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Review Article

Actors of necroptosis scenario in cell's scene

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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-α 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.

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1. Background

In the middle of 19th 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
Necroptosis*	RIP1 RIP3	MLKL, Na^+ and Ca^{2+} channels and H_2O pore formation
Parthanatos&	PARP1	PAR synthesis, NAD^+ and ATP depletion
Ferroptosis#	GPX4	ROS and Fe^{2+}
Pyroptosis†	caspase-1	inflammation
MPT-mediated regulated necrosis•	CypD	Ca^{2+}

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^+, nicotinamide adenine dinucleotide; ATP, adenosine 3-phosphate; ROS, reactive oxygen species;

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

&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^+ and ATP that eventually results in necrotic cell death (Fatokun et al., 2014).

#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).

†Pyroptosis occurs after caspase-1 activation which results in the maturation of pro-inflammatory cytokines like IL-1β 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-α (TNF-α) 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-α, necroptosis can be triggered by other death ligands like Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL). TNF-α 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-α 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

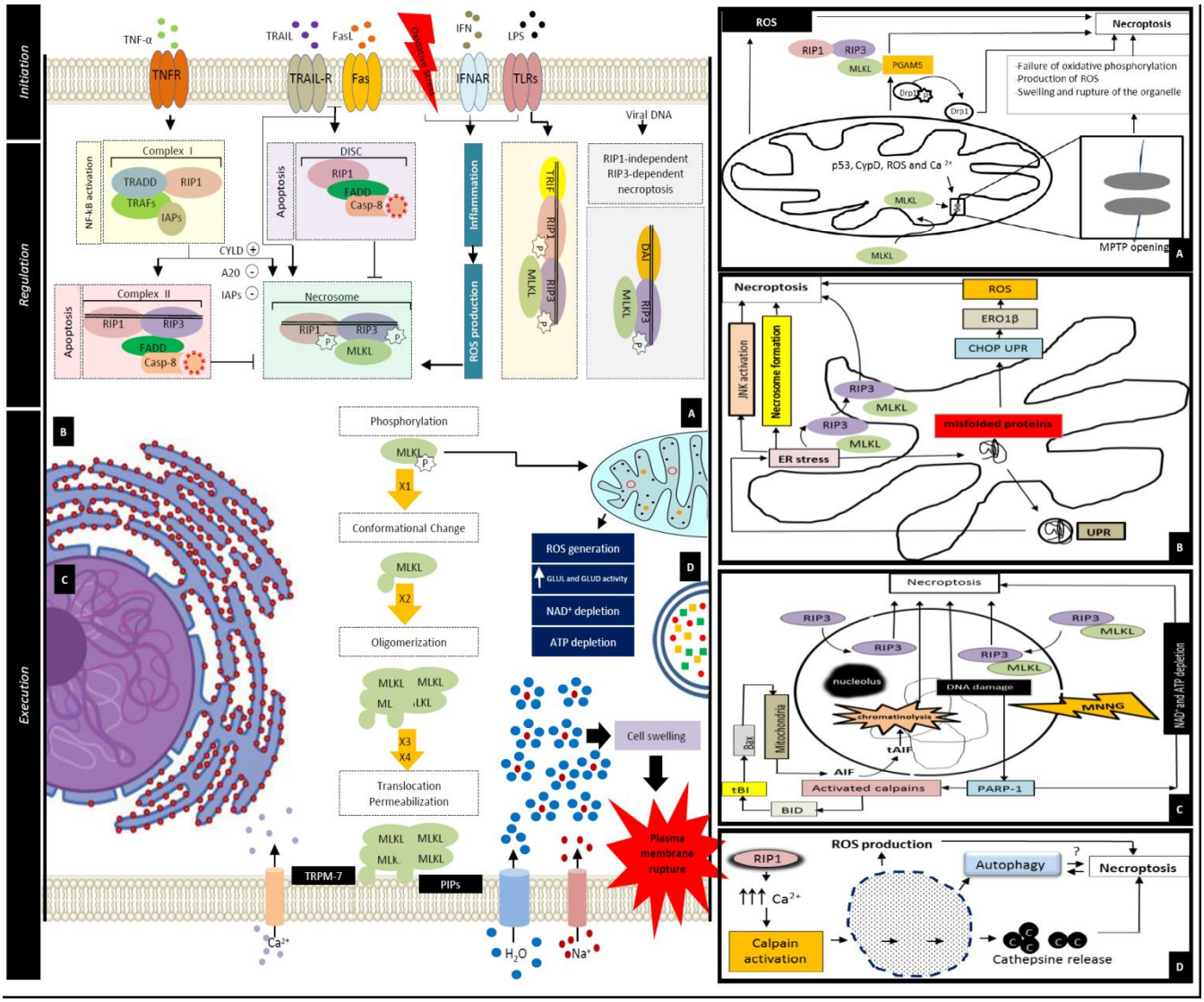

Fig.1. Molecular mechanism of necroptosis; initiation, regulation and execution. Necroptosis/programmed necrosis is induced by many factors ranging from ligands of death receptors to oxidative stress. A couple of kinases are main regulators of necroptosis; RIPK1 (receptor interacting protein kinase-1) 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-α (tumor necrosis factor-α), 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 (IFN)-β), 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), ERO1β (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 (α/β) and type II (γ) 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-β), 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 Ca^{2+} influx triggers plasma membrane damage (Cai et al., 2014). Besides 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 NAD^+, and NAD^+ reduction makes cell consume ATP to replete the 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 α-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 programed 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 TNFα-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 neoalbaconol. Neoalbaconol 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 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 MTPT 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 Molkentin, 2013). Responding to various insults, including oxidative stress and 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-1β 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 PGAM5S 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-α 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 (ERO1β), an enzyme to produce ROS during UPR stress. Overexpression of ERO1β 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-nitroso-guanidine (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 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 (Vancompernolle 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 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 (Krumschnabel 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.

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Conflict of interest

The authors have no conflict of interest to declare.

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