Cellular mechanisms of necrosis

XExecution mechanisms

Sun et al. in 2012 revealed that the RIP3-MLKL interaction is indispensable for the necroptotic pathway execution. In the same experiment, they figured that MLKL expressing cells without the capability to phosphorylate with RIP3 at Thr ^{357} and Ser ^{358} , or with defect RIP3 kinase domain do not undergo necroptosis. One year later, Wu et al. further proposed that MLKL deficient mice cells are capable of enduring apoptosis, while being unresponsive to certain necroptotic cell death stimuli, such as TNF-α. Taking their findings into consideration, as well as the fact that the RIP1-RIP3-MLKL complex formation is not a cytotoxic event, it should be deduced that the pre-mentioned complex cannot be the endpoint of the necroptotic path activation, and that additional proteins must be associated with the process.

On the other hand, necrosis has been known for a long time. Unlike necroptotic intracellular phenomena, the necrotic ones, such us the ROS (reactive oxygen species) formation and the ionic homeostasis disruption had been extensively observed and studied. Thus, the corresponding active participation of these necrotic incidents in the necroptotic cascade was ambiguous, as was the mitochondrial and membrane involvement. Recently, though, both organelles were accredited as necroptotic process dynamic members.

XMLKL oligomerization and ionic homeostasis disruption

Cell volume change is a morphologic phenomenon inextricably linked with cell death and distinctive for every type. Kerr et al. in 1972 highlighted cell volume loss as a notable dissimilarity between apoptosis and necrosis. They

indicated that cell shrinkage is a diverse aspect discriminating the former from the latter, and confirmed that apoptotic cell loses its volume, as water accompanied with minerals (K^+, Cl^-) migrates through its membrane to extracellular compartments. Necrosis, formerly characterized solely as a pathologic cell death type, was already distinguishable by cell swelling and cell membrane physical disruption, leading to intracellular contents leakage into extracellular space. An inflammatory response was later inducted, as a simultaneous immune cell infiltration was promoted. In 2005, when necroptosis was first described as a programmed cell death type, the issue concerning its interference in cellular ionic homeostasis was rationally developed.

Interestingly, recent findings correlated MLKL with the plasmatic membrane permeability regulation. Two independent researches done by Cai et al. and Chen et al. in 2014 refer to an enforcement mechanism, characterized by MLKL oligomeric structure formation, and successive membrane permeability to certain ions. Both claimed that MLKL oligomerizes through its amino-terminal four-helix bundle, thus provoking its translocation towards plasma membrane. MLKL oligomerization is secured by the RIP3 kinase mediated phosphorylation at the Thr ^{357} and Ser ^{358} residues, found on MLKL kinase-like domain. Cai et al. specifically stated that both divergent MLKL coiled-coil domains are crucial for necroptosis, although for different reasons. The first is implicated in the MLKL recruitment to membranes, whereas the second is in control of its oligomerization.

The explicit MLKL function in membranes is not completely illustrated, though, as intra-experimental variations regarding the oligomers number and the ionic influx nature exist. Cai et al., after experimenting on HT29 (human colorectal adenocarcinoma) cells, stated that MLKL forms a homotrimeric structure through its amino-terminal coiled-coil domain during TNF-α induced necroptosis and zVAD. fmk presence. They further declared that MLKL binds to the TRMP7 (transient receptor potential melastatin 7) ion channel located in the plasma membrane, which subsequently leads to Ca^{2+} influx and cell death. On the other hand, Chen et al. experimented on L929 mice cells and proposed that MLKL forms a homotetramer under the same conditions, and that its influence in membranes is derived from its Na^{+} channels regulation, which provokes Na^{+} ions entry, osmotic pressure increase and plasma membrane rupture. To make matters more complicated, a third experiment performed by Wang et al. in the same year respectively showed that oligomerized MLKL binds with membrane lipids through the positively charged amino acids, placed in its amino-terminal, and that MLKL might directly induce pore formation and membrane disruption.

Taking all these into serious consideration, we can assume that the exact execution mechanism responsible for the ionic homeostasis disruption and MLKL oligomerization during necroptosis induction is currently not adequately described, and need to be further defined in the near future.

XROS formation

Endogenous ROS (reactive oxygen species) suggest a natural cellular byproduct, created by oxygen metabolism in mitochondria, and its

significance is proven in cell death among others. Their formation has been thoroughly examined in the apoptotic phenomenon throughout the years and rationally their existence in non-apoptotic cases, such as necroptosis was questioned, thus resulting to various independent studies

For instance, Lin et al. in 2004 suggested that TNF-α induced non-apoptotic cell death necessitates ROS accumulation and that the RIP, TRAF2 and FADD proteins are capable of mediating it. To prove their point, they exposed MEF (mouse embryonic fibroblasts) cells to TNF-α, and then respectively measured the ROS presence extent. They further confirmed ROS essentiality to the whole process by incubating MEF cells with BHA (butylated hydroxyanisole) antioxidant, which blocked their accumulation, and prevented the imminent cell death. Furthermore, Vanlangenakker et al. in 2005 observed elevated ROS production shortly after necroptotic pathway was introduced to L929 cells. The cells were subsequently treated with BHA, and shortly after cytoplasmic ROS formation inhibition through siRNA mediated NOX1 (NADPH oxidase 1) knockdown was detected. NOX1, respectively, did not influence TNF-α induced cell death. Their effort was in fact the first one explicitly implicating ROS formation in necroptosis. 2 years later, Kim et al showed that NOX1 can be involved in ROS generation, as it might couple with TRADD provided that RIP1 is present, and a preceding TNFR1 stimulation occurs. According to their research, NOX1 knockout presented reduced sensitivity towards necroptosis.

Three different teams, Zhang et al. in 2009, Davis et al. in 2010 and Wang et al. in 2012 implicated RIP1, RIP3, and MLKL in a feasible translocation to the mitochondria upon stimulation, thus indicating that ROS production can

actually be a significant member of the necroptotic cascade execution. More specifically, besides reporting RIP1 and RIP3 translocation to the mitochondria in MEF cells, Davis et al. managed to restrain necrosis in endothelial cells by using the mitochondrial antioxidant MnSOD (manganese superoxide dismutase). Zhang et al., while trying to investigate mitochondrial components likely to play a key role in TNFα-induced necroptosis, noticed that RIP3 translocates to the mitochondria, interacts with the GLUD1 (glutamate dehydrogenase 1), PYGL (glycogen phosphorylase) and GLUL (glutamate-ammonia lyase) mitochondrial proteins and elevates their activity. Taking this into account, they indicated that their knockdown may partially block TNFα-induced ROS production. Wang et al. found that the necrosome activates and moreover interacts with the mitochondrial phosphatase PGAM5 (phosphoglycerate mutase family member 5), after translocating to the mitochondria. PGAM5 can be presented in two forms, PGAM5 $_{L}$ and PGAM5 $_{S}$, where the former represents the long variant and the latter the short one. An attainable knockdown of either one can result to TNF-α mediated necrosis and ROS formation deterioration. In addition, the team proved that the necrosomal signaling through PGAM5 $_{S}$ gives birth to mitochondrial fragmentation in a Drp1 (dynamin-like related protein 1) manner, after experimenting in HeLa cells. They concluded their work by pointing out a definite interaction between RIP1, RIP3 and PGAM5, as siRNA mediated Drp1 knockdown, and mdivi-1 mediated Drp1 inhibition were both able to prevent TNF-α mediated necrosis.

Finally, Baines in 2010 proposed MPT (mitochondrial permeability transition) pore as a probable mitochondrial necroptotic mediator that might provide a

link to ROS production. The MPT pore is a non-specific, wide channel crossing the inner mitochondrial membrane, whose potential opening leads to ROS production, mitochondrial transmembrane potential loss, oxidative phosphorylation failure, and eventually organelle swelling and rupture.