Acta Pharmaceutica Sinica B 2020;10(10):1880–1903

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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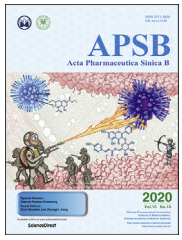

REVIEW

The role of lysosome in regulated necrosis

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Received 16 January 2020; received in revised form 29 March 2020; accepted 24 April 2020

KEY WORDS

Ferroptosis;
Lysosome;
Necroptosis;
Pyroptosis;
Regulated necrosis

Abstract Lysosome is a ubiquitous acidic organelle fundamental for the turnover of unwanted cellular molecules, particles, and organelles. Currently, the pivotal role of lysosome in regulating cell death is drawing great attention. Over the past decades, we largely focused on how lysosome influences apoptosis and autophagic cell death. However, extensive studies showed that lysosome is also prerequisite for the execution of regulated necrosis (RN). Different types of RN have been uncovered, among which, necroptosis, ferroptosis, and pyroptosis are under the most intensive investigation. It becomes a hot topic nowadays to target RN as a therapeutic intervention, since it is important in many patho/physiological settings and contributing to numerous diseases. It is promising to target lysosome to control the occurrence of RN thus altering the outcomes of diseases. Therefore, we aim to give an introduction about the common factors influencing lysosomal stability and then summarize the current knowledge on the role of lysosome in the execution of RN, especially in that of necroptosis, ferroptosis, and pyroptosis.

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Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2020.07.003

1. Introduction

Lysosome is a widespread intracellular organelle critical for cell homeostasis. It is once regarded as a simple housekeeping organelle owing to its critical impact on the turnover of the intracellular proteins and organelles[1]. However, more and more functions of lysosome have been explored nowadays, indicating that lysosome is a command-and-control center for cellular signaling, metabolism, and quality control[2,3]. Furthermore, it is generally acknowledged nowadays that lysosome plays key roles in cell death both in physiological and pathological conditions. Unlike the previous cognition, a novel method about the classification of cell death recommended by Nomenclature Committee on Cell Death (NCCD) has been commonly accepted[4,5] (Fig. 1). Apart from accidental cell death (ACD), at least 20 different types of programmed cell death have been enumerated, including apoptosis, autophagic cell death, and regulated necrosis (RN)[4–10]. In the past, the investigations concerning lysosome and cell death largely focused on apoptosis and autophagic cell death[3,11–13]. Nonetheless, tremendous studies gradually verified the capability of lysosomal alterations in dominating the outcomes of a new type of cell death: RN.

Instead of being accidental and uncontrolled, the occurrence of necrosis is now believed to be highly regulated in contrast to ACD[5]. RN, sometimes also called programmed necrosis, consists of a series of subtypes of cell death, including necroptosis[14], parthanatos[15], ferroptosis[16], cyclophilin D-mediated necrosis[6], oxytosis[17], NETosis (neutrophil extracellular trap, NET)[18], pyronecrosis[19], pyroptosis[20], mitochondrial permeability transition (MPT)-driven necrosis[21], poly-[adenosine diphosphate (ADP)-ribose] polymerase (PARP)-mediated cell death[9]. Lysosome takes part in different types of RN in different manners. Triggered by various inducers, lysosomal destabilization with ensuing lysosomal contents (cathepsins) release is observed both in necroptosis and pyroptosis, although they activate different signaling pathways[22,23]. Nevertheless, lysosomal membrane permeabilization (LMP) is not the only way for lysosome to influence RN. In ferroptosis, lysosome mainly functions in an autophagy-dependent manner including ferrintinophagy, clockophagy, and chaperon-mediated autophagy (CMA), exacerbating oxidative injury by promoting production of iron-dependent reactive oxygen species (ROS)[24,25]. In spite of this, other roles of lysosome in controlling RN are emerging.

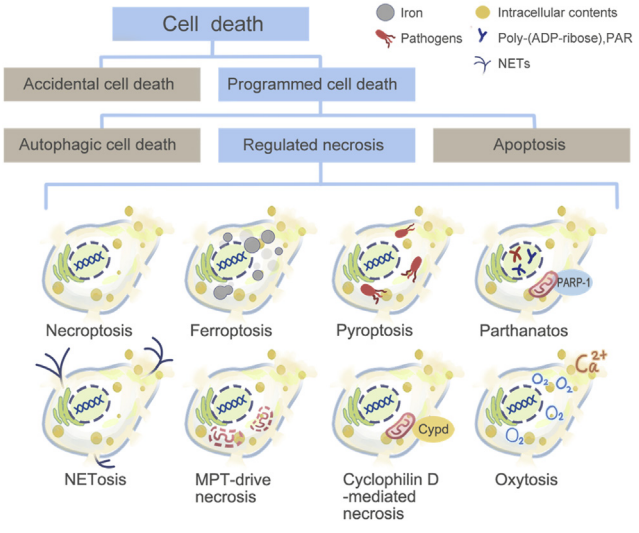

Figure 1 Classification of cell death. Cell death can be divided into ACD and programmed cell death (PCD). PCD includes apoptosis, autophagic cell death, and RN. RN comprises necroptosis, ferroptosis, pyroptosis, parthanatos, oxytosis, NETosis, pyronecrosis, cyclophilin D-mediated necrosis, and MPT-driven necrosis, etc.

Due to apoptosis defects and drug resistance, RN is considered as a novel target to overcome many diseases, especially cancers which show apoptosis- and therapy-resistance. Targeting lysosome may be a valuable approach for therapy, either by triggering cell death or by promoting therapeutic drug release that target the endocytic pathway[23]. Here we aim to give an introduction upon some common factors that influence the stability of lysosome and shed light on the current knowledge of how lysosome participates in the process of RN, especially in the three major forms of RN: necroptosis, ferroptosis, and pyroptosis. Table 1 [24–78] summarizes the key lysosomal factors that are involved in RN. Finally, we describe how interfering with lysosome can end up with enhanced prognosis of diseases by affecting RN.

2. Lysosomal stability

The functions of lysosome mainly depend on the hydrolases it contains, of which cathepsins B and D are the most important ones that can retain their activity both in the acid environment of lysosome and in the physiological pH value of cytosol[26]. LMP with subsequent hydrolases release is an essential stage during the occurrence of RN in most cases. Current knowledge suggests that LMP is either an initiator or a proteolytic amplifier that causes the final executioner phase of RN[6]. Although the underlying mechanisms by which the lysosomal lipid membrane gets altered remains obscure, several various pathways have been revealed to induce LMP[79].

Firstly, mitochondria-originated, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-generated ROS production causes lipid peroxidation (LPO) in lysosomal membrane thus promoting LMP. Secondly, increased cellular Ca^{2+} concentration owing to Ca^{2+} influx from outside or elevated Ca^{2+} release from inside stores, activates calcium-dependent enzymes (calpains and phospholipase in particular) and the phosphatidylinositol 3 kinase (PI3K) pathway to influence LMP. Thirdly, activated caspase-3 and -8 are closely related to LMP. Fourthly, tumor necrosis factor (TNF) induces LMP by activating neutral sphingomyelinase (N-SM), an initiator of sphingosine generation that inserts into lysosomal membrane. Fifthly, STAT3 promotes LMP through negatively regulating endogenous inhibitors of cathepsins, serine protease inhibitor 2A (Spi2A), or controlling the formation of lysosomal vacuoles that contain triglyceride. Triglyceride is metabolized to free fatty acids, resulting in destroyed lysosomal membrane with ensuing leakage of contents thus promoting lysosome-mediated necrotic cell death. Sixthly, the activation of *P53* and *Bax* are also reported to induce LMP[26,27,79–87]. Finally, several synthetic compounds, like methyl esters and lysosomotropic detergents can also cause LMP[23]. On the contrary, cholesterol accumulation, metallothionein 2 (MT2) and heat shock protein 70 (HSP70) are well-known protectors of lysosomal membrane[79,88].

HSP70, a highly conserved molecular chaperone located in lysosomal membrane lipids, is reported to inhibit LMP and prevent cell death in HSP70−bis-monoacylglycero phosphate

Table 1 Key lysosomal factors that are involved in regulated necrosis.

Type of regulated necrosis	Key lysosomal factor
Necroptosis	1. Lysosomal membrane permeabilization (LMP) with subsequent cathepsins release: (1) Increased intracellular Ca^{2+} concentration activated Ca^{2+}-dependent enzymes, including calpains and phospholipase, causing LMP[26–28]. (2) Enhanced ROS generation may also induce LMP under certain circumstances[29–32]. (3) Cathepsins (mainly B and D) released from lysosome as a result of LMP display hydrolytic functions. They interact with cytosolic signaling proteins, gradually self-digest certain cellular constituents, enhance LMP, induce mitochondrial and plasma membrane permeabilization, and finally cause cell death[26,27,33–39]. 2. Lysosomal regulation of RIPK1 and RIPK3: Lysosome maintains the normal homeostasis of RIPK1 and RIPK3 through their post-translational modification in TNF-induced necroptosis. Ubiquitylated RIPKs co-localize to lysosome with carboxyl-terminus of HSP70-interacting protein (CHIP) and get degraded in a lysosome-dependent pathway[40,41]. 3. Others: Lysosomal integrity takes part in the membrane repair system of plasma membrane during necroptosis, which consists of lysosomal degradation of endocytic MLKL and the exocytosis of MLKL on plasma membrane[42].
Ferroptosis	1. Lysosome can regulate iron homeostasis: (1) Lysosome has the highest abundance of active catalytic Fe^{2+} among all the organelles[43]. It can liberate active iron from ferritin (the main storage form of iron) in an autophagic process called ferritinophagy with the help of the cargo receptor nuclear receptor coactivator 4 (NCOA4). Autophagic degradation of ferritin is mediated by lysosomal acid hydrolases like cathepsin B inside lysosome. Increased free iron levels enhance lipid peroxides and ROS generation, which are prerequisite for ferroptosis[44–49]. (2) Lysosome stores iron imported by endocytosis of transferrin. Lysosome disrupters sir-amesine along with lapatinib increase transferrin expression and attenuate ferriportin-1 expression[50–52]. 2. Chaperon-mediated autophagy (CMA): Lysosome mediates the degradation of GPX4 in a manner of CMA, with an increased level of LAMP-2A[53,54]. 3. Clockophagy: Lysosome affects ferroptosis by selective autophagic degradation of core circadian clock protein ARNTL, named clockophagy which facilitates the expression of egl nine homolog 2 (EGLN2), thus destabilizing HIF1A and ultimately promoting LPO and subsequent ferroptosis[55,56].
Pyroptosis	1. Lysosome promotes NLRP3 inflammasome activation: (1) Lysosomal disrupters like crystalline materials, chemical compounds, nanomaterials, and rare earth oxide cause lysosomal rupture[23,39,57–59]. (2) Cathepsin B release as a result of lysosomal disruption attributes to NLRP3 activation. It binds to and activates NLRP3. Other cathepsins including cathepsins D, L, and X may also stimulate pyroptosis[59–65]. (3) ROS production and K^+ efflux as downstream events of lysosomal rupture contribute to the activation of inflammasome as well[66–68]. 2. Lysosomal permeabilization caused by HMGB1 avoided the lysosomal degradation of LPS, allowing LPS to stimulate pyroptosis by activating non-canonical inflammasome in endotoxemia[69,70]. 3. In pyroptosis induced by the endocytosis of HMGB1, lysosome destabilization and cathepsin B release are necessary for pyroptosome formation[71–73]. 4. The activation of NLRP3 inflammasome, non-canonical inflammasome, or pyroptosome further activates corresponding caspase and GSDMD, leading to final cell death.
Parthanatos	The occurrence of parthanatos may be accompanied by the activation of calpains, LMP, and cathepsin B/D release[74,75].
NETosis	NETs contains lysosomal enzymes. And instability of lysosome can cause the immediate formation of NETs and promote NETosis[18,76,77].
Cyclophilin D-mediated necrosis	Mitochondrial ROS induced generation of lysosomal ceramide activates cytosolic protein BAX which triggers a cascade of reaction with ultimate necrosis[78].

(BMP)−acid sphingomyelinase (ASM)−ceramide pathway[89−91]. HSP70 can bind to an endolysosomal phospholipid, BMP, enhancing the activity of ASM[89,91,92]. By binding to BMP, ASM promotes the production of ceramide which contributes to updated lysosomal membrane composition and increased membrane volume[91,93−95]. Downregulation of this pathway by inhibiting HSP70 or ASM could end up with destabilized lysosomal membranes and increased RN in cancer and neuronal cells[89,91,92,96−98]. On the contrary, upregulation or administration of HSP70 inhibits cell death and promotes neuroprotection[99,100]. It is noteworthy that calpains, another important inducer of LMP, can mediate the cleavage of oxidized HSP70 in hippocampal region of brain then induce lysosomal cell death and neurodegeneration[89,101,102].

3. Lysosome and necroptosis

Necroptosis is defined as a programmed form of lytic cell death in which receptor-interacting protein kinase 3 (RIPK3) activation leads to subsequent activation of the mixed lineage kinase domain-like protein (MLKL) and acute permeabilization of the plasma membrane[103]. As a prototype of RN[6], necroptosis shows morphological features similar to necrosis, namely ACD[104]. Therefore, it becomes hampered to distinguish necroptosis from ACD morphologically. Nevertheless, the discovery of MLKL which participates in the late event of necroptosis helps us better identify molecules that solely mediates necroptosis, thus providing probes for better assessing the role of necroptosis[103]. Unlike apoptosis, in which dying cells are cleared by phagocytes nearby before plasma membrane altered[105], cell death in necroptosis causes cell-membrane rupture with subsequent release of intracellular components that can stimulate an innate immune response[106].

3.1. *The molecular mechanisms of necroptosis*

When first being observed in 1990s, necroptosis was discovered to be a kind of TNF-induced necrotic cell death negatively regulated by caspase-1 and -8[5]. To date, except for TNF, an array of other stimuli has been discovered to induce necroptosis as well, followed by a set of well-understood signally pathway. Those identified stimuli include CD95 ligand [CD95L, also known as FAS ligand (FASL)], tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), tumor necrosis factor-related weak inducer of apoptosis (TWEAK), genotoxic stress, polyclonal stimulation of T-cell receptors, DNA-dependent activator of interferon regulatory factors (DAI), anticancer drugs, pathogen-associated molecular patterns (PAMPs), RIG-I-like receptors (RLRs), lipopolysaccharide (LPS), interferons (IFNs), and smac mimetic, etc.[6,107] However, death receptor-induced necroptosis, especially TNF-induced necroptosis, is still the best-understood among all these triggers in various backgrounds. Intriguingly, necroptosis can also be triggered in a receptor-independent manner[108].

The molecular mechanism of death receptor-induced necroptosis is a representative of all the triggers. Furthermore, TNF is the most frequently used death receptor activator to study necroptotic cell death. However, TNF can induce not only necroptosis, but also caspase-dependent apoptosis[6,109]. In the presence of caspase-8, TNF tends to induce apoptosis since caspase-8 inhibits the function of RIPK[110] while inactive caspase-8 contributes to necroptosis[111]. Thus, it is of vital importance to eliminate the disturbance of apoptosis while studying necroptosis. Notably, caspase-8 can be inhibited by Z-VAD-fmk (a pan-caspase inhibitor), FAS-associated death domain-like interleukin-1β-converting enzyme (FLICE)-like inhibitory protein (FLIP), caspase-8 knockout or FAS-associated death domain *(Fadd)* knockout, thus inhibiting apoptosis[79,112].

Under the circumstance of caspase-8 elimination, upon binding to death receptors on the membrane, TNF receptor 1 (TNFR1) signaling complex (TNF-RSC, also called complex I) recruits RIPK1 together with some other signaling molecules within minutes, forming a super-molecular complex that allows RIPK1 to recruit and activate its homologue RIPK3 by phosphorylating it[6]. It is observed that RIPK3, as an energy metabolism regulator related to the regulation of metabolic enzymes-glycogen phosphorylase (PYGL), glutamate-ammonia ligase (GLUL), glutamate dehydrogenase 1 (GLUD1) and subsequent ROS production, plays an essential role in the switch between apoptosis and necroptosis[111]. The complex I is reported to activate RIPK1 *via* transforming growth factor-β-activated kinase 1 (TAK1)-mediated phosphorylation or regulating the ubiquitination status of RIPK1[113−116]. Ubiquitylation and deubiquitylation of RIPK1 critically regulates the life and death of cells[117]. When caspase is inhibited, non-ubiquitinated RIPK1 dissociates from the complex I to the cytoplasm and gets phosphorylated[26,118]. Later on, activated RIPK1 interacts with RIPK3 stably to form a heterodimeric filamentous complex called necrosome, the process of which is mediated by RIP homotypic interaction motifs (RHIMs) of RIPK1 and RIPK3[119,120]. *Ripk3* knockout in animals results in resistance to necrosis and absent in inflammation[121].

MLKL, a critical downstream effector of RIPK3, is phosphorylated by RIPK3 and recruited to necrosome through its interaction with RIPK3, ending up with membrane permeability and cell death[122,123]. Still, how exactly phosphorylated MLKL leads to necropoptic cell death remains controversial. Chen et al.[124] asserted that activated MLKL will tetramerize and translocate to lipid rafts of plasma membrane, causing Na^+ influx afterwards, which increases osmotic pressure and results in membrane rupture eventually. Conversely, Cai et al.[122] and Schappe et al.[125] declared that MLKL trimerizes and translocates to plasma membrane, thereby promoting transient receptor potential melastatin-like 7 (TRPM7)-dependent Ca^{2+} influx rather than sodium influx, which is an early event of TNF-induced necroptosis. The oligomerization and/or translocation of MLKL to the plasma membrane relies on HSP90[126].

Generally speaking, lysosome can trigger the progression of necroptosis in two pathways: (1) LMP induced by Ca^{2+}-dependent enzymes and/or ROS promotes the release of cathepsins B and D into cytoplasm which finally causes necroptosis; (2) lysosome can regulate the key signaling molecules of necroptosis, RIPK1 and RIPK3 (Fig. 2).

3.2. *LMP with subsequent cathepsins release triggers necroptosis*

Like what's observed in apoptosis, lysosomal membrane damage like LMP with subsequent release of hydrolytic enzymes into cytosol occurs during the process of necroptosis[22,127]. A variety of cell death stimuli can lead to LMP, causing cathepsins release from lysosomal lumen into cytoplasm, thus inducing caspase-dependent or caspase-independent cell death[36,96,128,129]. Vanden Berghe et al.[29] demonstrated that lysosomal damage is a late event in TNF-induced necroptosis, whereas inhibition of lysosomal acidification led to decrease in the level of

TNF-induced necroptosis, although it has no difference or contrarily cause increase in the level of TRAIL-induced necroptosis[130]. It is described that ATPase H^+ transporting V1 subunit G2 (ATP6V1G2, H^+-ATPase), a ubiquitous multisubunit pump prerequisite for lysosomal acidification as well as activation of hydrolytic enzymes inside, is a hit required for necroptosis[128]. Besides, it is reported that granulysin, a saposin-like protein in human natural killer (NK) and cytotoxic T lymphocyte cells, can cause the disruption of lysosomal membranes in tumor cells and release lysosomal contents, especially cathepsin B, resulting in necroptotic cell death with single strand DNA (ssDNA) nicks[131]. Likewise, polyinosinic-polycytidylic acid (poly IC) can induce lysosomal destabilization and cathepsin D release into cytoplasm and then promote RLRs-induced necroptosis in dendritic cells. This may contribute to the cleavage of caspase-8 by cathepsin D and ubiquitination of RIPK1 within a cathepsin D−caspase-8−IPS-1−RIP-1 complex (IPS-1: an adaptor molecule for RLRs) formed in mitochondria after cathepsin D release[132].

Sharapova et al.[26] declared that activated MLKL contributes to an increase of intracellular Ca^{2+} concentration which activates Ca^{2+}-dependent enzymes, including calpains, phospholipase (*e.g.*, cytosolic phospholipase A2, cPLA2), and protease family members in FAS-dependent necroptosis of K562 cells. Inhibition of calpains and cPLA2 results in decreased cell death[26]. It is found that activation of calpains is also an essential stage in TNFR1-mediated necroptosis of tumor cells[133]. Calpains participate in the process of necroptosis *via* interfering with lysosome and subsequent cathepsins release[27,28]. Activated calpains cause LMP and release of cathepsins B and D, resulting in mitochondrial membrane depolarization, ROS production, and final cell death[27]. Fifteen members of the calpains family have been identified up to now, of which the best two characterized distinct, heterodimeric subtypes are μ- and m-calpains[28]. The localization of activated μ-calpains on lysosomal membrane was observed to be prior to the release of cathepsins while inhibition of calpains by inhibitors causes reduced necroptosis[134,135]. And it has been proven directly that calpains induce lysosomal rupture by the results of treating isolated lysosome with calpains[136].

Calpains are a group of Ca^{2+}-dependent proteases that are widely expressed in different tissues and organisms[28]. When activated, these enzymes truncate specific proteins by limited proteolysis to modify their structure and activity unlike the overall protein degradation mediated by proteasomes or lysosome[137]. Inactivated calpains exist in the cytoplasm whereas activated ones translocate to different cellular areas including nuclei, mitochondria, and lysosome, etc., and function distinctly[28,138]. Later on, in the presence of phospholipids, activated calpains cleave substrate proteins on membranes or in the cytoplasm when released from membranes, which may explain how activated calpains end up with LMP in cells undergoing necroptosis[138,139]. Intriguingly, calpains, with the downstream STAT3, are reported to regulate the expression of RIPK3 and phosphorylation of MLKL then induce endoplasmic reticulum (ER) stress and mitochondrial calcium dysregulation during ischemic/reperfusion induced lung injury which offer a positive feedback and amplify the function of calpains[135]. Moreover, upon initiation of necroptosis, activated calpains can also lead to phingomyelinase-mediated ceramide generation, and activation of c-Jun N-terminal kinase (JNK) besides inducing LMP[140].

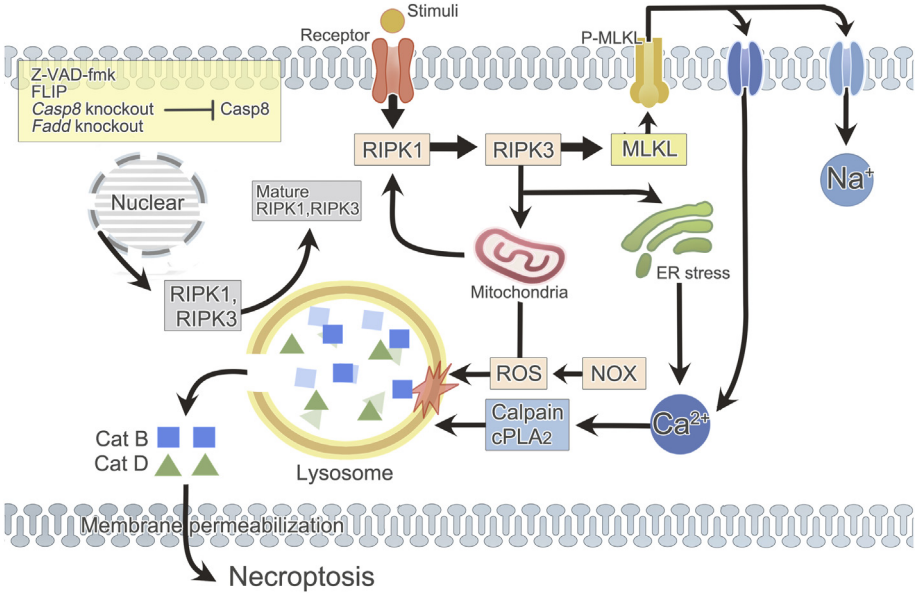

Figure 2 Lysosome and necroptosis. Necroptosis occurs in the context of caspase-8 inhibition (Z-VAD-fmk, FLIP, *Casp8* knockout, or *Fadd* knockout). Activated receptors on the membrane attract and activate RIPK1, which consecutively recruits and phosphorylates RIPK3 and MLKL. Phosphorylated MLKL translocates to plasma membrane, ending up with Na^+ or Ca^{2+} influx. ER stress, as a result of RIPK3 and MLKL phosphorylation, also causes Ca^{2+} dysregulation. Increased Ca^{2+} concentration activates Ca^{2+} dependent enzymes in cytosol, particularly calpains and cPLA2, which can later cause LMP. In addition, mitochondria- and NOX-dependent ROS production is another source of LMP inducer. The generation of ROS relies on necrosomal RIPK3 and it in turn facilitates RIPK1 autophosphorylation. Subsequently, disturbed lysosome releases acid hydrolases (especially cathepsins B and D) into cytoplasm, resulting in plasma membrane permeability and eventual necroptosis. Besides, lysosome is discovered to be essential for the post-translation of RIPK1 and RIPK3 which guarantees the occurrence of necroptosis. Cat: cathepsin; Casp: caspase.

Latest data also indicate the involvement of ROS in necroptosis in a context- and cell type-dependent manner. Although the significance of ROS in the induction of necroptosis have been proven in both *in vitro* and *in vivo* experiments, either in a direct or indirect method of inhibiting ROS generation[30,141,142]. However, He et al.[121] conversely showed that elimination of ROS makes no difference to necroptosis. As shown in recent study, the enhancement of ROS generation during necroptosis mainly lies in three distinct pathways which is mitochondria- and NADPH oxidase (NOX)-dependent, rather than Fenton reaction-reliant. The first is cathepsins-dependent mitochondria membrane potential reduction. The second is physical interactions between RIPK3 and enzymes of glycolysis which lead to abnormal energy metabolism and increase ROS production. The third is the interaction of TNFR and cell surface NOX enzymes which provides a significant source of death-inducing ROS in TNF-stimulated necroptosis[26,29,111,143,144]. Regardless of the sources of ROS, the downstream pathway between ROS and necroptosis has not been fully understood yet.

Current studies with respect to the relationship between ROS and necroptosis focus on the positive feedback circle which indicates that the production of ROS relies on necrosomal RIPK3, whereas ROS facilitates RIPK1 autophosphorylation on serine residue 161 and promotes the recruitment of RIPK3, thus enhancing the formation of necrosome and the occurrence of necroptosis[111,142,145−147]. Although ROS is a critical inducer of LMP, the direct evidence of ROS interfering with lysosome during ROS-induced necroptosis is still insufficient. Researchers found that lysosomal damage is a cPLA2-and ROS-dependent event and there is a steady increase of ROS generation in cells with necroptotic features which is followed by mitochondrial hyperpolarization, LMP, oxidative burst, and eventually plasma membrane permeabilization (PMP)[29]. Cai et al.[30] demonstrated that ROS-mediated LMP is involved in bupivacaine-induced necroptosis in rabbit intervertebral disc cells as well. Similarly, RIPK1-induced mitochondrial-ROS generation is followed by LMP and mitochondrial dysfunction as a result of a positive feedback loop between Ca^{2+} and JNK, culminating in necroptosis of A549 and H1299 cells[31,32]. Nevertheless, it is worthwhile to identify the specific types of ROS involved in necroptosis and how they participate in the occurrence of necroptosis.

ROS and Ca^{2+} trigger LMP synergistically rather than respectively. Upregulated-RIPK3 in necroptosis evokes endoplasmic reticulum stress which increases cellular Ca^{2+} concentration and the expression of xanthine oxidase leading to an enhanced ROS generation[148]. P2X7, an ATP-gated, nonselective cation channel, is possibly related to the execution of necroptosis after hemorrhagic stroke by stimulating Ca^{2+} and ROS rise in cytoplasm[146,149]. In post-ischemic neuronal necrosis, lysosomal rupture is induced in two different pathways at different times[150]. For one thing, in previous study of ischemic brain model of monkey, Yamashima et al.[33,151,152] formulated the "calpains−cathepsin" hypothesis to illustrate the execution of programmed neuronal necrosis affecting lysosomal membrane. μ-Calpains at the lysosomal membrane of cornu ammonis 1 (CA1) neurons gets activated when Ca^{2+} homeostasis is broken during transient brain ischemia. The activated form of μ-calpains was localized at the vacuolated or disrupted membrane of lysosome causing lysosomal membrane disruption and ensuing spillage of cathepsins from lysosome[33,151]. For another, during reperfusion, ROS oxidizes membrane fatty acids (*e.g.*, linoleic and arachidonic acid) to generates 4-hydroxy-2-nonenal which can cause carbonylation of HSP70[150]. Oxidative stress-induced carbonylated HSP70 at the lysosomal membrane is efficiently cleaved by activated μ-calpains followed by lysosomal rupture/permeabilization[102,150].

Lysosome contains around 70 kinds of broad-spectrum enzymes which can be released into cytosol as a consequence of lysosomal membrane damage. However, only cathepsins B and D display their enzymatic activity at physiological pH values[26]. The mechanisms of necroptotic cell death signaling owing to lysosomal activation have not been thoroughly illustrated up to now, but the release of lysosomal enzymes into cytoplasm is regarded as an indispensable event. That means in most cases, cathepsins are released into cytoplasm from lysosome after LMP, and display their hydrolytic functions which directly lead to cell death[37−39]. Leaked cathepsins interact with cytosolic signaling proteins, gradually self-digest certain cellular constituents, enhance LMP, induce mitochondrial membrane permeabilization (MMP), and finally cause cell death[27,33−36]. Cathepsins can induce MMP by directly interacting with membrane proteins or by indirectly processing the cytosolic proteins Bid, ending up with pores formation in the outer membrane of mitochondria[26,27,131].

Cathepsins B and D are the major functional contents released from impaired lysosome during the process of cell death[30,132]. Cytotoxin-1-induced necroptosis in leukemia cells is relevant to LMP and cathepsin B release[153]. And the formation of necrosome, phosphorylation and recruitment of MLKL with the ensuing cathepsin D activation, augmented the necroptotic pathway[154]. Using specific inhibitors of cathepsins B and D respectively can only partially reduce the execution of necroptosis whereas simultaneous pretreatment with both inhibitors completely blocked this type of cell death[26,27,155]. *Ripk1* knockdown decreased not only RIPK1−RIPK3 necrosome formation, but also LMP and the levels of active cathepsin B, thus reducing necroptosis in ischemic stroke model[156]. Similarly, administration of necrostatin-1 (Nec-1, an inhibitor of necroptosis) decreased the release of cathepsin B from lysosomes, which would explain at least partly why Nec-1 could protect hippocampal neuronal cells from necroptosis induced by ischemia/reperfusion injury[157]. The neuroprotective effect of Nec-1 also involves cathepsin D inhibition in its underlying mechanism[158].

Apart from cathepsins B and D, cathepsin L is also reported to be involved in certain context. Docosahexaenoic acid (DHA) attenuates TNF-induced necroptosis in L929 cells by reducing ROS generation, ceramide production, lysosomal dysfunction, and cathepsin L activation[159]. It is noteworthy that a positive feedback loop exists between LMP and cathepsins as well. As mentioned before in this paper, cathepsins are important inducers of LMP. However, the process of necroptosis is not always cathepsin-dependent. McComb et al.[160] demonstrated that cathepsins B and S directly cleaved RIPK1 and limited macrophage necroptosis. It is also reported that in L929Ts fibrosarcoma cells (a modified L929 subline sensitive to TRAIL), inhibitors of cathepsin B (Ca074Me), cathepsin L (zFF-fmk), or co-inhibitors of cathepsin B/L (zFA-fmk), failed to protect cells from TNF-induced necroptosis[161,162]. Therefore, the attendance of cathepsins in necroptosis is cell type- and stimuli-dependent.

3.3. *Lysosomal regulation of RIPK1 and RIPK3*

Recently, we discovered that lysosome also plays a central role in maintaining normal homeostasis of RIPKs (RIPK1 and RIPK3) through post-translational modification in TNF-induced necroptosis. It is demonstrated that CHIP (also known as STUB1) is a

direct E3 ligase which negatively regulates RIPKs by ubiquitylating them, thus inhibiting necroptosis in the mice model. Notably, ubiquitylated RIPKs co-localize to lysosome with CHIP and get degraded in a lysosome-dependent pathway other than proteasome-dependent pathway independent of HSP90[40,41]. On the contrary, lysosomal dysfunction after spinal cord injury may lead to decreased lysosomal localization and degradation of RIPKs, inducing rapid accumulation of RIPKs and MLKL in neurons, both *in vitro* and *in vivo*, thus inducing necroptosis that contributes to neuronal and glial cell death. Interestingly, inhibited lysosome may be enough to trigger necroptosis alone. In PC12 cells treated with Baf-A1 (bafilomycin A1, an inhibitor of lysosome's functions) and pan-caspase inhibitor, necroptotic cell death occurs even without the stimuli TNF[163]. Then, how did accumulated necroptotic mediators get activated without the stimuli? It is once demonstrated that RIPK1 is connected with autocrine production of TNF[164]. But whether this has happened in the context of lysosomal inhibition remains to be affirmed.

3.4. Others

Apart from what mentioned above, some various functions of lysosome during the initiation of necroptosis have been announced as well. Firstly, it is observed that necroptosis is usually accompanied by autophagy. Cells undergoing necroptosis show an increase in the number and size of lysosome[165]. RIPK3 overexpression in combination with chloroquine (CQ, which causes accumulation of vesicles but inhibits autophagy) enhanced LMP through the increase in autophagic flux and induced necroptosis in colon cancer cells[166]. Secondly, as discussed before, phosphorylated MLKL translocates to plasma membrane, then causes membrane rupture and the subsequent cell death. Lately, it is discovered that lysosomal integrity takes part in the membrane repair system of plasma membrane, which consists of lysosomal degradation of endocytic MLKL and the exocytosis of MLKL on plasma membrane, thus suppressing the occurrence of necroptosis[42]. Thirdly, lysosomal dysfunction plays an important role in the occurrence of necroptosis. In Parkinson's disease models, high levels of ROS may contribute to the activation of necroptosis *in vitro* with a decreased number of active lysosome and intralysosomal cathepsin D, increasing amount of total P62 protein which is degraded by lysosome, as well as the downregulated lysosomal genes and master regulator transcription factor EB (TFEB)[167]. However, 2-methoxy-6-acetyl-7-methyljuglone induced necroptosis in human colon cancer cells is reported to be independent of TNFα, MLKL, and LMP, instead dependent on cytosolic calcium accumulation-triggered JNK activation and mitochondrial ROS production[168]. Therefore, the role of lysosome in the execution of necroptosis may be stimuli type-dependent.

4. Lysosome and ferroptosis

Ferroptosis is a type of iron- and ROS-dependent, oxidative form of RN, which shows morphological, biochemical, and genetic distinction from other types of cell death like apoptosis and necroptosis, etc.[5,16,169]. It was first observed in 2003 to selectively kill oncogenic *Ras* mutated cells and was first formally termed by Dixon et al.[16] in 2012 using erastin and RAS-selective lethal small molecule 3 (RSL3)[170]. The morphology of ferroptotic cells is characterized by smaller mitochondrial membrane densities with reduced/devoid crista and ruptured outer mitochondrial membrane[171].

4.1. The molecular mechanisms of ferroptosis

Ferroptosis is initiated as a consequence of the imbalance between iron accumulation-induced ROS generation and antioxidant defense system that prevents LPO. Normally, on the one hand, as the pivotal protective mechanism against peroxidation damage, glutathione peroxidase 4 (GPX4) is activated relying on cystine (Cys2) import that is mediated mainly by system x_c^- (the glutamate/cystine antiporter) or by other routes like transsulfuration pathway[169,172,173]. When translocated into cells, Cys2 is reduced into cysteine which is used to synthesize glutathione (GSH), the recycling of which requires NADPH[174]. Subsequently, GPX4 uses GSH to eliminate phospholipid hydroperoxides (PLOOH) by converting it into non-toxic lipid alcohols (L-OH), so that iron can no longer react, thus reducing ROS accumulation[5,169,175]. In addition to GPX4, some other proteins including the transcription factor nuclear factor erythroid 2 like 2 (NFE2L2) and certain HSPs, like HSP25, HSP beta 1(HSPB1), and HSP family A member 5 (HSPA5) play antioxidant roles as well[5,176]. On the other hand, as for oxidative damage, it is demonstrated that lysophosphatidylcholine acyltransferase 3 (LPCAT3), acyl-CoA synthetase long-chain family member 4 (ACSL4), and arachidonate lipoxygenases (ALOXs, like ALOX12 and ALOX15) are involved in the catalytic process during the oxidation of polyunsaturated fatty acids (PUFAs) in lipid, which is critical for lethal lipid ROS accumulation[5,173,177]. Although it is a consensus that iron is indispensable in the process of ferroptosis, the precise role of iron still remains elusive. One tentative speculation is that iron regulates ferroptosis by influencing ROS accumulation *via* iron-dependent enzymatic and non-enzymatic (Fenton reaction) mechanisms[5]. The former refers to enzymes containing active iron site, like lipoxygenase (LOX) family enzymes which catalyzes the peroxidation of phospholipid PUFAs and hypoxia-inducible factor prolyl-4-hydroxylase isoform 1 (PHD1, also known as EGLN2)[169,178].

A plenty of reagents and genes have been discovered to induce or inhibit ferroptosis with distinct targets mentioned above to break the balance between oxidative damage and antioxidant system[179], among which the Raf kinase inhibitor sorafenib has already become a clinically-approved inducer of ferroptosis[180]. First of all, inactivation of GPX4 is the central stage. Regularly, we can use system x_c^- inhibitors (*e.g.*, erastin, sulfasalazine, sorafenib, and high extracellular glutamate) to deplete GSH thus reduce the activity of GPX4, which are referred to as class I ferroptosis inducers. What's more, we can also directly inhibit GPX4 utilizing its inhibitors such as (1*S*,3*R*)-RSL3, ML162, FINO2, or knockdown of *Gpx4* which causes overwhelming LPO and the eventual ferroptosis. And GPX4 inhibitors are regarded as class II ferroptosis inducers[5,169,173,181,182]. Of note, it is also observed that GPX4 depletion improves sensitivity to apoptosis, necroptosis, and pyroptosis, suggesting that LPO can accelerate multiple distinct RN modalities[5,108,183–185]. In comparison, vitamin E, ferrostatin-1, and coenzyme Q10, etc. can eliminate LPO and inhibit ferroptosis[5,16,179]. Nowadays, iron overload is considered to promote ferroptosis *via* stimulating ROS accumulation. Increasing iron concentration by using iron chloride, hemoglobin, and ferric ammonium citrate, etc. is reported to sensitize cells to ferroptosis whilst iron chelators, like deferoxamine (DFO), cyclipirox (CPX), or deferiprone can depletes iron and prevents

iron-dependent lipid ROS accumulation and ferroptosis[5,16,186]. In addition, it is lately discovered that IFN-γ produced by $CD8^+$ T cells in immunotherapy can induce ferroptosis to kill cancer cells[187,188]. Furthermore, P53 is found out to suppress tumor in a way of inhibiting cystine uptake and sensitizing cells to ferroptosis[189].

4.2. *Lysosome in regulating iron homeostasis*

Increasing evidence indicates the participation of lysosome in the process of ferroptosis nowadays[46,190] (Fig. 3). What is the most focused at present is that lysosome regulates ferroptosis by interfering with iron metabolism. Iron metabolism mainly consists of three parts: iron uptake, storage, and efflux. Ferritin is the main storage form of iron. Iron uptake is mediated by transferrin and transferrin receptor on the plasma membrane to transport iron into cells. On the contrary, ferroportin is the only known iron efflux pump to export iron out of cells[191,192]. Ordinarily, these three parts achieve a balance so that intracellular iron maintains a stable concentration which guarantees its normal function. However, lysosome contributes to ferroptosis by modulating iron equilibria and causing ensuing burst of ROS expression in lysosome[46,193]. It has been revealed that elevated level of labile Fe(II) both in lysosome and ER are responsible for erastin-induced ferroptosis in HT1080 cells, which is consistent with the intracellular location of ROS generation during ferroptosis[194].

Lysosomal ROS production attributes to the low pH and high iron content within lysosome unlike that originated from mitochondria and NADPH[5,195]. Subsequent LMP may be involved in ferroptosis in response to iron overload and ROS accumulation which causes peroxidation of the lipid components in lysosomal membrane[53,127,190]. In this way, LMP results in heightened Fenton radical generation, cell membrane denaturation, and GSH consumption[127]. DFO are also shown to block ferroptosis *via* inhibiting oxidative stress-induced LMP[196]. In spite of this, some dissenting voice put forward that LMP is not observed in ferroptosis[197]. Likewise, the reaction of downstream effector including cathepsin B varies in diverse cell types. In breast cancer cells, cathepsin B is discovered to be irrelevant to the ferroptosis process[50]. On the contrary, Gao et al.[190] presented that ferroptosis at least requires the activation of STAT3-mediated cathepsin B expression since its inhibition ended up with blocked ferroptosis in pancreatic ductal adenocarcinoma cells (PDACs). Therefore, the involvement of LMP−cathepsin axis in ferroptosis is context-dependent.

Among the organelles, the lysosome has the highest abundance of active catalytic Fe^{2+} probably owing to the acid environment inside lysosome where higher amounts of iron can be solubilized[43]. Carbonic anhydrase 9 inhibition causes intracellular iron overload co-localized both in lysosome and mitochondria in malignant mesothelioma cells, inducing oxidative stress and triggering LPO with final ferroptosis[198]. The lysosomal disrupter siramesine, combined with lapatinib (a dual tyrosine kinase inhibitor) or alone, induces a prompt rise in lysosomal pH followed by destabilization of lysosomal membrane, resulting in increased reactive iron and ROS production causing ferroptosis due to blocked iron transport and iron release by lysosomal disruption[52,96,199,200]. It is reported that lysosomal catalytic Fe^{2+} induced ferroptosis is critical for the efficacy of non-thermal plasma on killing oral squamous cell carcinoma[201]. Targeting

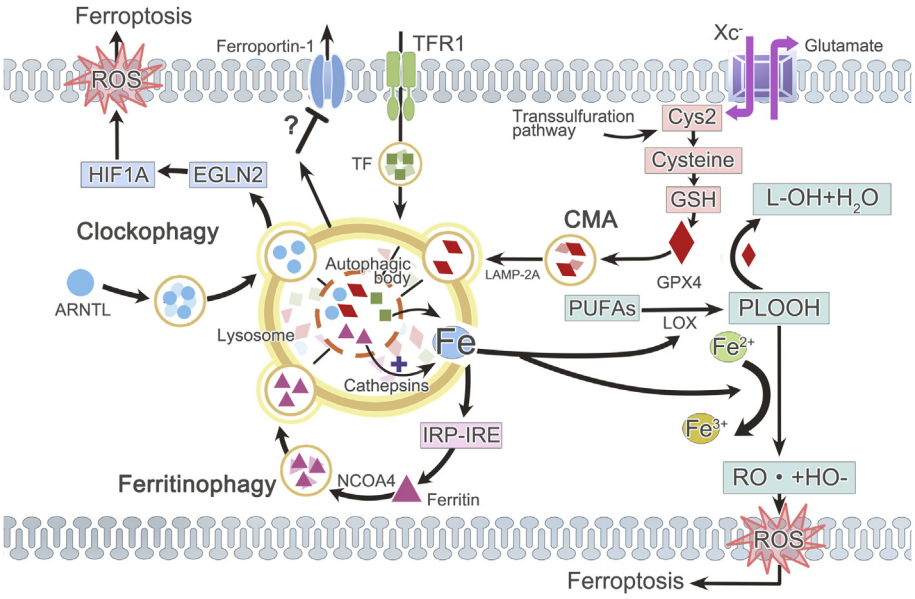

Figure 3 Lysosome and ferroptosis. Ferroptosis occurs owing to the imbalance between ROS generation and antioxidant defense system. Iron promotes ROS accumulation by enhancing the enzymetic activity of LOX or through Fenton reaction. But GPX4, coming from increased Cys2, reduces oxidative injury. Lysosome affects ferroptosis *via* regulating iron homeostasis, CMA (lysosomal degradation of GPX4) or clockophagy (lysosomal degradation of circadian clock protein ARNTL, which facilitates EGLN2 expression, thus destabilizing HIF1A and promoting LPO with subsequent ferroptosis). Lysosome increases the concentration of intracellular active iron, and NCOA4-dependent ferritinophagy is the most important form. Ferritin is sequestered into autophagosomes and then delivered to lysosome for degradation, liberating iron from ferritin. Increased labile iron concentration mediated by ferritinophagy in turn engages the IRP−IRE iron homeostatic system which leads to a continuous increase of ferritin synthesis, thus forming a positive feedback. Moreover, lysosome may also be related to increased transferrin and attenuated ferroportin-1 expression. Cys2: cystine; TFR1: transferrin (TF) receptor 1.

the lysosomal pool of iron provides an unprecedented therapeutic strategy to eradicate cancer stem cells[49]. Moreover, the iron chelator DFO likely protects against ferroptosis by chelating iron accumulation in lysosome[51]. And DFO is observed to be eventually localized within the lysosome, further confirming the localization of active iron in lysosome[196,202]. Artesunate is also able to induce ferroptosis in a lysosomal iron-dependent manner, since it can be fully blocked by lysosomal DFO whereas increasing lysosomal free iron by co-treatment with iron-saturated, diferric holo-transferrin significantly increased ferroptosis in PDACs[203,204].

But it is still elusive how lysosome enhances active iron to regulate ferroptosis. One possible explanation is through the connection with autophagy, so called ferritinophagy[24,25]. This process has been proven both *in vitro* and *in vivo*[205] and in various types of cells including fibroblasts cells, cancer cells[44], hepatic stellate cells[25], and breast cancer cells[205], etc. Moreover, some common inducers of ferroptosis, including erastin[206], cysteine depletion[207], artesunate (a water-soluble hemisuccinate derivative of artemisinin)[25,208], salinomycin, and its synthetic derivative ironomycin[49,205], are indicated to function *via* stimulating ferritinophagy with consequent iron overload. On the contrary, ascorbate can delay lysosomal ferritinophagy and stabilize ferritin, thus attenuate cell death by ferroptosis in relation to inflammatory pathology and anti-cancer therapy[209]. However, it is still debatable that whether another derivative of antimalarial drug artemisinin: dihydroartemisinin (DAT) induces ferroptosis by activating ferritinophagy[210,211] or not[206].

4.3. *Ferritinophagy*

Ferritinophagy refers to an autophagic process of ferritin being sequestered into autophagosomes and delivered to lysosome for degradation, which is essential for liberating active iron from ferritin and thus for maintaining cellular iron homeostasis[47,48]. This lysosomal degradation of ferritin is of great importance to maintain normal iron metabolism. Elevated intracellular iron concentrations can trigger ferroptosis by enhancing the production of lipid peroxides, which can be reverted by iron chelators, such as DFO[46]. However, the entry of ferritin into lysosome is independent of the presence of lysosome-associated membrane protein 2A (LAMP-2A), suggesting that ferritin entry has nothing to do with CMA[212]. Instead, the cargo receptor nuclear receptor coactivator 4 (NCOA4) is a significant regulator of ferritinophagy which binds to ferritin and selectively transports it into autophagosome, forming mature autophagosome that merges with the lysosome for degradation[47]. Activation of ferritinophagy followed by enhanced ferritin degradation is required for regulating ferroptosis[213]. For example, overexpression of NCOA4 increased ferritin degradation and promoted ferroptosis[44]. Contrarily, depletion of ferritinophagy either by knockdown of *Ncoa4* or by inhibition of autophagy using specific lysosomal lumen alkalizer inhibitor-CQ blocked the accumulation of cellular labile (or free) iron and lipid ROS, thus eventually suppressing ferroptosis[25,44,214].

Ferritin, composed of ferritin light polypeptide 1 (FTL1) and ferritin heavy polypeptide (FTH1), is the major intracellular iron storage protein[215]. It has been shown that ferritin expression and ferroptosis are negatively correlated[44,45]. Namely, increased ferritin expression limits ferroptosis and *vice versa*. That is because the initiation of ferroptosis at least partly relies on the autophagic degradation of ferritin in lysosome with subsequent increased labile iron levels and increased ROS, both of which are prerequisite for ferroptosis[44,45]. The lysosomal degradation of ferritin is mediated by lysosomal acid hydrolases like cathepsin B inside lysosome[44,49]. Lysosomal dysfunction that occurs in senescent cells causes ferritin accumulation and a deficiency in labile iron as a result of impaired ferritinophagy, inducing resistance to ferroptosis[216]. However, re-building the function of ferritinophagy with rapamycin averted the iron accumulation phenotype of senescent cells, yet still unable to sensitize senescent cells to ferroptosis. So, it is noteworthy that in those cells, lysosome is pivotal but not unique in regulating ferroptosis[216]. In contrast, activated ferritinophagy increases labile iron pool (LIP) and promotes ferroptosis[213,214].

Notably, increased cellular labile iron concentration mediated by ferritinophagy can in turn engages the IRP−IRE iron homeostatic system (IRE: iron-responsive element) which leads to a continuous increase of ferritin synthesis, thus forming a positive feedback with continued free iron increase and ROS enhancement[206]. This efficient pathway has been observed, including but not limited to erastin- and DAT-induced ferroptosis[206]. Furthermore, unlike autophagy, NCOA4-mediated ferritinophagy can be done directly in lysosome rather than relying on autophagic vacuoles[44]. But inhibitor of autophagy can suppress this process[44]. The underlying mechanisms of this merit need further investigation.

Ferrintinophagy with subsequent release of free iron and ROS generation may underlie ferroptosis in a sum of disease such as neurodegenerative diseases (*e.g.*, Alzheimer's, Parkinson's, and Huntington's diseases)[217] and liver fibrosis[25,213]. A recent study proposed that the upregulated mRNA binding protein ELAVL1/HuR (ELAV like RNA binding protein 1) in activated hepatic satellite cells was shown to enhance autophagy and meanwhile promote ferritinophagy and ferroptosis, contributing to liver fibrosis[213]. Treatment with sorafenib or artesunate inhibited this pathway and ameliorated liver fibrosis[25,213]. Although these data require further confirmation, including the newly discovered HuR pathway, understanding the role of ferritinophagy in regulating ferroptosis may provide a promising insight into overcoming ferroptosis-related diseases.

Lysosome also has impact on iron metabolisms *via* regulating transferrin and ferroportin-1. Under normal conditions, lysosome stores iron imported by endocytosis of transferrin, a critical process for ferroptosis[51]. It is demonstrated that lysosome disrupting agent, siramesine, synergistic with lapatinib, is capable of increasing transferrin expression and conversely attenuating ferroportin-1 expression, leading to increased iron concentration and causes ferroptosis in breast cancer cells[50]. This result is further confirmed in their research again later in 2017[52] with increased transferrin and decreased ferritin partly due to increased autophagy. In contrast, transferrin receptor expression is observed to be reduced during impaired ferroptosis owing to the high mobility group box 1 (*HMGB1*) gene knockdown in HL-60 cells[46].

4.4. *Chaperon-mediated autophagy and clockophagy in ferroptosis*

CMA may not relate to iron metabolism[212], but it can affect ferroptosis through another pathway rather than affecting iron homeostasis. It is reported that the induction of ferroptosis is accompanied with an increase in the levels of LAMP-2A to promote HSP90−CMA which in turn, mediate the degradation of GPX4 and glyceraldehyde 3-phosphate dehydrogenase

(GAPDH)[53,54]. On the contrary, using lysosome inhibitors (*e.g.*, CQ, NH_4Cl, and Baf-A1) or HSP90 inhibitor 2-amino-5-chloro-*N*,3-dimethylbenzamide (CDDO), or otherwise knockdown of *Lamp-2a* is able to restore GPX4 levels and reduces ferroptosis significantly[54]. Apart from regulating iron homeostasis or CMA, recently we found that lysosome can also affect ferroptosis by selective autophagic degradation of the core circadian clock protein aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL), named clockophagy[55,56]. The autophagy-mediated degradation of ARNTL can facilitate the expression of egl nine homolog 2 (EGLN2), thus destabilizing the pro-survival factor hypoxia-inducible factor 1 subunit α (HIF1A) and ultimately promote LPO and subsequent ferroptosis[55,56].

4.5. *Others*

Interestingly, we observed that most ferrostatins (an inhibitor of ferroptosis) are located within lysosome, mitochondria, and the endoplasmic reticulum which blocks ferroptosis through inhibition of LPO[51,197]. What's more, the lysosome-located ferrostatins are observed with alleviated potency whereas utilizing lysosome disrupter CQ to prevent its accumulation in lysosome can increase the potency of ferrostatin-1[51]. Therefore, it is not hard to understand that lysosome can enhance the sensitivity of cells to ferroptosis both in inductive or inhibitive environment. Moreover, the lysosomal acid environment is essential for the release of sorafenib from nanoparticles where it is loaded to inhibit GPX4 enzyme for ferroptosis initiation and enhances the efficacy of cancer treatment[218]. Similarly, iron-based nanoparticles could release ferrous (Fe^{2+}) or ferric (Fe^{3+}) ions inside acidic lysosome, thus rapidly increase the generation of ROS in tumor cells[191]. Ultrasmall nanoparticles, which reside in lysosome, can induce ferroptosis in nutrient-deprived cancer cells *via* a novel pathway and suppress tumor growth[219]. However, lysosome can sometimes entrap nanocarriers of ferroptosis inducers after internalization. On the contrary, nanoparticles modified to escape from lysosome and arrive at their subcellular targets can induce ferroptosis more efficiently and enhance the therapy efficacy in tumor cells[220]. Therefore, we come to a conclusion that the association of lysosome with nano-therapy of ferroptosis relies on the various types and targets of nanoparticles.

In summary, lysosome initiates ferroptosis by increasing iron-uptake (transferrin receptor) or decreasing iron efflux (ferroportin-1) and iron storage (ferritin), dependent/independent of NCOA4-mediated ferritin autophagy (ferritinophagy)[198]. Moreover, it is not hard to understand that ferroptosis is closely related to autophagy, referring to the involvement of ferritinophagy, clockophagy, and CMA.

5. Lysosome and pyroptosis

Pyroptosis is a lytic form of regulated cell death (RCD) that is driven by the activation of inflammasome and critically depends on the formation of plasma membrane pores mediated by gasdermin protein family[5,7]. It was first termed by Cookson and Brennan[20] in 2001 to describe an atypical caspase-1-dependent cell death of macrophages infected with *Salmonella* or *Shigella*. But now we found that bacterial infection is far from the only inducer of this type of cell death. The assembly of inflammasome can also be activated in the context of tissue injury, metabolism in macrophages, monocytes, and other cells[69]. Mostly, although involving different caspase sub-types[221], pyroptosis is considered as a kind of caspase-dependent cell death[103] like apoptosis, yet pyroptosis simultaneously shares morphological features with both necrosis and apoptosis[222]. Pyroptosis is characterized by the absence of DNA fragmentation, instead by the presence of a specific form of chromatin condensation coupled to cellular swelling and PMP as a result of the membrane pores formation, forming an apoptotic body-like cell protrusions (termed pyroptotic bodies)[223,224].

5.1. *The molecular mechanisms of pyroptosis*

Gasdermin D (GSDMD) to pyroptosis is exactly what MLKL to necroptosis. That is to say, GSDMD is the key effector for inducing pyroptosis, which is cleaved and activated by the inflammatory caspase-1 and -11 (along with its human homologs caspase-4 and -5)[69,224,225]. When activated, GSDMD generates C (GSDMD-C) and N-terminal (GSDMD-N) fragments of GSDMD, the latter of which forms oligomer and is translocated to plasma membrane to form non-selective pores on it, coordinating membrane lysis and the release of inflammatory cytokines, interleukin-1β (IL-1β), and IL-18 included[69,223,224,226]. In contrast, GSDMD-C is thought to fold back on and inhibit GSDMD-N, which is removed by the cleavage of activated inflammasome[227]. Except GSDMD, it is discovered that other gasdermin family members like gasdermin A (GSDMA), GSDMA3, and gasdermin E (GSDME, also called DFNA5) share the GSDMD-N domains and show similar pore-forming activity[227,228]. Pyroptosis can be pharmacologically inhibited by a direct inhibitor of GSDMD, necrosulfonamide, which offers a new insight into artificial manipulation of this type of cell death[223].

Caspase-1 is stimulated by canonical inflammasome to activate GSDMD. It is a multiprotein complexes constituted by pro-caspase-1, pattern recognition receptors (PRRs), and the apoptosis speck-like adaptor protein (ASC)[149]. The PRRs are composed of two sub-types. The first type refers to nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) family pyrin domain (NLRP) containing 1−3, 6, and 7 (NLRP1−3, NLRP6, andNLRP7), NLR family caspase recruitment domain (CARD) domain-containing 4 (NLRC4) among which NLRP3 is the most intensively investigated one. And the second type is non-NLR, such as absent in melanoma 2 (AIM2) and tripartite motif-containing (TRIM)[69,229,230]. They act as a sensor that NLR recognizes a wide variety of danger-associated molecular patterns (DAMPs) and PAMPs whereas non-NLR including AIM2 recognizes double-stranded DNA from bacteria or host cells[69,229,231,232]. ASC, however, functions as a bridge between the upstream sensor and pro-caspase-1, which contains two death-fold domains including pyrin domain (PYD) and CARD[69]. When this inflammasome is activated, it would in turn activate pro-caspase-1 into mature caspase-1 which triggers pyroptosis and cleaves pro-IL-1β and pro-IL-18 into active IL-1β and IL-18, respectively[233]. Recently, some studies reported that during the pyroptotic process, caspase-1 could be activated by both inflammasome and pyroptosome (composed of oligomerized ASC dimers)[72].

On the contrary, the other activator of GSDMD, caspase-11 (as well as its homologs caspase-4 and -5 in human) is stimulated by non-canonical inflammasome which is activated by cytosolic LPS from invading Gram-negative bacteria[69,234]. HMGB1 and outer membrane vesicles (OMVs) produced by Gram-negative bacteria are critical for the delivery of extracellular LPS for caspase-11 dependent pyroptosis[70,235,236]. However, the canonical and non-

canonical pathways are not absolutely isolated. Instead, there are some amplifying interconnections between canonical (NLRP3- and AIM2-dependent) and non-canonical pathways[237,238]. Non-canonical caspase-11 can induce activation of canonical NLRP3 by activating downstream pennexin 1 channel and P2X7 receptor causing potassium efflux[229,239,240]. Similar to necroptosis, endosomal sorting complexes required for transport III (ESCRT-III) dependent membrane repair system negatively regulates pyroptosis upon GSDMD activation[241].

5.2. *The role of lysosome in pyroptosis*

The role of lysosome in the execution of pyroptosis has been briefly introduced by Repnik et al.[23]. Thus, here we would supplement with most recent findings (Fig. 4). Similar to that of necroptosis, LMP with subsequent cathepsins release, particularly cathepsin B, is a central regulator of inflammasome-dependent pyroptosis. This has been proven in numerous experiments on non-epithelial cells, especially in macrophages exposed to infectious agents[59,64,65]. Activation of pyroptosis during lysosomal damage could be alleviated through inhibition of lysosomal acidification or cathepsins. Lysosomal destabilization induced by dipeptidyl methyl ester Leu-Leu-OMe (LLME) triggers caspase-1 mediated pyroptosis whilst blocking lysosomal disruption abrogates the cytotoxicity of LLME[242]. Moreover, overexpression of cathepsin B by ablation of cystatin C (endogenous inhibitor of cathepsin B) significantly exacerbates, whereas the cathepsin B inhibition either by genetically knockout of cathepsin B or cathepsin B-selective inhibitor Ca074Me attenuates caspase-1 dependent pyroptosis[60,62,243,244]. Functional lysosomal cathepsins are important for downstream signaling since Baf-A1 (a lysosomal disrupter) prevents the activation of caspase-1[245]. In tamoxifen-treated retinal pigment epithelial cells, blockade of the activity of cathepsins B and L leads to a significant decrease not only in apoptosis and necroptosis, but also in pyroptosis[246].

Lysosomal rupture is one of the common stimulators of NLRP3 inflammasome which results in direct activation of caspase-1[247]. In macrophages, various crystalline materials (*e.g.*, silica or alum) or chemical compounds (*e.g.*, LLME) can cause LMP and cathepsin B release, which is an important mechanism of NLRP3 inflammasome and subsequent caspase-1 activation and then pyroptosis[23,245]. In addition, NLRP3-mediated pyroptosis was observed after viral infection or 27-hydroxycholesterol-, cholesterol crystals-, rare earth oxide-induced lysosomal damage in other cell types, like nerve cells and Kupffer cells[39,57–59]. Nanomaterials, like nano-silver and nano rare earth metal oxide, are capable of stimulating inflammasome activation owing to lysosomal disruption which is closely related to the morphological characteristics of nanoparticles for needle-shaped nanoparticles are more prone to pierce lysosomal membrane[248–251].

Cathepsin B's release, as a result of lysosomal disruption, attributes to NLRP3 activation. Unlike necroptosis, cathepsins function as an upstream initiator of pyroptotic cell death rather than an executor. In Table 2, a comparison about the functions of cathepsins in necroptosis and pyroptosis is summarized. Nano-therapy based on magnetic intra-lysosomal hyperthermia involves an enhanced production of ROS through lysosomal Fenton reaction which subsequently induced the release of LPO, LMP, and cathepsin B that in turn activates caspase-1 (either by directly or indirectly activating upstream inflammasome), and ultimately induces pyroptosis in pancreatic endocrine, pancreatic exocrine, and gastric cancer cells without IL-1β secretion[252]. Cathepsin B binds to NLRP3 as verified

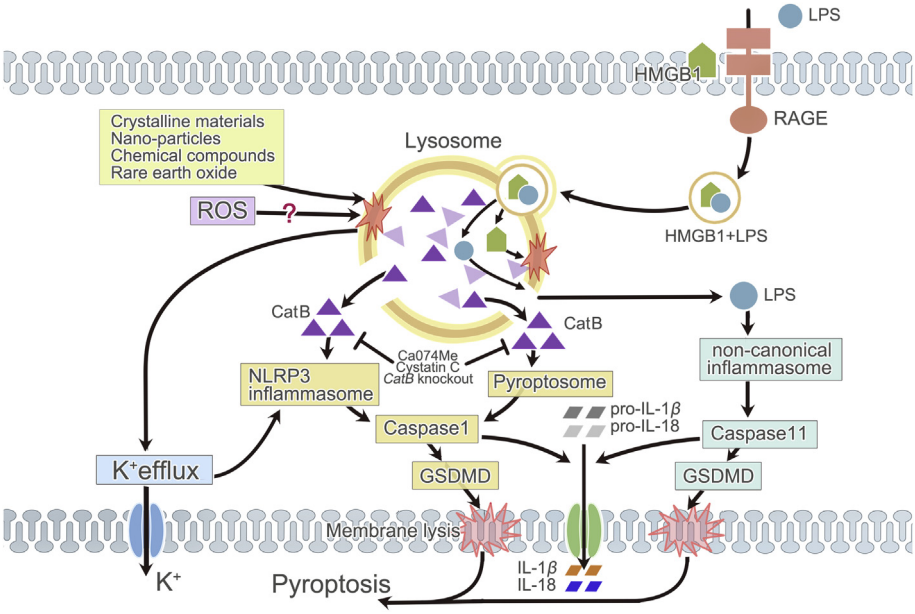

Figure 4 Lysosome and pyroptosis. In pyroptosis, lysosomal rupture can be initiated by various crystalline materials, nano-particles, chemical compounds, rare earth oxide, and maybe ROS. Subsequent CatB release and K^+ efflux activate NLRP3 inflammasome. But in pyroptosis induced by the endocytosis of HMGB1, lysosome destabilization and CatB release are necessary for pyroptosome formation. Activated NLRP3 inflammasome or pyroptosome stimulates caspase-1. CatB can be inhibited by Ca074Me, endogenous cystatin C, or genetic knockout of *CatB*. Lysosome destabilization caused by HMGB1 is also indispensable for LPS-induced activation of non-canonical inflammasome and caspase-11, since it allows LPS internalized through HMGB1−RAGE signaling pathway to escape from lysosomal degradation. Activated caspase-1 and -11 drive the cleavage of pro-IL-1β/18 and GSDMD, coordinating membrane lysis, mature IL-1β/18 release and ultimate pyroptosis. CatB: cathepsin B.

by co-precipitation using either an anti-NLRP3 or anti-cathepsin B antibody[60]. Cathepsin B may activate NLRP3 inflammasome through mitogen-activated protein kinase (MAPK) and TAK1−JNK pathway[61]. This mechanism of activating NLRP3 accounts for the cytotoxicity of cathepsin B to coxsackie virus B3-induced myocarditis[243]. In Kawasaki disease, cathepsin B's release induced endothelial cell pyroptosis by activating NLRP3 inflammasome[253]. 27-Hydroxycholesterol, which initiates pyroptosis procedures in neurons and causes neurodegenerative diseases, functions through inducing LMP and cathepsin B release, leading to NLRP3 inflammasome activation as well[59,254,255]. We also found that multi-walled carbon nanotubes induced pyroptosis and inflammasome activation in primary human bronchial epithelial cells can be distinctly decreased by cathepsin B inhibitor Ca074Me[256]. Uptake of boron nitride nanotubes caused lysosomal destabilization, cathepsin B increase, inflammasome activation, and pyroptosis both *in vitro* and *in vivo*[257].

However, some studies revealed that the execution of pyroptotic cell death stimulated by particle and pyroptosis inducers like nigericin and ATP relies on multiple cathepsins rather than just cathepsin B. Because cathepsin B's deficiency does not prevent the induction of pyroptosis under this circumstance[59,62,63]. This includes another type of cathepsin, cathepsin X[59,63]. Moreover, inhibition of cathepsins B and L suppresses inflammasome activation and significantly reduced pyroptotic cell death in primed retinal pigment epithelial cells which revealed the involvement of both cathepsins in pyroptosis[258]. Similarly, the death of retinal pigment epithelium cells activated by all-*trans* retinal is related to cathepsins B and D, as well as NLRP3 inflammasome activation[259]. But the cytosolic flagellin-induced cathepsin B release in macrophage may induce cell death by activating neuronal apoptosis inhibitory protein 5 (NAIP5)/NLRC4 inflammasome rather than NLRP3 inflammasome[64].

In addition to the cathepsin release, ROS production and K^+ efflux are common stimulators for inflammasome activation as well[66−68]. When exposed to excess all-*trans* retinal, both ROS (including mitochondria ROS) and cathepsins released from lysosome contribute to NLRP3 inflammasome activation and the subsequent pyroptosis in human ARPE-19 cells[259]. Notably, ROS appears to be upstream of lysosomal damage in the context of iron oxide nanoparticles-induced inflammasome activation, as ROS inhibitor significantly reduced lysosomal damage, while cathepsin B's inhibitor shows no difference to ROS production[251]. Moreover, polymers induce lysosomal rupture followed by the concurrent K^+ efflux from the cells, which leads to subsequent pyroptosis as observed by the propidium iodide stain[260]. Cytosolic DNA-induced IL-1β maturation and pyroptosis are essential for the anti-viral immunity in human myeloid cells, during which activated stimulator of interferon genes (STING, a component of DNA detection axis) would translocate to lysosome and trigger LMP, initiating K^+ efflux upstream of NLRP3 inflammasome activation[261]. Some other studies indicated that lysosome rupture induces Ca^{2+} release from the lysosome to the cytosol and then promotes complete NLRP3 inflammasome activation through the oligomerization of ASC *via* TAK1−JNK pathway[61]. However, it is reported that only low level of lysosome disruption activates NLRP3 inflammasome initiating by K^+ efflux nevertheless extensive lysosome rupture is inhibitory[262]. Variously, *Shigella* infection-induced pyroptosis in macrophage influences K^+ metabolism in a unique manner. This infection can cause the internalization of the invasion plasmid antigen B channels which permit K^+ influx within endolysosomal compartments followed by vacuolar destabilization, endolysosomal leakage, and the ultimate caspase-1-dependent pyroptosis[263].

Therefore, it is conceivable that lysosomal rupture can regulate the occurrence of pyroptosis *via* cathepsins release, ROS pathway or by reducing the K^+ concentration in cytoplasma. At first, it is believed that lysosomal rupture is an upstream activator of NLRP3 inflammation independent of caspase-11[234,245,264]. Recently, there are a few dissenting voice claims that lysosomal rupture occurs either upstream or downstream of inflammasome activation. They presented that NLRP1b/NLRP3 inflammasome ignited by pyroptosis agonists was prior to lysosomal disruptsion[64,265]. Furthermore, it is postulated that activated caspase-1 contributes to lysosomal acidification in systemic lupus erythematosus[266]. Therefore, further investigation requires to be done to clarify the relationship between lysosomal rupture and NLRP3 activation.

Lysosomal permeabilization also takes part in the activation of non-canonical inflammasome by allowing LPS stimulation. Deng et al.[70] demonstrated that extracellular LPS molecules are internalized into the acidic lysosome of macrophages and endothelial cells by physically binding to hepatocyte-released HMGB1 through HMGB1-RAGE (receptor for advanced glycation end-products) signaling pathway. HMGB1 is a nuclear protein which can activate multiple cell surface receptors including RAGE or Toll-like receptors to initiate innate immune response when released into extracellular fluid[267]. When internalized, HMGB1 can directly permeabilize the phospholipid bilayer of the lysosome under acidic conditions, thus allowing LPS to escape from lysosomal degradation and then leak out into its key pathogenic cytosolic receptor, caspase-11, which culminates in the activation of non-canonical inflammasome and pyroptosis in endotoxemia[69,70]. Inhibition of HMGB1/RAGE mediated endocytosis prevented caspase-11 dependent pyroptosis in endotoxemia and bacterial sepsis[70,268]. Cathepsin G mediated caspase-4 activation by a bacterial surface protein in human gingival fibroblasts and

Table 2 The functions of cathepsins in necroptosis and pyroptosis.

Content	Necroptosis	Pyroptosis
Similarities	1. Released from lysosome into cytoplasm after lysosomal damage. 2. Promote necroptosis or pyroptosis in most cases.	
Types involved	Mainly: B and D; others: L	Mainly: B; others: D, L, and X
Role	Downstream executor	Upstream trigger
Functions	Interact with cytosolic signaling proteins, gradually self-digest certain cellular constituents, enhance LMP, induce mitochondrial and plasma membrane permeabilization, and finally cause cell death.	1. Bind to and activate NLRP3 inflammasome, ending up with caspase-1 activation. 2. In pyroptosis induced by the endocytosis of HMGB1, cathepsin B release is necessary for pyroptosome formation, culminating in caspase-1 activation.

induced pyroptosis[269]. Nevertheless, by inducing lysosomal rupture with ensuing lysosomal contents release including cathepsin D, HMGB1 can also activate NLRP3 inflammasome independent of caspase-11, subsequently inducing caspase-1 activation through Toll-like receptors[70,72].

However, we should notice that in pyroptosis induced by the endocytosis of HMGB1, lysosome destabilization and cathepsin B release are necessary for pyroptosome formation. Acting through the RAGE and dynamin-dependent signaling, the endocytosis of HMGB1 triggers a cascade of intracellular molecular events, including lysosomal rupture with cathpsin B release followed by pyroptosome assembly and NLRP3 inflammasome-independent caspase-1 activation and pyroptosis both *in vitro* and *in vivo*[71−73,270]. Furthermore, these events are reported to be indispensable for LPS-induced pyroptosis. Yet it is indicated that HMGB1 endocytosis and subsequent cathepsin B activation is only required for late-phase of caspase-1 activation unlike that NLRP3 inflammasome accounts for the early-phase[73].

To sum up, lysosomal rupture triggers the execution of pyroptosis either by activating NLRP inflammasome or by promoting pyroptosome assembly.

6. Lysosome and other forms of RN

6.1. *Parthanatos*

Parthanatos is a form of RN characterized by hyperactivation of the nuclear protein PARP1 and consequent apoptosis inducing factor (AIF)- or macrophage migration inhibitory factor (MIF)-dependent DNA degradation[7]. PARP1 is essential for restoring cellular homeostasis by taking part in DNA repair, chromosome stability, and the inflammatory response[6,10]. But overactivated PARP1 stimulated by a wide array of stimuli such as DNA damage, oxidative stress, hypoxia, hypoglycemia, or inflammatory cues ends up with parthanatotic cell death by transferring PARP1 from nicotinamide adenine dinucleotide (NAD^+) and binding to AIF or hexokinase1 (HK1), which mediates the release of AIF from mitochondria into cytosol and its translocation into the nucleus where it promotes large-scale DNA fragmentation and chromatin condensation, leading to cell death[5,7,10]. Through binding to PARP1, HK1 inhibits glycolysis to cause the energy depletion and precipitate parthanatos[271]. Recently, MIF emerges as the main nuclease that binds to AIF and produces large DNA fragment to induce pathanatos[272]. On the contrary, PARP1 inhibition by pharmacological or genetic modification provides cytoprotective effects in multiple animal models of diseases[10].

The relationship of lysosome and parthanatos is largely unknown. Porte Alcon et al.[74] discovered that the occurrence of parthanatos in microglial cell death triggered by manganese is accompanied by LMP and cathepsin B/D release. Inhibition of both cathepsins B and D results in partial decrease of cell death combined pretreatment retinal photoreceptor with calpains and cathepsin inhibitors, rather than cathepsin inhibitors alone, reduced parthanotic cell death[75].

6.2. *NETosis*

NETosis, also called NETotic cell death, is a ROS-dependent modality of RN which is driven by NETs release in response to infection or injury in cells of hematopoietic derivation like neutrophils[7,273]. ROS generation in NETosis is dependent on NOX activation and can promote the translocation of granular enzymes including elastase, neutrophil expressed (ELANE), myeloperoxidase, and peptides of the cathelecidin family from cytosol to nucleus[274,275]. Later on, the myeloperoxidase-dependent proteolytic activity of ELANE gets promoted, which culminates in histone citrullination, chromatin decondensation, extrusion of chromatin fibers along with cytoplasmic and nuclear components, ultimately resulting in plasma membrane rupture and necrosis[5,276,277].

It has been demonstrated that NETs contains unfolded chromatin and lysosomal enzymes[18]. Moreover, instability of the lysosomal compartment and fast damage of plasma membranes in various cell types and tissues cause the immediate formation of NETs in the context of 10−40 nm sized nanoparticles[76]. Similarly, it is observed that LLME induced crystal-triggered NETosis in a dose-dependent fashion, suggesting that lysosomal disruption is involved in the execution of NETosis as well[77].

6.3. *Cyclophilin D-mediated necrosis*

Cyclophilin D-mediated necrosis is another form of RN that relies on the cyclophilin D-dependent formation of mitochondrial permeability transition pore[10]. Most recently, researchers found that cyclophilin D-mediated necrosis in tuberculosis-infected macrophage relies on a mitochondrial−lysosomal−endoplasmic reticulum circuit[78]. During this process, mitochondrial ROS induced generation of lysosomal ceramide is a critical activator of the cytosolic protein BAX which triggers a cascade of reaction with ultimate necrosis.

Nevertheless, the evidence indicating a direct connection between lysosome and parthanatos, cyclophilin D-mediated necrosis, and NETosis is far from sufficient. And up to now, no direct proof has referred to the connection between lysosome and oxytosis or MPT-driven necrosis. Therefore, a broad area about the relationship between lysosome and RN can be explored in the future.

7. Physiological and pathological significance

Cell death is essential for living organisms and is a hallmark of numerous disorders. It also has a significant influence on the treatment of certain diseases, for example, cancer[278]. In general, RN also occurs both under physiological and pathological conditions. On the one hand, it is of great importance for embryogenesis, lymphocyte function, and the involution of post-lactation mammary gland[85,87,279]. On the other hand, RN is a genetically determined process that contributes to plenty of human diseases. Different types of RN may account for various pathological injuries either simultaneously or respectively. For example, during ischemic brain injuries and neurodegenerative diseases, RN rather than apoptosis seems to compose the major cell death mode[152]. Analogously, necroptosis, mitochondrial-mediated necrosis, ferroptosis, parthanatos, and pyroptosis are probably the main contributors of renal and myocardial ischemic-reperfusion injury and renal transplantation[8,54,280,281].

It is noteworthy that the pathways of RN can be therapeutically targeted[10,16,282,283]. What's more, targeting lysosome may provide a novel insight into controlling RN thus further improve the outcomes of diseases. A large number of experiments have confirmed that reagents or genetic modifications of lysosome and relevant factors showed positive efficacy in controlling RN, and some of

Table 3 Lysosomal reagents or genetic modifications in regulating RN and relevant diseases.

Name	Target	State	Diseases/model	Result	Effect on injury
Desipramine	(−) ASM	*In vitro*	TB infection	Reduced susceptibility to necroptosis	Reduce bacterial burdens, making zebrafish hyperresistant[295]
ALLN	(−) Calpain	*In vitro* and *in vivo*	IRI in hippocampal neurons	(−) Necrosis	Neuroprotection[101]
		In vitro	IRI in lung	(−) Necroptosis	(−) Cell death after IRI in lung[135]
Calpastatin overexpression	Endogenous calpain inhibitor	*In vitro* and *in vivo*		(−) Caspase-independent neuronal cell death	Neuroprotection[296]
DPI	(−) NOX	*In vitro*	Human umbilical vein endothelial cells	(−) Apoptosis and necroptosis	ND[297]
GKT137831 *Nox4* siRNA	(−) NOX4	*In vitro*		(−) Apoptosis and necroptosis	ND[297]
N-Acetyl-L-cysteine	(−) ROS	*In vitro*	Bupivacaine-induced cytotoxicity of IVD cells	(−) LMP and necroptosis	Reduced cytotoxity[30]
CDDO	(−) HSP90	*In vitro*		(−) Ferroptosis and necroptosis	ND[54]
CQ, NH4Cl, and Baf-A1	Lysosome inhibitor	*In vitro*		(−) Ferroptosis	ND[54]
Baf-A1 and CQ	Lysosome inhibitor	*In vitro*	In certain cancer cells (*e.g.*, HT1080 and BJeLR)	Can't modulate ferroptosis	ND[16]
		In vitro	MEFs and HT1080 cells	(−) Ferroptosis	ND[214]
Baf-A1	Lysosome inhibitor	*In vitro*	PDAC	(−) Ferroptosis	ND[190]
		In vitro and *in vivo*	Cytosolic flagellin stimulated macrophages	(−) Pyroptosis	ND[64]
Lysosomal lumen alkalizer-CQ	Lysosome inhibitor	*In vitro* and *in vivo*	Liver fibrosis	Impair ferritinophagy and ferroptosis	Inhibited anti-fibrosis function of artesunate[25]
LLME	Lysosomal disrupter	*In vitro*	AMD	(+) Pyroptosis	ND[242]
		In vitro	Acutely inflamed joints of gout	(+) NETosis	ND[77]
		In vitro and *in vivo*	murine BMDC	(+) Pyroptosis	ND[262]
Ca074Me	(−) Cat B	*In vivo*	Ischemic injury of hippocampus	Inhibit 67% of programmed necrosis	Neuroprotection[33]
		In vitro and *in vivo*	endotoxemia	(−) Pyroptosis	ND[73]
		In vitro	SH-SY5Y and C6 cells	Partially prevented pyroptosis	ND[254]
		in vitro and *in vivo*	CLP sepsis	(−) Pyroptosis	Attenuate inflammatory responses[71]
		In vitro	KUP5 cells and macrophages	(−) Pyroptosis	ND[57]
		In vitro		(−) Necroptosis	ND[298]
		In vitro	Promyelocytic leukemia cells	(−) Necroptosis	ND[299]
		In vitro	PDAC	(−) Ferroptosis	ND[190]
		In vitro	Cancer stem cells	(−) Ferroptosis	ND[49]
Genetic knockout of *CatB*	(−) Cat B	*In vitro* and *in vivo*	Coxsackievirus B3-induced viral myocarditis	(−) Pyroptosis	Attenuate[243]
Genetic deletion of cystatin C	(+) Cat B	*In vitro* and *in vivo*		(+) Pyroptosis	Aggravate[243]
Ca074Me and pepstatin A	(−) Cats B and D	*In vitro*	Bupivacaine-induced cytotoxicity of IVD cells	(−) Necroptosis	Reduced cytotoxity[30]
		In vitro	ARPE-19 cells	(−) Apoptosis and pyroptosis	ND[259]
		In vitro	Mn^{2+} exposed BV-2 cells	Prevented a ~13% parthanatos	Alleviates toxicity[74]
		In vitro and *in vivo*	Cytosolic flagellin stimulated macrophages	Halved pyroptosis	ND[64]
Ca074Me/ pepstatin A alone	(−) Cat B or D	*In vitro* and *in vivo*	Cytosolic flagellin stimulated macrophages	No effect on pyroptosis	ND[64]
Ca074Me and Z-FY(*t*-Bu)FMK	(−) Cats B and L	*In vitro*	Tamoxifen treated human RPE cells	(−) Apoptosis, necroptosis, pyroptosis	ND[246]

(−): Inhibit; (+): promote; CAD: cationic amphiphilic drugs; MDR: multidrug resistance; TB: tuberculosis; DPI: diphenyleneiodonium; IRI: ischemia−reperfusion injury; IVD: intervertebral disc; Cat: cathepsin; (A)RPE cells: (acute) retinal pigment epithelial cells; CLP: cecal ligation and puncture; AMD: age-related macular degeneration; BMDC: bone marrow-derived dendritic cells; ND: not determined.

them can significantly enhance the prognosis of various diseases. Numerous lysosomotropic agents showed positive efficacy in treating various cancers including breast cancers, lymphoma, leukemia, and those showing resistance to traditional forms of treatment. Many of those agents are U.S. Food and Drug Administration (FDA)-approved drugs already or under clinical trials[284,285]. Cathepsins and calpains are promising therapeutic targets, especially for cancers and neurological diseases. Inhibitors of cathepsins have reached clinical trials in osteoporosis and cancer. E-64, an inhibitor of both calpains and cathepsins, is in clinical trials of Alzheimer's disease[286]. What's challenging now is that clinical trials targeting this complex regulating network of lysosome in RN is still insufficient. Here we would describe the known human diseases related to each kind of regulated necrosis and the potential roles of lysosome in these diseases *via* dominating the occurrence of RN.

7.1. *Necroptosis*

As an alternative cell death pathway in apoptosis-defective cells, necroptosis is implicated in a wide range of pathological cell death paradigms, including infectious diseases, ischemic−reperfusion injury in brain, inflammatory diseases, tumorigenesis, myocardial infarctions, acute lung/kidney/liver injury, amyotrophic lateral sclerosis, and chemotherapy-induced cell death, etc.[118,287−289] Notably, necroptotic cell death pathway becomes a novel therapeutic target in the treatment of many diseases nowadays, particularly in the treatment of apoptosis- and therapy-resistant cancers such as breast, colon, and liver cancers[79,85,287,290,291]. Many cancers contain mutations that inactivate apoptotic pathway and cause drug-resistance. However, the idea that tumors may be sensitive to RN can hopefully offer another direction for overcoming the resistance[292]. Researches regarding the necroptosis in cancer therapy mainly focus on two aspects. The first is that the induction of necroptosis can eliminate therapy-resistant cancer cells or enhance surveillance and protective immunity against cancer. On the contrary, inhibition of necroptosis reduces cancer-promoting inflammation[287]. The inhibitor of RIPK1, benzoxazepinone, has already reached phase 2 clinical trial[293]. RIPK3 is now considered as a potential therapeutic target for Gaucher's disease, a lysosomal storage disease with insufficient lysosomal enzyme β-glucocerebrosidase[294]. Table 3 summarized the reagents or genetic modifications of lysosome and relevant factors which could affect RN and change the outcomes of various diseases.

In a zebrafish model of tuberculosis infection, necroptosis of macrophage releases residual bacteria into permissive extracellular milieu and causes continuous infection. However, desipramine, a tricyclic antidepressant that promotes the degradation of ASM and inhibits ceramide production which is an essential step for LMP, decreased the susceptibility of macrophage to necroptosis and reduced bacterial burdens, making zebrafish hyperresistant to infection[295]. Using *N*-acetyl-Leu-Leu-norleucinal (ALLN) to block calpain (another inducer of LMP) inhibited the occurrence of necroptosis which occurs after ischemia−reperfusion injury in lung[135]. Similarly, overexpression of endogenous calpain inhibitor, calpastatin, inhibited caspase-independent neuronal cell death and promoted neuroprotection in calpastatin mutant mice[296]. Diphenyleneiodonium (DPI), GKT137831 or *Nox4* siRNA inhibited the function of NOX, reduced the production of ROS, and inhibited the execution of both apoptosis and necroptosis *in vitro*[297]. Inhibition of ROS directly by *N*-acetyl-L-cysteine effectively blocked bupivacaine-induced LMP and necroptosis, reducing cytotoxicity to intervertebral disc (IVD) cells[30].

CDDO, an inhibitor of lysosomal membrane protector HSP90, effectively blocked necroptosis of HT-22, 661W, HT29, and human leukemia *Fadd*-deficient Jurkat cells by inhibiting the activation of RIPK1[54]. The activity of the lysosomal protease cathepsin B was observed to be increased on Days 3−5 after ischemia injury in hippocampus of monkeys. However, blockade of lysosomal activity rescued neurons from death which showed mild central chromatolysis. A specific inhibitor of cathepsin B, Ca074Me, saved around 67% of CA1 neurons from programmed necrosis and showed a promising capability of neuroprotection after ischemic injury[33]. Similarly, Ca074Me promoted the survival of U937 cells and promyelocytic leukemia cells dying from necroptosis[298,299]. Apart from cathepsin B, other cathepsins also count in the process of necroptosis. Ca074Me along with pepstatin A (cathepsin D inhibitor) prevented necroptotic cell death and reduced bupivacaine-induced cytotoxicity of IVD cells[30]. What's more, Ca074Me combined with cathepsin L inhibitor, Z-FY(*t*-Bu)FMK, blocked apoptosis, necroptosis, and pyroptosis which occur in tamoxifen-treated human retinal pigment epithelial cells[246].

7.2. *Ferroptosis*

Ferroptotic cell death is discovered to be involved in many diseases including cancers[50,190], neurodegenerative disorders[300], acute renal injury, renal IRI[301], and infectious diseases, etc.[44] It is noteworthy that pharmacological inhibition or inducement of this type of cell death may be a novel therapeutic approach for those diseases[187,188,206,302]. In some cases, the metabolic reprogramming in cancer cells heightens their sensitivity to ferroptosis, therefore opening up a novel insight for overcoming tumors, particularly the therapy-resistant ones[171,302−304]. Sorafenib, a multikinase inhibitor drug for the treatment of kidney/liver/thyroid cancer, has already become a clinically-approved inducer of ferroptosis[10,180]. Moreover, it is reported that ferroptosis, which is triggered by $CD8^+$ T cells *via* releasing IFN-γ, can boost the effectiveness of immune checkpoint inhibitors[305].

Similar to necroptosis, CDDO could also inhibit ferroptosis of HT-22, 661W, and human HT-1080 cells[54]. What's more, lysosome pathway inhibitor CQ, NH_4Cl, or Baf-A1 inhibited GPX4 degradation and subsequent ferroptosis induced by glutamate or erastin *in vitro*[54]. Although Baf-A1 and CQ could block ferroptosis in various cell lines, like MEFs, HT1080 cells, and PDAC, they failed to modulate ferroptosis in certain cancer cells (*e.g.*, HT1080 and BJeLR)[16,190,214]. Artesunate alleviates liver fibrosis by triggering activated hepatic stellate cell (HSC) ferroptosis. However, the lysosomal lumen alkalizer CQ, which disturbs the fusion of lysosomes with autophagosomes, impaired ferritinophagy and ferroptosis both *in vitro* and *in vivo*, thus inhibiting antifibrosis function of artesunate[25]. The inhibitor of cathepsin B, Ca074Me, could block ferroptosis in PDAC and cancer stem cells as well[49,190].

7.3. *Pyroptosis*

Pyroptosis is crucial for immune defenses (*e.g.*, against infection) and numerous diseases including hemorrhagic stroke and sepsis[71,149,227,230,306]. Of note, it is reported that except apoptosis, pyroptosis accounts for over 95% of quiescent $CD4^+$ T cells death

in HIV-1 infection[307]. Interestingly, chemotherapy drugs can induce pyroptosis through caspase-3 cleavage of GSDME[228].

The usage of lysosomal inhibitor Baf-A1 showed a significant inhibitory effect on cytosolic flagellin-induced pyroptosis in macrophages[64]. The lysosomotropic agent LLME which is used to disrupt lysosome and induce lysosomal destabilization, triggered pyroptosis in ARPE-19 cells of age-related macular degeneration patients and murine bone marrow-derived dendritic cells[242,262]. Suppression of cathepsin B with its inhibitor Ca074Me prevented pyroptosis in bone marrow-derived macrophages of endotoxemia[73], in SH-SY5Y and C6 cells (partially prevented)[254], in macrophages of a mouse sepsis model and attenuate inflammatory responses[71] and in Kupffer cells[57]. Analogously, genetic knockout of cathepsin B also inhibited pyroptosis and attenuated coxsackievirus B3-induced myocarditis. But genetic deletion of cystatin C (endogenous inhibitor of cathepsin B) contrarily promoted cell death and aggravated myocarditis[243]. However, in cytosolic flagellin stimulated macrophages, pyroptosis seems to be promoted by both cathepsins B and D since inhibiting both cathepsins simultaneously with Ca074Me and pepstatin A can inhibit pyroptosis. Ca074Me or pepstatin A alone failed to have effect on pyroptosis[64].

7.4. *Other forms of regulated necrosis*

Other regulated forms of necrosis have been involved in various pathological conditions and provide a potential for drug development. For instance, parthanatos has been studied mostly under the background of neuronal damage and neurodegeneration such as Parkinson disease[10,308]. Plenty of pathological conditions are reported to attribute to parthanatos, including neurodegeneration, some cardiovascular and renal disorders, diabetes, and cerebral ischemia[7]. NETosis reportedly contributes to a wide range of human pathologies, including diabetes, cancers, allergic asthma systemic, autoimmune diseases, and renal diseases etc.[309–313] Another type of cell death, cyclophilin D-mediated necrosis, has been reported to be involved in cardiac and renal IRI[314], muscular dystrophy[315], thrombosis[316], and diabetes[317]. Cathepsin inhibitors Ca074Me and pepstatin A prevented around 13% parthanatos and reduced the cytotoxicity of Mn^{2+} to BV-2 cells[74]. Lysosomal disrupter LLME induced NETosis in a dose-dependent manner *in vitro*[77].

8. Conclusions, perspective, and challenges

The role of lysosome in regulating necroptosis, ferroptosis, and pyroptosis shares both similarities and differences. The release of hydrolytic enzymes as a result of LMP, especially cathepsin B, is closely involved in the occurrence of both necroptosis and pyroptosis. However, in cells undergoing necroptosis, LMP, and cathepsins release, triggered by various inducers like Ca^{2+} and ROS, are more prone to be a frequent downstream occurrence of MLKL and can directly cause cell death. In contrast, LMP and cathepsin B release is the initiator of pyroptotic cell death. In pyroptosis, cathepsin B functions by promoting the assembly of the pivotal complex, canonical inflammasome, or pyroptosome, thus activates caspase-1 and initiates pyroptotic cell death. Diversely, the role of lysosome in ferroptosis is rather different. Lysosome acts in an autophagy-dependent manner to control the degradation of ferritin and cause iron accumulation, namely ferrintinophagy. It is noteworthy that the roles of lysosome in regulating RN are far from just these. Instead, some other functions are surfacing, including lysosomal post-translation of RIPK1 and RIPK3 in necroptosis, CMA-dependent degradation of GPX4, or clockophagy in ferroptosis and lysosome-dependent delivery system of LPS in pyroptosis, etc. Utilizing agents or genetic methods to affect lysosome or lysosomal molecules can vigorously dominate the fate of cells and even influence the outcomes of diseases. Therefore, targeting lysosome and relevant events may provide a promising therapeutic strategy to alter the hallmarks of RN, thus further improve the prognosis of diseases, especially of cancers which show resistance to apoptosis and traditional treatments.

In the past, the researches concerning RN have been principally biased towards necroptosis, ferroptosis, and pyroptosis, and consequently lysosome was supposedly thought to be responsible for these three types of RN mainly. Therefore, the evidence for lysosomal involvement in other forms of RN like parthanatos, PARP-mediated cell death, etc., is still insufficient. However, we now noticed that other forms of RN also play an essential role in many physiological and pathological situations, hence it is necessary to expand our study to uncover the role of lysosome in a wider range of RN. Moreover, due to the diversity and abundance of cathepsins in lysosome, as well as the complexity of stimuli and molecular mechanisms in necroptosis and pyroptosis, the functions of different types of cathepsins in these two forms of cell death remain ambiguous, although most studies agreed that cathepsins promote these processes. Some results are even contradictory in different context. Considering that cathepsins are validated targets for the treatment of human diseases, it's worthwhile to further investigate the complicated interactions of cathepsins and RN. Finally, lysosomal factors are emerging as therapeutic targets for many diseases. Strategies that alter the status of lysosome provided a method of dominating the occurrence of RN and showed therapeutic effects in certain diseases. Future studies should explore the therapeutic effects of lysosome through interfering with RN in different diseases background.

Acknowledgments

This work is supported by the National Major Scientific and Technological Special Project for "Significant New Drugs Development" (No. 2018ZX09733001, China), the Development Program of China (No. 2016YFA0201402), and by the Excellent Youth Foundation of Sichuan Scientific Committee Grant in China (No. 2019JDJQ008).

Author contributions

Aqu Alu wrote the initial manuscript and made the tables. Xuejiao Han contributed writing materials and new ideas. Xuelei Ma created the figures and revised the manuscript. Min Wu,Yuquan Wei and Xiawei Wei revised the manuscript as well and approved the final version. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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