wave1 pathway reduces amyloid-beta production. *Nat Med* 2015; 21: 1054-9.
- Ch'en IL, Beisner DR, Degterev A, Lynch C, Yuan J, Hoffmann A, et al. Antigen-mediated t cell expansion regulated by parallel pathways of death. *Proc Natl Acad Sci U S A* 2008; 105: 17463-8.
- Ch'en IL, Tsau JS, Molkentin JD, Komatsu M, Hedrick SM. Mechanisms of necroptosis in t cells. *J Exp Med* 2011; 208: 633-41.
- Chan FK, Luz NF, Moriwaki K. Programmed necrosis in the cross talk of cell death and inflammation. *Annu Rev Immunol* 2015; 33: 79-106.
- Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, et al. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. *J Biol Chem* 2003; 278: 51613-21.
- Chang CR, Blackstone C. Cyclic amp-dependent protein kinase phosphorylation of drp1 regulates its gtpase activity and mitochondrial morphology. *J Biol Chem* 2007; 282: 21583-7.
- Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H, et al. A regulatory signaling loop comprising the pgam5 phosphatase and ck2 controls receptor-mediated mitophagy. *Mol Cell* 2014a; 54: 362-77.
- Chen W, Zhou Z, Li L, Zhong CQ, Zheng X, Wu X, et al. Diverse sequence determinants control human and mouse receptor interacting protein 3 (rip3) and mixed lineage kinase domain-like (mlkl) interaction in necroptotic signaling. *J Biol Chem* 2013; 288: 16247-61.
- Chen X, Li W, Ren J, Huang D, He WT, Song Y, et al. Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. *Cell Res* 2014b; 24: 105-21.
- Chtourou Y, Slima AB, Makni M, Gdoura R, Fetoui H. Naringenin protects cardiac hypercholesterolemia-induced oxidative stress and subsequent necroptosis in rats. *Pharmacol Rep* 2015; 67: 1090-7.
- Clarke R, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, et al. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. *Cancer Res* 2012; 72: 1321-31.
- Coustry F, Posey KL, Liu P, Alcorn JL, Hecht JT. D469del-comp retention in chondrocytes stimulates caspase-independent necroptosis. *Am J Pathol* 2012; 180: 738-48.
- Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol* 2005; 1: 112-9.
- Delavallee L, Cabon L, Galan-Malo P, Lorenzo HK, Susin SA. Aif-mediated caspase-independent necroptosis: A new chance for targeted therapeutics. *IUBMB Life* 2011; 63: 221-32.
- Deniaud A, Sharaf el dein O, Maillier E, Poncet D, Kroemer G, Lemaire C, et al. Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene* 2008; 27: 285-99.
- Devalaraja-Narashimha K, Padanilam BJ. Parp-1 inhibits glycolysis in ischemic kidneys. *J Am Soc Nephrol* 2009; 20: 95-103.
- Dey A, Mustafi SB, Saha S, Kumar Dhar Dwivedi S, Mukherjee P, Bhattacharya R. Inhibition of bmi1 induces autophagy-mediated necroptosis. *Autophagy* 2016; 12: 659-70.
- Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, et al. Mlkl compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep* 2014; 7: 971-81.
- Dubois A, Ginet C, Furstoss N, Belaid A, Hamouda MA, El Manaa W, et al. Differentiation inducing factor 3 mediates its anti-leukemic effect through ros-dependent drp1-mediated mitochondrial fission and induction of caspase-independent cell death. *Oncotarget* 2016; 7: 26120-36.
- Dunai Z, Bauer PI, Mihalik R. Necroptosis: Biochemical, physiological and pathological aspects. *Pathol Oncol Res* 2011; 17: 791-800.
- Elrod JW, Molkentin JD. Physiologic functions of cyclophilin d and the mitochondrial permeability transition pore. *Circ J* 2013; 77: 1111-22.
- Fakharnia F, Khodaghali F, Dargahi L, Ahmadiani A. Prevention of cyclophilin d-mediated mptp opening using cyclosporine-a alleviates the elevation of necroptosis, autophagy and apoptosis-related markers following global cerebral ischemia-reperfusion. *J Mol Neurosci* 2017; 61: 52-60.
- Fan H, Tang HB, Kang J, Shan L, Song H, Zhu K, et al. Involvement of endoplasmic reticulum stress in the necroptosis of microglia/macrophages after spinal cord injury. *Neuroscience* 2015; 311: 362-73.
- Fatkun AA, Dawson VL, Dawson TM. Parthanatos: Mitochondrial-linked mechanisms and therapeutic opportunities. *Br J Pharmacol* 2014; 171: 2000-16.
- Giorgio V, Bisetto E, Soriano ME, Dabbeni-Sala F, Basso E, Petronilli V, et al. Cyclophilin d modulates mitochondrial f0f1-atp synthase by interacting with the lateral stalk of the complex. *J Biol Chem* 2009; 284:

- 33982-8.
- Golstein P, Kroemer G. Cell death by necrosis: Towards a molecular definition. *Trends Biochem Sci* 2007; 32: 37-43.
- Gonatas NK, Stieber A, Gonatas JO. Fragmentation of the golgi apparatus in neurodegenerative diseases and cell death. *J Neurol Sci* 2006; 246: 21-30.
- Guicciardi ME, Leist M, Gores GJ. Lysosomes in cell death. *Oncogene* 2004; 23: 2881-90.
- Gurung P, Man SM, Kanneganti TD. A20 is a regulator of necroptosis. *Nat Immunol* 2015; 16: 596-7.
- Halestrap AP. What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol* 2009; 46: 821-31.
- Hanus J, Anderson C, Wang S. Rpe necroptosis in response to oxidative stress and in amd. *Ageing Res Rev* 2015; 24: 286-98.
- He S, Wang L, Miao L, Wang T, Du F, Zhao L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to tnf-alpha. *Cell* 2009; 137: 1100-11.
- Hetz C. The unfolded protein response: Controlling cell fate decisions under er stress and beyond. *Nat Rev Mol Cell Biol* 2012; 13: 89-102.
- Hicks SW, Machamer CE. Golgi structure in stress sensing and apoptosis. *Biochim Biophys Acta* 2005; 1744: 406-14.
- Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase rip as effector molecule. *Nat Immunol* 2000; 1: 489-95.
- Hoozemans JJ, Scheper W. Endoplasmic reticulum: The unfolded protein response is tangled in neurodegeneration. *Int J Biochem Cell Biol* 2012; 44: 1295-8.
- Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV. Tnf-dependent recruitment of the protein kinase rip to the tnf receptor-1 signaling complex. *Immunity* 1996; 4: 387-96.
- Iurlaro R, Munoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. *FEBS J* 2015; 283: 2640-2652.
- Jain MV, Paczulla AM, Klonisch T, Dimgba FN, Rao SB, Roberg K, et al. Interconnections between apoptotic, autophagic and necrotic pathways: Implications for cancer therapy development. *J Cell Mol Med* 2013; 17: 12-29.
- Jouan-Lanhouet S, Riquet F, Duprez L, Vanden Berghe T, Takahashi N, Vandenabeele P. Necroptosis, in vivo detection in experimental disease models. *Semin Cell Dev Biol* 2014; 35: 2-13.
- Kaiser WJ, Sridharan H, Huang C, Mandal P, Upton JW, Gough PJ, et al. Toll-like receptor 3-mediated necrosis via trif, rip3, and mlkl. *J Biol Chem* 2013; 288: 31268-79.
- Kaku Y, Tsuchiya A, Kanno T, Nishizaki T. Huhs1015 induces necroptosis and caspase-independent apoptosis of mkn28 human gastric cancer cells in association with amid accumulation in the nucleus. *Anticancer Agents Med Chem* 2015; 15: 242-7.
- Kanamaru Y, Sekine S, Ichijo H, Takeda K. The phosphorylation-dependent regulation of mitochondrial proteins in stress responses. *J Signal Transduct* 2012; 2012: 931215.
- Karch J, Kanisicak O, Brody MJ, Sargent MA, Michael DM, Molkentin JD. Necroptosis interfaces with momp and the mptp in mediating cell death. *PLoS One* 2015; 10: e0130520.
- Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007; 87: 99-163.
- Krumschnabel G, Ebner HL, Hess MW, Villunger A. Apoptosis and necroptosis are induced in rainbow trout cell lines exposed to cadmium. *Aquat Toxicol* 2010; 99: 73-85.
- Kubli DA, Gustafsson AB. Mitochondria and mitophagy: The yin and yang of cell death control. *Circ Res* 2012; 111: 1208-21.
- Leon-Annicchiarico CL, Ramirez-Peinado S, Dominguez-Villanueva D, Gonsberg A, Lampidis TJ, Munoz-Pinedo C. Atf4 mediates necrosis induced by glucose deprivation and apoptosis induced by 2-deoxyglucose in the same cells. *FEBS J* 2015; 282: 3647-58.
- Li J, McQuade T, Siemer AB, Napetschnig J, Moriwaki K, Hsiao YS, et al. The rip1/rip3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. *Cell* 2012; 150: 339-50.
- Li Z, Scott MJ, Fan EK, Li Y, Liu J, Xiao G, et al. Tissue damage negatively regulates lps-induced macrophage necroptosis. *Cell Death Differ* 2016; 23: 1428-47.
- Liu X, Zhang C, Zhang C, Li J, Guo W, Yan D, et al. Heat shock protein 70 inhibits cardiomyocyte necroptosis through repressing autophagy in myocardial ischemia/reperfusion injury. *In Vitro Cell Dev Biol Anim* 2016; 52: 690-8.
- Lo SC, Hannink M. Pgam5, a bcl-xl-interacting protein, is a novel substrate for the redox-regulated keap1-dependent ubiquitin ligase complex. *J Biol Chem* 2006; 281: 37893-903.
- Lo SC, Hannink M. Pgam5 tethers a ternary complex containing keap1 and nrf2 to mitochondria. *Exp Cell Res* 2008; 314: 1789-803.
- Lu W, Sun J, Yoon JS, Zhang Y, Zheng L, Murphy E, et al. Mitochondrial protein pgam5 regulates mitophagic protection against cell necroptosis. *PLoS One* 2016; 11: e0147792.
- Ma YM, Peng YM, Zhu QH, Gao AH, Chao B, He QJ, et al. Novel chop activator lgh00168 induces necroptosis in a549 human lung cancer cells via ros-mediated er stress and nf-kappab inhibition. *Acta Pharmacol Sin* 2016; 37: 1381-1390.
- Maeda A, Fadeel B. Mitochondria released by cells undergoing tnf-alpha-induced necroptosis act as danger signals. *Cell Death Dis* 2014; 5: e1312.
- Marsh BJ, Howell KE. The mammalian golgi--complex debates. *Nat Rev Mol Cell Biol* 2002; 3: 789-95.
- Marshall KD, Baines CP. Necroptosis: Is there a role for mitochondria? *Front Physiol* 2014; 5: 323.
- Mc Guire C, Beyaert R, van Loo G. Death receptor signalling in central nervous system inflammation and demyelination. *Trends Neurosci* 2011; 34: 619-28.

- McComb S, Cessford E, Alturki NA, Joseph J, Shutinoski B, Startek JB, et al. Type-i interferon signaling through isgf3 complex is required for sustained rip3 activation and necroptosis in macrophages. *Proc Natl Acad Sci U S A* 2014; 111: E3206-13.
- McGee AM, Baines CP. Complement 1q-binding protein inhibits the mitochondrial permeability transition pore and protects against oxidative stress-induced death. *Biochem J* 2011; 433: 119-25.
- Moquin D, Chan FK. The molecular regulation of programmed necrotic cell injury. *Trends Biochem Sci* 2010; 35: 434-41.
- Moriwaki K, Farias Luz N, Balaji S, De Rosa MJ, O'Donnell CL, Gough PJ, et al. The mitochondrial phosphatase pgam5 is dispensable for necroptosis but promotes inflammasome activation in macrophages. *J Immunol* 2016; 196: 407-15.
- Moujalled DM, Cook WD, Murphy JM, Vaux DL. Necroptosis induced by ripk3 requires mlk1 but not drp1. *Cell Death Dis* 2014; 5: e1086.
- Mukai S, Moriya S, Hiramoto M, Kazama H, Kokuba H, Che XF, et al. Macrolides sensitize egfr-tki-induced non-apoptotic cell death via blocking autophagy flux in pancreatic cancer cell lines. *Int J Oncol* 2016; 48: 45-54.
- Nikseresht S, Khodagholi F, Nategh M, Dargahi L. Rip1 inhibition rescues from lps-induced rip3-mediated programmed cell death, distributed energy metabolism and spatial memory impairment. *J Mol Neurosci* 2015; 57: 219-30.
- Nozawa K, Casiano CA, Hamel JC, Molinaro C, Fritzler MJ, Chan EK. Fragmentation of golgi complex and golgi autoantigens during apoptosis and necrosis. *Arthritis Res* 2002; 4: R3.
- Oettinghaus B, D'Alonzo D, Barbieri E, Restelli LM, Savoia C, Licci M, et al. Drp1-dependent apoptotic mitochondrial fission occurs independently of bax, bak and apaf1 to amplify cell death by bid and oxidative stress. *Biochim Biophys Acta* 2016; 1857: 1267-76.
- Osborn SL, Diehl G, Han SJ, Xue L, Kurd N, Hsieh K, et al. Fas-associated death domain (fadd) is a negative regulator of t-cell receptor-mediated necroptosis. *Proc Natl Acad Sci U S A* 2010; 107: 13034-9.
- Oshima R, Hasegawa T, Tamai K, Sugeno N, Yoshida S, Kobayashi J, et al. Esrt-0 dysfunction compromises autophagic degradation of protein aggregates and facilitates er stress-mediated neurodegeneration via apoptotic and necroptotic pathways. *Sci Rep* 2016; 6: 24997.
- Otera H, Mihara K. Molecular mechanisms and physiologic functions of mitochondrial dynamics. *J Biochem* 2011; 149: 241-51.
- Oyadomari S, Mori M. Roles of chop/gadd153 in endoplasmic reticulum stress. *Cell Death Differ* 2004; 11: 381-9.
- Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. *Nature* 2015; 517: 311-20.
- Pasupuleti N, Leon L, Carraway KL, Gorin F. 5-benzylglyciny-amiloride kills proliferating and nonproliferating malignant glioma cells through caspase-independent necroptosis mediated by apoptosis-inducing factor. *J Pharmacol Exp Ther* 2013; 344: 600-15.
- Persson HL, Yu Z, Tirosh O, Eaton JW, Brunk UT. Prevention of oxidant-induced cell death by lysosomotropic iron chelators. *Free Radic Biol Med* 2003; 34: 1295-305.
- Proskuryakov SY, Konoplyannikov AG, Gabai VL. Necrosis: A specific form of programmed cell death? *Exp Cell Res* 2003; 283: 1-16.
- Rizzi F, Naponelli V, Silva A, Modernelli A, Ramazzina I, Bonacini M, et al. Polyphenon e(r), a standardized green tea extract, induces endoplasmic reticulum stress, leading to death of immortalized pnt1a cells by anoikis and tumorigenic pc3 by necroptosis. *Carcinogenesis* 2014; 35: 828-39.
- Roberg K. Relocalization of cathepsin d and cytochrome c early in apoptosis revealed by immunoelectron microscopy. *Lab Invest* 2001; 81: 149-58.
- Saveljeva S, Mc Laughlin SL, Vandenabeele P, Samali A, Bertrand MJ. Endoplasmic reticulum stress induces ligand-independent tnfr1-mediated necroptosis in 1929 cells. *Cell Death Dis* 2015; 6: e1587.
- Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, et al. Cyclophilin d is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc Natl Acad Sci U S A* 2005; 102: 12005-10.
- Shahsavari Z, Karami-Tehrani F, Salami S. Shikonin induced necroptosis via reactive oxygen species in the t-47d breast cancer cell line. *Asian Pac J Cancer Prev* 2015; 16: 7261-6.
- Shen HM, Codogno P. Autophagy is a survival force via suppression of necrotic cell death. *Exp Cell Res* 2012; 318: 1304-8.
- Shindo R, Kakehashi H, Okumura K, Kumagai Y, Nakano H. Critical contribution of oxidative stress to tnfa-induced necroptosis downstream of ripk1 activation. *Biochem Biophys Res Commun* 2013; 436: 212-6.
- Shulga N, Pastorino JG. Retraction: Grim-19-mediated translocation of stat3 to mitochondria is necessary for tnf-induced necroptosis. *J Cell Sci* 2016; 129: 2686.
- Smirnova E, Griparic L, Shurland DL, van der Bliek AM. Dynamin-related protein drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell* 2001; 12: 2245-56.
- Sosna J, Voigt S, Mathieu S, Lange A, Thon L, Davarnia P, et al. Tnf-induced necroptosis and parp-1-mediated necrosis represent distinct routes to programmed necrotic cell death. *Cell Mol Life Sci* 2014; 71: 331-48.
- Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of rip3 kinase. *Cell* 2012; 148: 213-27.
- Sun X, Lee J, Navas T, Baldwin DT, Stewart TA, Dixit VM. Rip3, a novel apoptosis-inducing kinase. *J Biol Chem* 1999; 274: 16871-5.
- Sun X, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM. Identification of a novel homotypic interaction motif

- required for the phosphorylation of receptor-interacting protein (rip) by rip3. *J Biol Chem* 2002; 277: 9505-11.
- Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 2006; 7: 880-5.
- Tait SW, Ichim G, Green DR. Die another way--non-apoptotic mechanisms of cell death. *J Cell Sci* 2014; 127: 2135-44.
- Tanzer MC, Matti I, Hildebrand JM, Young SN, Wardak A, Tripaydonis A, et al. Evolutionary divergence of the necroptosis effector mlkl. *Cell Death Differ* 2016; 23: 1185-97.
- Thapa RJ, Basagoudanavar SH, Nogusa S, Irrinki K, Mallilankaraman K, Slifker MJ, et al. Nf-kappab protects cells from gamma interferon-induced rip1-dependent necroptosis. *Mol Cell Biol* 2011; 31: 2934-46.
- Thapa RJ, Nogusa S, Chen P, Maki JL, Lerro A, Andrade M, et al. Interferon-induced rip1/rip3-mediated necrosis requires pkr and is licensed by fadd and caspases. *Proc Natl Acad Sci U S A* 2013; 110: E3109-18.
- Upton JW, Kaiser WJ, Mocarski ES. Dai/zbp1/dlm-1 complexes with rip3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vira. *Cell Host Microbe* 2012; 11: 290-7.
- Vancompernelle K, Van Herreweghe F, Pynaert G, Van de Craen M, De Vos K, Totty N, et al. Atractyloside-induced release of cathepsin b, a protease with caspase-processing activity. *FEBS Lett* 1998; 438: 150-8.
- Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: An ordered cellular explosion. *Nat Rev Mol Cell Biol* 2010; 11: 700-14.
- Vaseva AV, Marchenko ND, Ji K, Tsrirka SE, Holzmann S, Moll UM. P53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell* 2012; 149: 1536-48.
- Wang JY, Xia Q, Chu KT, Pan J, Sun LN, Zeng B, et al. Severe global cerebral ischemia-induced programmed necrosis of hippocampal ca1 neurons in rat is prevented by 3-methyladenine: A widely used inhibitor of autophagy. *J Neuropathol Exp Neurol* 2011; 70: 314-22.
- Wang Z, Jiang H, Chen S, Du F, Wang X. The mitochondrial phosphatase pgam5 functions at the convergence point of multiple necrotic death pathways. *Cell* 2012; 148: 228-43.
- Welz PS, Wullaert A, Vlantis K, Kondylis V, Fernandez-Majada V, Ermolaeva M, et al. Fadd prevents rip3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* 2011; 477: 330-4.
- Wu CF, Hong C, Klauck SM, Lin YL, Efferth T. Molecular mechanisms of rosmarinic acid from *salvia miltiorrhiza* in acute lymphoblastic leukemia cells. *J Ethnopharmacol* 2015; 176: 55-68.
- Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: Process and function. *Cell Death Differ* 2016; 23: 369-79.
- Yamashima T, Oikawa S. The role of lysosomal rupture in neuronal death. *Prog Neurobiol* 2009; 89: 343-58.
- Yashin DV, Romanova EA, Ivanova OK, Sashchenko LP. The tag7-hsp70 cytotoxic complex induces tumor cell necroptosis via permeabilisation of lysosomes and mitochondria. *Biochimie* 2016; 123: 32-6.
- Ye YC, Wang HJ, Chen L, Liu WW, Tashiro S, Onodera S, et al. Negatively-regulated necroptosis by autophagy required caspase-6 activation in tnfalpa-treated murine fibrosarcoma 1929 cells. *Int Immunopharmacol* 2013; 17: 548-55.
- Ye YC, Wang HJ, Yu L, Tashiro S, Onodera S, Ikejima T. Rip1-mediated mitochondrial dysfunction and ros production contributed to tumor necrosis factor alpha-induced 1929 cell necroptosis and autophagy. *Int Immunopharmacol* 2012; 14: 674-82.
- Yin B, Xu Y, Wei RL, He F, Luo BY, Wang JY. Inhibition of receptor-interacting protein 3 upregulation and nuclear translocation involved in necrostatin-1 protection against hippocampal neuronal programmed necrosis induced by ischemia/reperfusion injury. *Brain Res* 2015; 1609: 63-71.
- Yoon S, Bogdanov K, Kovalenko A, Wallach D. Necroptosis is preceded by nuclear translocation of the signaling proteins that induce it. *Cell Death Differ* 2016; 23: 253-60.
- Yu T, Sheu SS, Robotham JL, Yoon Y. Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species. *Cardiovasc Res* 2008; 79: 341-51.
- Yu X, Deng Q, Li W, Xiao L, Luo X, Liu X, et al. Neoalbacanol induces cell death through necroptosis by regulating ripk-dependent autocrine tnfalpa and ros production. *Oncotarget* 2015; 6: 1995-2008.
- Zhang H, Zhong C, Shi L, Guo Y, Fan Z. Granulysin induces cathepsin b release from lysosomes of target tumor cells to attack mitochondria through processing of bid leading to necroptosis. *J Immunol* 2009; 182: 6993-7000.
- Zhang L, Jiang F, Chen Y, Luo J, Liu S, Zhang B, et al. Necrostatin-1 attenuates ischemia injury induced cell death in rat tubular cell line nrk-52e through decreased drp1 expression. *Int J Mol Sci* 2013; 14: 24742-54.
- Zhang M, Harashima N, Moritani T, Huang W, Harada M. The roles of ros and caspases in trail-induced apoptosis and necroptosis in human pancreatic cancer cells. *PLoS One* 2015; 10: e0127386.
- Zhang T, Zhang Y, Cui M, Jin L, Wang Y, Lv F, et al. Camkii is a rip3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. *Nat Med* 2016; 22: 175-82.
- Zhao J, Jitkaew S, Cai Z, Choksi S, Li Q, Luo J, et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of tnf-induced necrosis. *Proc Natl Acad Sci U S A* 2012; 109: 5322-7.
- Zhao M, Lu L, Lei S, Chai H, Wu S, Tang X, et al. Inhibition of receptor interacting protein kinases attenuates cardiomyocyte hypertrophy induced by palmitic acid. *Oxid Med Cell Longev* 2016; 2016: 1451676.
- Zhou W, Yuan J. Necroptosis in health and diseases. *Semin Cell Dev Biol* 2014; 35: 14-23.