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Autophagy-Dependent Ferroptosis: Machinery and Regulation

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Abstract

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved cellular process capable of degrading various biological molecules (e.g., protein, glycogen, lipids, DNA, and RNA) and organelles (e.g., mitochondria, endoplasmic reticulum, ribosomes, lysosomes, and micronuclei) via the lysosomal pathway. Ferroptosis is a type of oxidative stress-dependent regulated cell death associated with iron accumulation and lipid peroxidation. The recently discovered role of autophagy, especially selective types of autophagy (e.g., ferritinophagy, lipophagy, clockophagy, and chaperone-mediated autophagy), in driving cells towards ferroptotic

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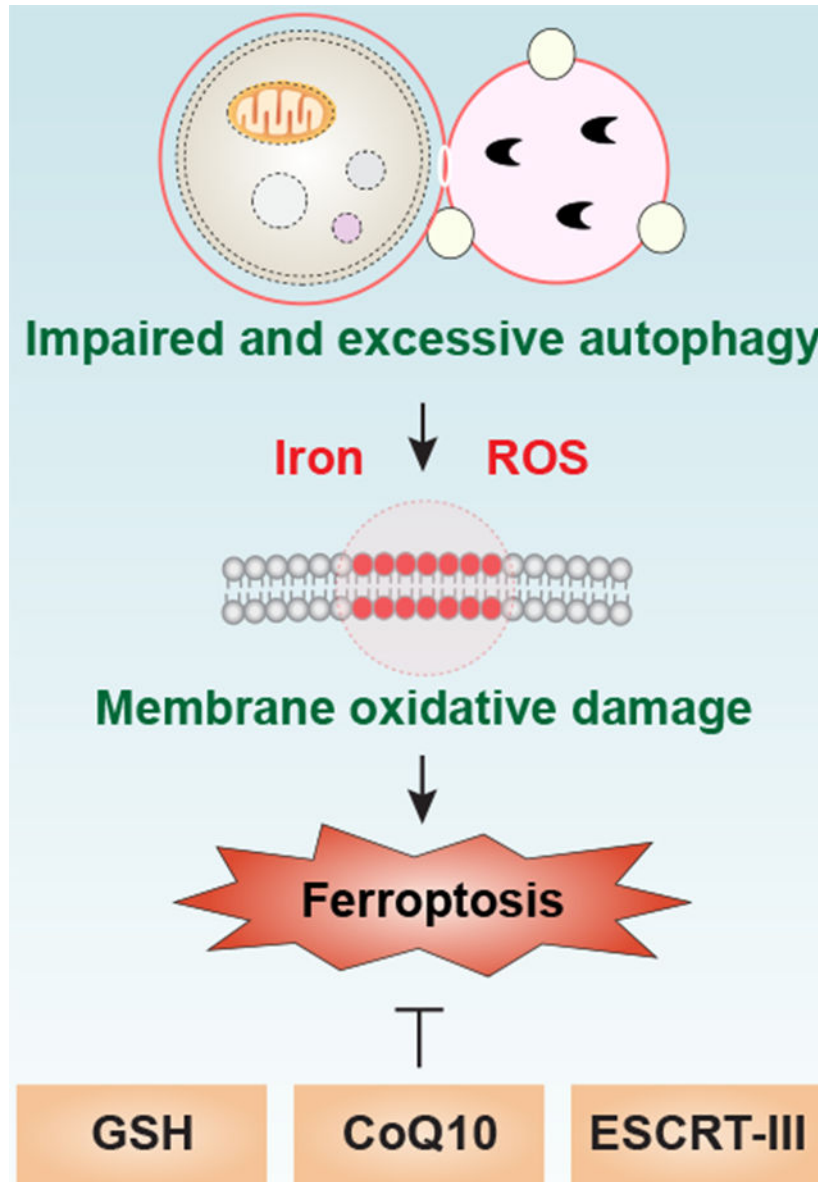
Declaration of interests

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death motivated us to explore the functional interactions between metabolism, immunity, and cell death. Here, we describe types of selective autophagy and discuss the regulatory mechanisms and signaling pathways of autophagy-dependent ferroptosis. We also summarize chemical modulators that are currently available for triggering or blocking autophagy-dependent ferroptosis and that may be developed for therapeutic interventions in human diseases.

Graphical Abstract



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Liu et al describes types of selective autophagy and discuss the regulatory mechanisms and signaling pathways of autophagy-dependent ferroptosis. A deeper understanding of the process

and function of autophagy-dependent cell death is critical for creating innovative therapeutic strategies for oxidative stress-related diseases.

Keywords

autophagy; ferroptosis; network; regulated cell death; selective autophagy

Introduction

Cell homeostasis is controlled by an integrated system that maintains the balance between biosynthesis (anabolism) and decomposition (catabolism). Macroautophagy (hereafter referred to as autophagy), which was first described in the 1950s, is a phylogenetically conserved catabolic process that plays a fundamental role in removing or clearing the accumulation of intracellular waste (Klionsky and Emr, 2000; Levine and Kroemer, 2019; Yang and Klionsky, 2010). After its breakdown by autophagy, cellular garbage, such as aggregated protein and damaged organelles, can be recycled for macromolecular synthesis and energy production. In addition, elements of the autophagic machinery can be used to eliminate invading pathogens or engulfed apoptotic cells via lysosomes. Thus, autophagy is generally beneficial for cellular survival in response to environmental stresses ranging from nutrient starvation to growth factor depletion, hypoxia, temperature change, and infectious pathogens (Kroemer et al., 2010; Murrow and Debnath, 2013). Increased autophagy appears to be widely observed during cell death, and the term “autophagic cell death” has been used to describe type II cell death according to morphological alterations (Kroemer and Levine, 2008). Excessive or dysfunctional autophagy can result in cellular killing. In particular, the term “autophagy-dependent cell death” was recommended by the Nomenclature Committee on Cell Death to describe a form of regulated cell death (RCD) that mechanistically depends on the autophagic machinery or components thereof (Galluzzi et al., 2018). Autophagy-dependent cell death has been implicated in several diseases such as cancer, neurodegeneration, and ischemia-reperfusion injury (Bialik et al., 2018; Denton and Kumar, 2019). Mechanistically, it involves the progressive cellular consumption or degradation of cytoprotective proteins, although the specific signaling cascade remains to be elucidated (Bialik et al., 2018; Denton and Kumar, 2019).

Cell death has been divided into two categories: accidental cell death and RCD (Galluzzi et al., 2018). Unlike accidental cell death that lacks clear molecular signals and regulators, RCD is an active process involving tightly controlled mechanisms that can be targeted by genetic manipulations or drugs. The interplay between autophagy and cell death decides cell fate by activating integrated signaling pathways and influencing gene expression programs. Autophagy can coincide with apoptosis, the most common form of the RCD, as well as with non-apoptotic forms of RCD including ferroptosis (Zhou et al., 2019). Ferroptosis was initially described as a unique type of RCD observed in oncogenic RAS-mutated cancer cells that is distinct from apoptosis, necrosis, and autophagy at the morphological, biochemical, and genetic levels (Dixon et al., 2012). However, increasing evidence challenges these early observations and suggests that the autophagic machinery, at least under certain conditions, contributes to ferroptotic cell death (Kang and Tang, 2017; Zhou et

al., 2019). Autophagy-dependent ferroptosis may contribute to anticancer therapies, inflammation responses, and tissue fibrosis (Kang and Tang, 2017; Zhou et al., 2019). In this review, we introduce the basic process of autophagy and ferroptosis, describe types of selective autophagy, and discuss recent advances in understanding the molecular mechanisms of autophagy-dependent ferroptosis and its regulation, placing special emphasis on the biomedical implications of these findings.

The molecular machinery of autophagy

Autophagy is a dynamic process that relies on the formation and maturation of specific membrane structures such as phagophores, autophagosomes, and autolysosomes (Dikic and Elazar, 2018) (Fig. 1). The cup-shaped phagophores (also known as the isolation membrane) can be generated from multiple sources (e.g., the endoplasmic reticulum [ER], mitochondria-ER associated membrane, Golgi complex, plasma membrane, and recycling endosomes) to engulf cytoplasm (Tooze and Yoshimori, 2010). The subsequent expansion and closure of the phagophores lead to the formation of typical double-membrane structures, which are termed autophagosomes. In a subsequent step, autophagosomes fuse with lysosomes to generate autolysosomes, where the degradation of the cargo proceeds by lysosomal hydrolytic enzymes acting at low pH. Mechanistically, autophagy-related (ATG) proteins are core components of the machinery that drives the initiation, progression, and maintenance of autophagy. Genetic screens in yeast have led to the identification of over 40 *ATG* genes, many of which have mammalian homologs. These ATG proteins can interact with other factors governing phagophore and autophagosome formation, which may rely on multiple posttranslational modifications (Dikic and Elazar, 2018; Xie et al., 2015). Although the molecular details are still not completely understood, members of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family (e.g., syntaxin 17 [STX17], synaptosome-associated protein 29 [SNAP29], and vesicle-associated membrane protein 8 [VAMP8]), the homotypic fusion and vacuole protein sorting (HOPS) complex, certain cytoskeleton motor proteins (e.g., dynein), and regulatory lipids (e.g., phospholipids) are involved in the formation of autolysosomes (Nakamura and Yoshimori, 2017).

The ULK1 complex

The unc-51 like autophagy activating kinase (ULK) family consists of five serine/threonine-specific protein kinases: ULK1, ULK2, ULK3, ULK4, and serine/threonine kinase 36 (STK36). ULK1 and ULK2, the mammalian orthologs of yeast Atg1, drive the formation of the phagophore through binding to ATG13, RB1-inducible coiled-coil 1 (RB1CC1/FIP200, ortholog of yeast Atg17), and ATG101. Binding to ATG13 or RB1CC1 increases the stability and kinase activity of ULK, which is partly regulated by ATG101-dependent ATG13 phosphorylation (Mercer et al., 2009). Moreover, ULK1 can phosphorylate itself as well as ATG13 and RB1CC1 during autophagy induction. ULK1 and ULK2 may have some functional redundancy in autophagy induction, although they share only 52% amino acid identity (Lee and Tournier, 2011). The formation of the ULK1-ATG13-ATG101-RB1CC1 complex is determined by the status of intracellular energy and nutrients. This complex can be activated under starvation via the inhibition of the mechanistic target of rapamycin kinase

complex 1 (MTORC1, containing MTOR; regulatory associated protein of MTOR complex 1 [RPTOR]; MTOR associated protein, LST8 homolog [MLST8]; DEP domain containing MTOR interacting protein [DEPTOR]; and AKT1 substrate 1 [AKT1S1/PRAS40]) or via the activation of the energy sensor 5'-AMP-activated protein kinase (AMPK) (Kim et al., 2011). Under starvation conditions, MTORC1 and AMPK directly regulate ULK1 (de)phosphorylation at multiple sites. In addition to phosphorylation, ULK1 undergoes acetylation and ubiquitination (Ub) as an alternative regulatory mechanism (Lin et al., 2012; Nazio et al., 2013). Many phosphorylation substrates of ULK1, such as ATG9A (a lipid-embedded protein for phagophore membrane cycling) (Zhou et al., 2017), Beclin 1 (BECN1; a mammalian homolog of yeast Vps30/Atg6) (Park et al., 2018; Russell et al., 2013), and autophagy and beclin 1 regulator 1 (AMBRA1) (Russell et al., 2013), have been identified to regulate the formation of phagophores or autophagosomes. The recruitment of ULK1 to the phagophore assembly site may involve the Golgi-localized WW domain containing adaptor with coiled-coil (WAC), a positive regulator of autophagy (Joachim et al., 2015). Thus, the ULK1 complex is an important signal node, translating multiple protein phosphorylation signals into the initiation of autophagy.

The PIK3C3 complex

The PIK3C3 complex contains the catalytic subunit phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3, an ortholog of yeast Vps34) that converts phosphatidylinositol (PtdIns) into PtdIns-3-phosphate, BECN1, and phosphoinositide-3-kinase regulatory subunit 4 (PIK3R4, a mammalian homolog of yeast Vps15), which can be activated by the ULK1 complex. This core PIK3C3 complex can further form several subcomplexes that involve distinct co-factors, such as the PI3KC3-C1 complex (containing ATG14) (Fan et al., 2011), the PI3KC3-C2 complex (containing UV radiation resistance associated [UVRAG]) (Liang et al., 2006), and the rubicon autophagy regulator (RUBCN)-containing complex (Tabata et al., 2010). Functionally, the PI3KC3-C1 complex is responsible for the formation of the phagophore assembly site, membrane nucleation, elongation, and autophagosome formation. The PI3KC3-C2 complex enhances, whereas the RUBCN complex suppresses, autophagosome maturation. Accordingly, there are multiple positive and negative feedback loops involved in the regulation of PIK3C3 complex activity through different BECN1-binding proteins, such as ULK1 (Park et al., 2018), BCL2 apoptosis regulator (BCL2) (Patingre et al., 2005), BCL2 like 1 (BCL2L1/BCLXL) (Oberstein et al., 2007), high mobility group box 1 (HMGB1) (Tang et al., 2010b), vacuole membrane protein 1 (VMP1) (Molejon et al., 2013), inositol 1,4,5-trisphosphate receptor type 3 (ITPR3/IP3R) (Vicencio et al., 2009), PTEN induced kinase 1 (PINK1) (Michiorri et al., 2010), baculoviral IAP repeat containing 5 (BIRC5/SURVIVIN) (Niu et al., 2010), and epidermal growth factor receptor (EGFR) (Wei et al., 2013). These findings suggest that diversity in the assembly of the PIK3C3 complex could be associated with different roles of the autophagic machinery, connecting it to other signaling networks. For example, BECN1 and its partners are implicated in the control of apoptosis and other types of RCD in both an autophagy-dependent and -independent manner, indicating the existence of a BECN1-centric network that orchestrates cell death and stress responses (Kang et al., 2011).

The ubiquitin-like conjugation systems

Two ubiquitin-like conjugation systems play key roles in the process of phagophore elongation and autophagosome formation. The first system involves the ATG12-ATG5-ATG16L1 pathway (Hanada et al., 2007). ATG12 was the first identified ubiquitin-like protein (Ubl) in autophagy and is covalently conjugated to ATG5 by the E1 enzyme ATG7 and then the E2 enzyme ATG10. The ATG12-ATG5 conjugate can further interact with ATG16L1, a homolog to yeast Atg16, to form a multimeric complex at a phagophore assembly site. Structurally, the homo-oligomers of ATG16L1 are required for the production of the ATG12-ATG5-ATG16L1 complex. The second system involves microtubule associated protein 1 light chain 3 (MAP1LC3, including MAP1LC3A, MAP1LC3B, and MAP1LC3C), the first identified protein to associate with autophagosomal structures (Kabeya et al., 2000). The turnover of MAP1LC3 between MAP1LC3-I (the cytosolic form) and MAP1LC3-II (the lipidated and membrane-associated form) is responsible for the formation of the autophagosome and the recruitment of cargo proteins destined for lysosomal degradation. This process is initiated by ATG4, the sole cysteine protease that cleaves MAP1LC3 at the C terminus to expose a glycine residue (Scherz-Shouval et al., 2007). MAP1LC3-I is further processed by the E1 enzyme ATG7 and then the E2 enzyme ATG3, finally resulting in the formation of phosphatidylethanolamine-conjugated MAP1LC3-II through the ATG12-ATG5-ATG16L1 system. As a recycling mechanism, the membrane-associated MAP1LC3-II can be cleaved by ATG4 to release free MAP1LC3-I for reuse in autophagosome formation (Nakatogawa et al., 2012). Although MAP1LC3-II is the most widely used autophagosome marker, other orthologs of yeast Atg8, such as GABA type A receptor-associated protein (GABARAP) and GABA type A receptor-associated protein like 2 (GABARAPL2/GATE-16) may play similar roles in the formation of the autophagosome in mammalian cells (Schaaf et al., 2016); the LC3 and GABARAP proteins are collectively referred to as Atg8-family proteins. Protein kinase A-mediated MAP1LC3 phosphorylation limits its autophagic activity (Cherra et al., 2010). In addition to its role in the bulk degradation process, MAP1LC3-II also interacts with cargo receptors (e.g., sequestosome 1 [SQSTM1/p62], calcium-binding and coiled-coil domain 2 [CALCOCO2/NDP52], and optineurin [OPTN]) to regulate the process of selective forms of autophagy. At the same time, MAP1LC3-II can be degraded in a lysosome-dependent manner (Mizushima and Yoshimori, 2007; Pankiv et al., 2007). Thus, autophagic flux capacity refers to the balance between the formation and degradation of autophagosomes or MAP1LC3-II during a certain time period (Yoshii and Mizushima, 2017). The measurement of autophagic flux is facilitated by the use of various inhibitors targeting the early (e.g., wortmannin, 3-methyladenine, LY294002, and spautin-1) or late (e.g., bafilomycin A₁ and chloroquine) stage of autophagy (Table S1), as denoted in specific guidelines (Klionsky et al., 2016).

The types of selective autophagy

Selective autophagy serves as an intracellular quality control mechanism to mediate the degradation of specific targets, such as invading pathogens, aggregated proteins, and damaged organelles. Mechanistically, specific autophagy receptors are used for cargo recognition and degradation, and thus better fulfill the catabolic necessities of the cell in response to various types of stress, including cell death stimuli (Gatica et al., 2018). The

autophagy receptors function by interacting simultaneously with cargoes and Atg8-family proteins. In general, the autophagy receptors interact with lipidated LC3 or GABARAP subfamily proteins by LC3-interacting region (LIR) motifs that bind to the LIR docking site (LDS) of Atg8 family proteins in a cargo-dependent manner (Johansen and Lamark, 2020). The LIR-LDS interaction is also modulated by the upstream regulators of autophagy, such as ULK1 and PIK3C3 (Turco et al., 2020). Here, we introduce different types of selective autophagy for the degradation of organelles in mammalian cells (Fig. 2) and emphasize the mechanisms of action of selective autophagy receptors in providing cargo specificity.

Mitophagy

Mitochondria are intracellular powerhouses that convert oxygen and nutrients into adenosine triphosphate (ATP), the chemical energy "currency" of the cell. These branch-like organelles, made up of an outer membrane, an intermembrane space, an inner membrane, and a matrix, are regulated by mitochondrial dynamics (including fusion and fission), biogenesis, and degradation, as well as communication with other organelles (e.g., the ER and nucleus). Mitophagy is a well-studied type of selective autophagy that segregates and degrades mitochondria through different mechanisms, depending on the context. Mutations in PINK1 (a serine-threonine protein kinase) and parkin RBR E3 ubiquitin protein ligase (PRKN/PARK2) are implicated in the development of Parkinson's disease. The PINK1-PRKN pathway plays a major role in the regulation of mitophagy through a Ub-dependent process. Oxidative injury and depolarization of mitochondria can increase PINK1 accumulation on the outer membrane, and subsequent PRKN recruitment from the cytosol to mitochondria. This dynamic change of PINK1 and PRKN leads to the assembly of phosphorylated Ub chains on proteins of the outer mitochondrial membrane, which in turn recruit multiple cargo receptors (e.g., SQSTM1 (Geisler et al., 2010), OPTN (Wong and Holzbaur, 2014), NBR1 (NBR1 autophagy cargo receptor) (Gao et al., 2015), CALCOCO2 (Lazarou et al., 2015), and Tax1 binding protein 1 (TAX1BP1) (Lazarou et al., 2015) for the degradation of mitochondria in a context-dependent manner. This process is significantly enhanced by the activation of TANK-binding kinase 1 (TBK1) because TBK1 can directly phosphorylate cargo receptors (e.g., SQSTM1, OPTN, CALCOCO2, and TAX1BP1), enhancing their binding to cargos and MAP1LC3 (Heo et al., 2015; Heo et al., 2018; Moore and Holzbaur, 2016). In contrast, de-ubiquitinating enzymes, such as ubiquitin specific peptidase 8 (USP8), USP15, USP30, and USP35, can limit PRKN-mediated mitophagy (Wang et al., 2015). In addition to PRKN, other E3 ubiquitin ligases (e.g., mitochondrial E3 ubiquitin protein ligase 1 [MUL1] (Yun et al., 2014), siah E3 ubiquitin protein ligase 1 [SIAH1] (Szargel et al., 2016), SMAD specific E3 ubiquitin protein ligase 1 [SMURF1] (Orvedahl et al., 2011), and autocrine motility factor receptor [AMFR/GP78]) may elicit mitophagy through poorly characterized mechanism (Fu et al., 2013). In addition to Ub-dependent receptors, Ub-independent receptors (e.g., BCL2 interacting protein 3 like [BNIP3L/NIX] (Sandoval et al., 2008), FUN14 domain containing 1 [FUNDC1] (Liu et al., 2012), BCL2 interacting protein 3 [BNIP3] (O'Sullivan et al., 2015), nipsnap homolog 1 [NIPSNAP1] (Princely Abudu et al., 2019), nipsnap homolog 2 [NIPSNAP2] (Princely Abudu et al., 2019), prohibitin 2 [PHB2] (Wei et al., 2017), two putative yeast Atg32 orthologs, BCL2 like 13 [BCL2L13] (Murakawa et al., 2015) and FKBP prolyl isomerase 8 [FKBP8] (Bhujabal et al., 2017)) and mitochondrial membrane phospholipids lipid (e.g.,

cardiolipin and ceramide) (Chu et al., 2013) contribute to mitophagy under different mitochondrial stresses (e.g., hypoxia or intrinsic stress signals). In addition, the inhibition of mitochondrial fission by the depletion of dynamin 1 like (DNM1L/DRP1) or fission, mitochondrial 1 (FIS1) reduces the levels of mitophagy, whereas mitophagy deficiency increases mitochondrial fragmentation (Dagda et al., 2009; Kageyama et al., 2014). Collectively, the molecular machinery involved in mitophagy is complex, and multiple cargo receptors and signals are required for the recognition of damaged mitochondria. Impaired mitophagy enhances cell death, tissue injury and inflammation in mouse models, suggesting a major homeostatic role for this type of selective autophagy (Kang et al., 2016; Li et al., 2018; Sliter et al., 2018).

Reticulophagy

The ER is a network of tubules and flattened sacs that connects to the nucleus. This organelle includes rough and smooth ER, which play a key role in the regulation of protein synthesis, folding and secretion, calcium homeostasis, and lipid biosynthesis. The ER also undergoes dynamic changes in its morphology and functional properties. ER stress results from the accumulation of misfolded proteins and causes the activation of a signaling network called the unfolded protein response (UPR). ER stress not only promotes cell survival, but also causes cell death when the UPR is overwhelmed. Autophagic structures are associated with the ER membrane. The removal of unwanted portions of the ER through autophagy, a process called reticulophagy/ER-phagy, is critical for the maintenance of ER homeostasis. The level of reticulophagy is remarkably upregulated by ER stress, starvation, MTOR inhibition, misfolded protein aggregates, or microbial infection. Up to now, seven autophagic cargo receptors, including cell cycle progression 1 (CCPG1) (Smith et al., 2018), reticulophagy regulator 1 (RETREG1/FAM134B) (Khaminets et al., 2015), reticulon 3 (RTN3) (Grumati et al., 2017), SEC62 homolog, preprotein translocation factor (SEC62) (Fumagalli et al., 2016), testis expressed 264, ER-phagy receptor (TEX264) (Chino et al., 2019), atlastin GTPase 3 (ATL3) (Chen et al., 2019), and SEC24 homolog C, COPII coat complex component (SEC24C) (Cui et al., 2019) specifically bind to the ER and autophagosomal membranes, thus contributing to reticulophagy. RETREG1 in sheets and RTN3 in tubules are essential reticulophagy receptors that promote the remodeling and scission of ER sheets through the reticulon domains (Grumati et al., 2017; Khaminets et al., 2015). SEC62 (an ER transmembrane protein) is required for the reticulophagy-mediated degradation of ER chaperones (e.g., calnexin [CANX], calreticulin [CALR], and heat shock protein family A [Hsp70] member 5 [HSPA5/BiP]), and folding enzymes (protein disulfide isomerase family A member 4 [PDIA4/ERp72] and protein disulfide isomerase family A member 3 [PDIA3/ERp57]) during cellular recovery from the UPR (Fumagalli et al., 2016). CCPG1 (an ER transmembrane protein) can deliver portions of ER carrying insoluble proteins to phagophores in pancreatic cells (Smith et al., 2018). TEX264 is an ER-resident protein responsible for nutrient starvation-induced reticulophagy and has a particular high affinity for Atg8-family proteins (Chino et al., 2019). ATL3 is a GABARAP-binding receptor (but not a MAP1LC3 receptor) and plays a major role in starvation-induced tubular ER turnover during reticulophagy (Chen et al., 2019). In contrast, SEC24C is required for the degradation of RETREG1 and RTN3 during reticulophagy (Cui et al., 2019). In-depth characterization of these reticulophagy pathways, which presumably regulate separate stress-

response pathways, will be important for the development of new approaches for the treatment of ER-related disorders, such as diabetes, nonalcoholic fatty-liver disease, and Alzheimer's disease (Smith and Wilkinson, 2017).

Pexophagy

Peroxisomes are membrane-bound organelles containing a wide variety of metabolic enzymes that regulate the redox status of specific biomolecules. Selective autophagy of peroxisomes, pexophagy, is initiated by the ubiquitination of peroxisomal biogenesis factor 3 (PEX3, a regulator of the assembly and maintenance of the peroxisomal membrane) (Yamashita et al., 2014) or PEX5 (a peroxisomal matrix protein carrying peroxisomal targeting signals) (Wang and Subramani, 2017). Unlike PEX5, which is required for peroxisome protein import, the functional contribution of PEX3 to peroxisomes remains controversial. ATM serine/threonine kinase (ATM)-mediated phosphorylation of PEX5 is required for monoubiquitination of PEX5, which in turn promotes the binding between PEX5 and SQSTM1 (Zhang et al., 2015). Either SQSTM1 (Zhang et al., 2015) or NBR1 (Deosaran et al., 2013) act as autophagic receptors in pexophagy. Other PEX members, such as PEX14 (Zutphen et al., 2008), PEX13 (Lee et al., 2017), or PEX2 (Sargent et al., 2016), may participate in pexophagy through PEX5-dependent or -independent circuitries. In particular, PEX14 can directly bind MAP1LC3 or ATG9A to trigger pexophagy (Li et al., 2017). It will be important to examine the consequences of dysfunctional pexophagy on oxidative stress-mediated human diseases.

Nucleophagy

The nucleus is the largest cellular organelle that stores genomic materials to control gene expression. The selective removal of the nucleus or nuclear components by autophagy, namely nucleophagy, was first observed in yeast under nutrient-rich conditions as well as during nitrogen deprivation. Nucleophagy also occurs in mammalian cells and can cause the degradation of nuclear lamina components upon DNA damage. Nucleophagy-associated lamin B1 (LMNB1) degradation is associated with oncogenic RAS activation, but not starvation (Dou et al., 2015). The SUMOylation of lamin A/C (LMNA) promotes molecular interactions between the lamina and MAP1LC3 during nucleophagy (Li et al., 2019). Micronuclei are nuclear bodies containing damaged chromosome fragments that appear after genomic stress or mitotic catastrophe and that are usually removed by autophagy (Rello-Varona et al., 2012). Autophagy deficiency increases the number of micronuclei, which may play a pathological role in aging, cancer, and neurodegenerative diseases (Dou et al., 2015; Papandreou and Tavernarakis, 2019). The impact of DNA sensors, such as cyclic GMP-AMP synthase (CGAS) and HMGB1, on the regulation of nucleophagy remains to be characterized.

Ribophagy

Ribophagy refers to the selective degradation of ribosomes via autophagy. MTOR plays a key role in protein synthesis and ribosome biogenesis. The inhibition of MTOR by starvation or pharmacological agents such as rapamycin or torin1 can trigger ribophagy in mammalian cells. Using an MTOR inhibition model, nuclear FMR1-interacting protein 1 (NUFIP1) was identified as a cargo receptor for ribophagy (Wyant et al., 2018). NUFIP1 and

its binding partner zinc finger HIT-type containing 3 (ZNHIT3) can bind ribosomes and promote their targeting to phagophores by interacting with MAP1LC3B, but not with other MAP1LC3 isoforms or GABARAP family members (Wyant et al., 2018). Regarding the wider role of MTOR inhibition in nonselective autophagy, it is important to identify the specific downstream signals responsible for NUFIP1 translocation from the nucleus to the cytoplasm during selective autophagy. In contrast, a recent study reported that agents that induce defects in the ribosomal quality control fails to induce robust ribophagy in HEK293 cells using a Ribo-Keima reporter system (An and Harper, 2018), indicating that there may be no specific autophagy receptor for the clearance of damaged ribosomes.

Lysophagy

Lysosomes are sac-like structures delimited by a single-layer membrane. This organelle contains a variety of hydrolases for the degradation of various kinds of macromolecules. The leakage of lysosomal constituents into the cytosol can trigger “lysosomal cell death”. A membrane-damaged lysosome itself can be a target of autophagy, and this type of selective autophagy is termed lysophagy. For example, agents causing lysosomal rupture (e.g., silica, monosodium urate, and LLOMe) can induce lysophagy in an ATG5-dependent manner. Moreover, the ubiquitination of the damaged lysosomes is a biomarker and contributor to lysophagy. The cargo receptors SQSTM1 (Hung et al., 2013) and TAX1BP1 (Koerver et al., 2019) play a context-dependent role in the recognition of damaged lysosomes during lysophagy. This process can be further regulated by galectin 8 (LGALS8) (Aits et al., 2015), galectin 3 (LGALS3) (Maejima et al., 2013), ubiquitin conjugating enzyme E2 Q family like 1 (UBE2QL1) (Koerver et al., 2019; Mizushima, 2019), F-box protein 27 (FBXO27) (Yoshida et al., 2017), and valosin containing protein (VCP/P97) (Papadopoulos and Meyer, 2017) through either ubiquitination-dependent or -independent mechanisms. Further research is needed to clarify the interplay between lysophagy and lysosomal cell death in the control of lysosome homeostasis.

Other selective autophagy pathways

In addition to degrading cellular organelles as discussed above, selective autophagy also contributes to intracellular homeostasis through eliminating invading pathogens (xenophagy for bacteria or viruses) (Sharma et al., 2018), lipid droplets (lipophagy) (Singh et al., 2009), zymogen granules (zymophagy) (Grasso et al., 2011), glycogen (glycophagy) (Reichelt et al., 2013), DNA (DNautophagy) (Fujiwara et al., 2015), RNA (RNautophagy) (Fujiwara et al., 2015), myelin (myelinophagy) (Gomez-Sanchez et al., 2015), stress granules (granulophagy) (Buchan et al., 2013), proteasomes (proteaphagy) (Marshall et al., 2015), protein aggregates (aggrephagy) (Overbye et al., 2007), and specific proteins, e.g., ferritin (ferritinophagy) (Mancias et al., 2014) and aryl hydrocarbon receptor nuclear translocator like (ARNTL/BMAL1 for clockophagy) (Yang et al., 2019a). These types of selective autophagy also require specific, and in some cases unknown, receptor-cargo interactions (Fig. 2), although the precise structural basis of this interaction and the ubiquitin substrates required for autophagic targeting remain to be further explored.

The molecular machinery of ferroptosis

Reactive oxygen species (ROS) are a key determinant of RCD when the integrated antioxidant systems are impaired. In general terms, ferroptosis is a form of ROS-dependent regulated necrosis that occurs through multiple signals and pathways. Among them, iron accumulation and lipid peroxidation seem to play a major role in the initiation of ferroptosis, although the specific downstream effector proteins (e.g., pore-forming proteins) and the precise mechanisms remain unidentified (Stockwell et al., 2017; Xie et al., 2016) (Fig. 3). Here, we summarize the key connections between ferroptosis and autophagy, including small molecules or drugs that can be used to trigger or inhibit ferroptosis (Table S1).

Iron overload and toxicity

Iron is an essential trace metal required by all living organisms. The basic process of iron metabolism in animals and humans includes iron absorption, in vivo distribution, the formation of various pools of protein-bound iron, iron storage, and iron excretion (Fig. 4). Many diseases are linked to abnormal iron metabolism, and altered iron metabolism itself can trigger a variety of diseases. The majority of iron come from nutritional or endogenous sources, the latter of which is mostly from hemoglobin in aging red blood cells. Most of the exogenous iron is directly absorbed into the bloodstream through the duodenal mucosal cells, and part of the iron is stored in ferritin in mucosal cells. Intestinal iron intake and endogenous iron re-use is balanced by iron excretion. When iron is deficient, the iron absorption rate and endogenous iron utilization rate increase, and iron storage decreases. When the iron level in food is high, iron absorption decreases while iron storage rises, thus allowing a homeostatic maintenance of physiological iron levels.

The control of cellular iron levels is a dynamic process. Iron exists in two oxidation states: ferrous (Fe^{2+} , Fe(II)) or ferric (Fe^{3+} , Fe(III)). Transferrin (TF)-bound ferric iron from serum is recognized by transferrin receptor (TFRC/TFR1), a carrier protein for TF in cell membranes. After uptake by TFRC, ferric iron is reduced to ferrous iron by STEAP3 metalloreductase (STEAP3) in the endosome and then released from the endosome to the cytoplasm by solute carrier family 11 member 2 (SLC11A2/DMT1). Ferrous iron serves numerous important functions in regulating multiple metabolic and biochemical processes, such as oxygen transport, DNA synthesis, and iron-sulfur production. Ferritin is the primary form of iron storage protein, which includes two subunits: ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1). Although the sequences of these subunits are highly homologous, FTL and FTH1 exhibit different functions in the regulation of iron storage and utilization, respectively. Ferritin degradation can occur by ferritinophagy, a type of selective autophagy (discussed below). Finally, the export of intracellular iron across the cell membrane requires the iron efflux pump SLC40A1/ferroportin-1 (solute carrier family 40 member 1), leading to the production of ferric iron from ferrous iron. Thus, iron transport across the plasma membrane determines the redox status.

Iron overload, resulting from excessive extracellular or intracellular iron, can cause cell death and tissue injury, which is involved in a number of diseases, such as neurodegeneration and cancer. In particular, intracellular iron overload can trigger ferroptotic cell death through at least two mechanisms (Fig. 3). On the one hand, iron can

generate excessive ROS via the Fenton reaction, which then increases oxidative DNA and lipid damage. On the other hand, iron can increase the activity of nonheme iron-containing enzymes, such as lipoxygenases responsible for lipid peroxidation. Indeed, increased iron uptake (e.g., due to TFRC overexpression), reduced iron storage (e.g., due to the knockdown of ferritin [FTL and FTH1] or induction of ferritinophagy), and impaired cellular iron export (e.g., resulting from the knockdown of SLC40A1/ferroportin) enhance the sensitivity to ferroptosis (Bogdan et al., 2016; Du et al., 2018; Gao et al., 2016; Geng et al., 2018; Hou et al., 2016; Yang and Stockwell, 2008). In contrast, intracellular iron chelators such as desferrioxamine block ferroptosis. The transport of iron between cytosol, mitochondria, and lysosome, as well as the activation of iron responsive element binding protein 2 (IREB2, a major regulator of iron homeostasis) also affect the onset of ferroptosis (Dixon et al., 2012). Collectively, iron is an intracellular second messenger promoting ferroptosis. It remains unknown whether, under certain conditions, iron-mediated ferroptosis may amplify or diminish the susceptibility of cells to other types of RCD such as apoptosis (Yang et al., 2017), necrosis (Yang et al., 2017), and pyroptosis (Zhou et al., 2018). Moreover, the relationship between systemic iron homeostasis (e.g., dietary iron absorption and the concentration of circulating iron) and ferroptosis is largely uncharacterized, although it is known that excessive heme can induce ferroptosis (NaveenKumar et al., 2018). Iron is essential for both the host and its pathogens, and the potential impact of gut microbiota on ferroptotic cell death in host cells remains to be clarified.

Lipid peroxidation and toxicity

Lipid peroxidation plays a major role in ferroptosis. Lipids are not only the building block of cellular membranes, but also the key players in energy storage, heat insulation, cell communication, and signal transduction. Cell membrane rupture that is associated with the breakdown of membrane lipids (phospholipids, glycolipids, and cholesterol) is the hallmark of regulated necrosis, including necroptosis, pyroptosis, alkaliptosis, and ferroptosis (Tang et al., 2019). Lipid peroxidation of polyunsaturated fatty-acid-containing phospholipids (PUFA-PLs) plays a major role in promoting ferroptotic cell death (Yang et al., 2016). This process is driven by the activation of specific enzymes, including acyl-CoA synthetase long chain family member 4 (ACSL4) (Doll et al., 2017; Kagan et al., 2017; Yuan et al., 2016b) and lipoxygenase (arachidonate lipoxygenase [ALOX]) enzymes (Yang et al., 2016). ACSL4 is required for the production of PUFA-PLs, whereas lipoxygenases oxidize PUFA-PLs to the corresponding hydroperoxy derivatives, namely lipid peroxides or lipid oxidation products. The mammalian ALOX family, consisting of six members (ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15, and ALOX15B), plays a tissue- or cell-dependent role in the induction of ferroptosis (Hinman et al., 2018; Liu et al., 2015; Shintoku et al., 2017; Wenzel et al., 2017). ACSL4 is not only a contributor to ferroptosis, but also may be a biomarker for it (Yuan et al., 2016b). Increased ACSL4 expression, but not that of other members of the ACSL family, has been observed in ferroptotic cancer cell death (Yuan et al., 2016b). In addition, the expression of lysophosphatidylcholine acyltransferase 3 (LPCAT3), a downstream effector of ACSL4, is upregulated during ferroptosis (Dixon et al., 2015); however, there is no direct evidence showing that the inhibition of LPCAT3 blocks ferroptosis.

This classification of the ferroptosis inducers has provided a useful framework for studying and understanding ferroptosis. Classical ferroptosis inducers inhibit either of the two antioxidant systems during lipid peroxidation. One is the antiporter SLC7A11/system x_c^- (solute carrier family 7 member 11), which imports cysteine (the oxidized form) into cells with a 1:1 counter-transport of glutamate (the most abundant neurotransmitter, an excess of which can cause neurotoxicity and oxidative injury) and then induces the production of glutathione (GSH), a major free radical scavenger. A number of small molecules or drugs such as erastin, sulfasalazine, and sorafenib induce ferroptosis by the inhibition of SLC7A11/system x_c^- (Dixon et al., 2012). Erastin, as the first-used ferroptosis inducer, has other direct binding targets, such as voltage dependent anion channel 2 (VDAC2) and voltage dependent anion channel 3 (VDAC3), which also contribute to ferroptosis (Yagoda et al., 2007). The second antioxidant system is the selenium-containing glutathione peroxidase 4 (GPX4), a phospholipid hydroperoxidase that can directly reduce phospholipid hydroperoxide production. The antioxidant activity of GPX4 requires GSH, the pharmacological inhibition of GPX4 by RSL3 or FIN56, causes ferroptotic cell death (Yang et al., 2014). Notably, the conditional depletion of GPX4 in tissues or cells can cause oxidative injury or cell death in both ferroptosis-dependent and -independent manners (Canli et al., 2016; Cole-Ezea et al., 2012; Friedmann Angeli et al., 2014; Kang et al., 2018a; Matsushita et al., 2015; Ran et al., 2006; Ran et al., 2004; Ran et al., 2003; Seiler et al., 2008), indicating broad roles for GPX4 in different RCD scenarios. In contrast, antioxidants, including N-acetyl cysteine and vitamin E, can protect against ferroptosis caused by the classical ferroptosis inducers (e.g., erastin or RSL3) or GPX4 depletion in vivo and in vitro. In addition to GPX4, apoptosis inducing factor mitochondria associated 2 (AIFM2) (Bersuker et al., 2019; Doll et al., 2019) or endosomal sorting complexes required for transport (ESCRT)-III (Dai et al., 2020b; Dai et al., 2020c) also limits membrane damage through increased CoQ10 production or plasma membrane repair during ferroptosis.

Other components of an integrated stress response, such as transcription factors (e.g., nuclear factor, erythroid 2 like 2 [NFE2L2/NRF2] (Chen et al., 2017; Shin et al., 2018; Sun et al., 2016; Wu et al., 2011) and tumor protein p53 [TP53] (Gnanapradeepan et al., 2018; Jiang et al., 2015; Kang et al., 2019; Xie et al., 2017)), heat shock response (Sun et al., 2015), the UPR (Sauzay et al., 2018; Zhu et al., 2017), cell adhesion (Wu et al., 2019a; Yang et al., 2019b), and autophagy (discussed below) also play a context-dependent role in the control of oxidative injury-mediated ferroptosis.

Role of autophagy in ferroptosis

Increased autophagy flux is observed in various cells in response to classical ferroptosis activators, such as erastin and RSL3. While appropriate autophagy has likely evolved as a pro-survival response, excessive autophagy, especially selective autophagy, and impaired lysosomal activity may promote ferroptotic cell death. Here, we outline the key types of selective autophagy or regulators of autophagic machinery in driving ferroptosis (Fig. 5).

NCOA4-dependent ferritinophagy

Ferritinophagy is the process of autophagic degradation of the iron-storage protein ferritin, which is critical for the regulation of cellular iron levels. Ferritin is composed of 24 subunits of FTH1 and FTL, and can store up to 4500 atoms of iron. The combination of autophagosome isolation and quantitative proteomics has identified nuclear receptor coactivator 4 (NCOA4) as a cargo receptor responsible for autophagy-dependent ferritin degradation, likely because the C terminus of NCOA4 binds a conserved surface arginine (R23) on FTH1 in phagophores, and subsequently in autophagosomes and autolysosomes (Mancias et al., 2014). NCOA4-dependent ferritinophagy promotes ferroptosis through releasing free iron from ferritin. The depletion or inhibition of NCOA4 or ATG protein (e.g., ATG3, ATG5, ATG7, and ATG13) inhibits ferritin degradation and therefore reduce free iron levels and thus limit subsequent oxidative injury during ferroptosis (Gao et al., 2016; Hou et al., 2016). As a feedback mechanism, the level of intracellular NCOA4 is regulated by cellular iron (Mancias et al., 2014). When cellular iron levels are high, NCOA4 can be degraded subsequent to its ubiquitination by HECT and RLD domain containing E3 ubiquitin protein ligase 2 (HERC2, a ubiquitin E3 ligase) through the ubiquitin-proteasome pathway (Mancias et al., 2015), suggesting a negative correlation of HERC2 with ferroptosis induction. Moreover, deficient ferritinophagy may increase the activity of iron responsive element binding protein 2 (IREB2/IRP2), a central posttranscriptional regulator of iron metabolism, and subsequently upregulate TF as a feedback mechanism (Dixon et al., 2012). Mitochondrial ferritin and iron uptake also limit or amplify the iron toxicity and ferroptosis (Wang et al., 2016; Yuan et al., 2016a). Other than NCOA4, it remains unknown whether additional mitochondrial factors are required for the selective autophagic degradation of ferritin in mitochondria. Ferritinophagy-mediated ferroptosis is implicated in hepatic fibrosis and neurodegenerative diseases (Kong et al., 2019; Zhang et al., 2018) and can be used to kill cancer under specific circumstances (Du et al., 2018; Lin et al., 2016). A further understanding of different pathways leading to ferritin degradation, including autophagy-independent lysosomal degradation of ferritin (Goodwin et al., 2017), and their contribution to intracellular and systemic iron homeostasis will be important for the therapeutic manipulation of ferroptosis.

RAB7A-dependent lipophagy

Free fatty acids can be esterified into triglycerides and cholesterol esters in cells to reduce their toxicity. This anabolic reaction occurs in the ER, causing the deposition of neutral lipids in a lenticular microdomain between the bilayer membranes of the ER, ultimately leading the outer leaves of the layer to expand to form a unique spherical organelle, the lipid droplet (Liu and Czaja, 2013). Lipid droplets are complex organelles that were previously thought to be inert sites for fat fixation and are now known to have a unique, dynamic proteome. The lipid droplet proteome fluctuates with changes in cellular metabolic status, thus influencing the composition of neutral lipids in organelles. Lipophagy, the autophagic digestion of lipid droplets can release free fatty acids, which then serve as a fuel for mitochondrial beta-type oxidation. The process by which intracellular lipid droplets are selectively transported by autophagosomes for lysosomal decomposition, lipophagy, provides another potential pathway for regulating cellular lipid levels, and therefore the propensity to cell death. The level of lipid droplets is negatively related to oxidative stress-

induced ferroptosis (Bai et al., 2018). Increased lipid droplet formation by the upregulation of tumor protein D52 (TPD52) suppresses RSL3-induced ferroptosis in hepatocytes (Bai et al., 2018). The main function of RAB7A (a member of the RAS oncogene family) is to mediate the recruitment of lipid droplets by multivesicular bodies and lysosomes during lipid phagocytosis. In contrast, increased RAB7A-dependent lipophagy promotes lipid droplet degradation and therefore increases lipid peroxidation-mediated ferroptosis (Bai et al., 2018). It remains unknown whether other regulators of lipophagy, such as glycine N-methyltransferase (GNMT), transcription factor EB (TFEB), and forkhead box O1 (FOXO1), contribute to lipophagy-mediated ferroptosis in diseases caused by fat accumulation.

SQSTM1-dependent clockophagy

Circadian clocks are endogenous, entrainable rhythmic oscillations with a periodicity of approximately 24 hours that play a fundamental role in internal cycles of behavior, physiology, and metabolism. The core oscillation mechanism is a transcriptional-translational negative feedback loop, in which ARNTL and clock circadian regulator (CLOCK) are core nuclear factors driving circadian clocks through regulating the expression of a number of genes via the binding of E-box motifs in their promoters. Clockophagy is a recently identified type of selective autophagy for the degradation of ARNTL during ferroptosis induced by type-2 ferroptosis inducers (e.g., RSL3 and FIN56), but not type-1 ferroptosis inducers (e.g., erastin, sulfasalazine, and sorafenib) (Liu et al., 2019; Yang et al., 2019a). Mass spectrometric mechanistic investigation of ARNTL-binding protein indicates that the cargo receptor SQSTM1 (but not NBR1, OPTN, CALCOCO2, or NCOA4) makes a major contribution to autophagic ARNTL degradation (Yang et al., 2019a). Although clockophagy requires several ATG proteins, such as ATG5 and ATG7, it is mechanistically distinct from classical autophagy, in thus far that clockophagy does not appear to require ATG9A (Yang et al., 2019a). Autophagic ARNTL degradation promotes ferroptotic cell death through blocking hypoxia inducible factor 1 subunit alpha (HIF1A)-dependent fatty acid uptake and lipid storage. HIF1A, one of the major transcription factors regulating hypoxic response, plays a pro-survival role in ferroptosis through the upregulation of fatty acid binding protein 3 (FABP3, muscle and heart) and fatty acid binding protein 7 (FABP7, brain) for lipid storage (Yang et al., 2019a). The level of HIF1A is further regulated by egl-9 family hypoxia inducible factor 2 (EGLN2/PHD1), an ARNTL target gene in RSL3-induced ferroptosis (Yang et al., 2019a). In addition to HIF1A, endothelial PAS domain protein 1 (EPAS1/HIF2A) regulates ferroptosis in kidney cancer (Zou et al., 2019). It also remains unclear how the disruption of circadian rhythms might affect ferroptosis sensitivity.

Chaperone-mediated autophagy

Chaperone-mediated autophagy (CMA) is a type of selective autophagy that uses molecular chaperones to deliver certain cytosolic proteins to lysosomes for degradation based on the recognition of specific amino acid sequences. Heat shock protein family A (Hsp70) member 8 (HSPA8/HSC70), the constitutively expressed member of the Hsp70 family, is a major molecular chaperone responsible for the recognition of cytosolic proteins that contain a KFERQ-like motif within the sequence of a protein. GPX4 protein degradation by CMA seems to be a universal event in response to various ferroptosis activators (Muller et al.,

2017; Shimada et al., 2016; Zhu et al., 2017). HSPA5, an ER stress-associated molecular chaperone, can limit erastin-induced GPX4 degradation and therefore protects against ferroptotic cell death in pancreatic cancer cells (Zhu et al., 2017). HSPA8-dependent CMA also contributes to erastin-induced GPX4 degradation in breast cancer cells (Wu et al., 2019b). Moreover, HSP90 can increase the protein stability of lysosomal associated membrane protein 2A (LAMP2A), a CMA receptor, to enhance GPX4 degradation during ferroptosis (Wu et al., 2019b). These findings establish a model of interaction between CMA and autophagy to determine GPX4 protein stability in ferroptosis. However, the structural basis of GPX4 degradation remains poorly understood.

BECN1-mediated oxidative injury

BECN1 was originally identified as a BCL2 (an anti-apoptotic protein)-binding protein through yeast two-hybrid assays (Liang et al., 1998). Later, BECN1 was reported as the mammalian homolog of yeast Vps30/Atg6, and is involved in autophagy induction and tumor suppression (Liang et al., 1999). Autophagy-independent functions of BECN1 were also demonstrated. The multifunctional roles of BECN1 in cell survival and death appear to rely on its binding partners. In the case of ferroptosis, BECN1 was identified as a novel SLC7A11/system x_c^- -binding protein to inhibit transport activity in cancer cells in response to type 1 ferroptosis inducers (e.g., erastin, sulfasalazine, and sorafenib) (Kang et al., 2018b; Song et al., 2018) (Fig. 6A). This process is controlled by AMPK-mediated BECN1 phosphorylation at S90 and S93. Thus, the mutation of these BECN1 phosphorylation sites or inhibition of AMPK activity limits the formation of a BECN1-SLC7A11 / system x_c^- complex and blocks ferroptosis (Song et al., 2018). In contrast, the administration of the BECN1 activator peptide Tatbeclin 1 enhances the anticancer activity of the SLC7A11 / system x_c^- inhibitor through the induction of lipid peroxidation-dependent ferroptosis (Song et al., 2018). In addition to protein-protein interactions, BECN1-mediated ferroptosis is regulated by *BECN1* mRNA stability. Consistent with this notion, the RNA-binding protein ELAV like RNA binding protein 1 (ELAVL1) can increase *BECN1* mRNA stability to trigger subsequent ferritinophagy in hepatic stellate cells (Zhang et al., 2018) (Fig. 6B). These findings indicate that BECN1 regulates ferroptosis through both autophagy-dependent and -independent mechanisms. *becn1* knockout mice exhibit late-onset lung carcinomas, hepatocellular carcinomas, and lymphomas (Liang et al., 1999), and hence may constitute a convenient model for studying the impact of autophagy-dependent ferroptosis on tumorigenesis.

HMGB1-mediated danger signals

HMGB1 plays a dual role in the regulation of stress responses. Most intracellular HMGB1 localizes in the nucleus and functions as a DNA chaperone to regulate gene transcription, replication, and recombination. Moreover, HMGB1 is a damage-associated molecular pattern (DAMP) and can be secreted by immune cells or passively released from dead or dying cells. Extracellular HMGB1 acts as a danger signal to trigger immune responses during various types of cell death, including ferroptosis (Wen et al., 2019). Various ferroptosis activators (e.g., erastin, sorafenib, RSL3, and FIN56) can induce HMGB1 release, and this process is inhibited by pharmacological (using ferrostatin-1, liproxstatin-1, baicalein, bafilomycin A₁, or chloroquine) or genetic (using *Acs14* shRNA or *atg5*^{-/-} or

atg7^{-/-} cells) inhibition of autophagy or ferroptosis (Wen et al., 2019). Moreover, HMGB1 release mediates ferroptotic cell death-induced inflammatory responses in macrophages through the activation of advanced glycosylation end-product specific receptor (AGER/RAGE), but not toll like receptor 4 (TLR4) (Wen et al., 2019) (Fig. 7A). HMGB1 also plays a location-dependent role in promoting autophagy. Cytosolic HMGB1 is a BECN1-binding protein for the induction of autophagosome formation (Tang et al., 2010b). Nuclear HMGB1 regulates heat shock protein family B (small) member 1 (HSPB1) expression for actin filament assembly that is involved in the regulation of autophagy membrane dynamics (Tang et al., 2011). Extracellular HMGB1 induces autophagy through the activation of the PIK3C3 complex (Tang et al., 2010a). Interestingly, intracellular HMGB1 positively regulates erastin-induced ferroptosis in leukemia cells through the upregulation of TFRC expression downstream of the activation of mitogen-activated protein kinase (MAPK, e.g., MAPK/JNK and MAPK/p38) (Ye et al., 2019) (Fig. 7B). The broader interconnectivity of HMGB1 in autophagy, ferroptosis, and immune response requires further elucidation.

Conclusions and perspectives

In the past 5 years, studies of ferroptosis have been vastly expanded and the knowledge of the mechanism for ferroptosis has been ever increasing, revealing a surprising degree of complexity. Ferroptosis was originally recognized as a novel cell death modality that is morphologically, biochemically, and genetically distinct from other forms of cell death including apoptosis, necrosis, and autophagy. However, recent studies indicate that ferroptosis may occur while sharing common signals or regulators with other types of RCD such as apoptosis (Hong et al., 2017; Huang et al., 2018), necroptosis (Muller et al., 2017), autophagy (Gao et al., 2016; Hou et al., 2016), and lysosomal cell death (Gao et al., 2018; Torii et al., 2016). Indeed, mixed types of cell death appear to be more prevalent in human disease than “pure” types, although one type of cell death may dominate over others at a particular stage. In this review, we highlighted the regulation of autophagy-dependent ferroptosis and the involvement of a diverse array of molecular factors in this process. While certain types of selective autophagy (e.g., ferritinophagy, lipophagy, clockophagy, and CMA) play a significant role in promoting ferroptosis, the molecular, structural, and metabolic basis of autophagy-dependent ferroptosis still remains largely unknown. Although mitochondrial dysfunction and ER stress are related to ferroptosis in some cases (Dixon et al., 2014; Gao et al., 2019; Lee et al., 2018; Zhu et al., 2017), the functional role of mitophagy, reticulophagy, or the associated autophagy receptor in ferroptosis remains poorly understood. It is also a challenge to define the threshold or checkpoints associated with pro-survival and pro-death autophagy in ferroptosis. More recently, autophagy-dependent ferroptosis has been related to pancreatic tumor growth and progression through the released KRAS protein-mediated tumor-associated macrophage polarization (Dai et al., 2020a), indicating a dual role of ferroptosis in tumorigenesis and cancer therapy. More connections between autophagy-dependent ferroptosis and human disease will likely be demonstrated in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

Autophagy is a lysosome-dependent cellular degradation mechanism.

Ferroptosis is an iron-dependent form of oxidative cell death.

Ferroptosis requires the autophagy machinery for its induction and execution.

Autophagy-dependent ferroptosis is implicated in diseases and pathological conditions.

Significance

Autophagy plays a fundamental role in controlling of cellular homeostasis, thus determining the cellular fate. In many cases, autophagy is presumably activated by dying cells as a part of the programmed cell survival mechanism in response to stress. However, impaired and excessive autophagy promotes cell death, namely autophagy-dependent cell death. Of note, ferroptosis has been recognized as a form of autophagy-dependent cell death in some conditions. The ties between autophagy and ferroptosis could potentially allude to a complex interplay between metabolism dysfunction and oxidative stress. As a result, it is important to monitor autophagic activity or flux during ferroptosis. There are multiple compounds or drugs that inhibit the different types of autophagy-dependent cell death. A deeper understanding of the process and function of autophagy-dependent ferroptosis is critical for creating innovative therapeutic strategies for oxidative stress-related diseases, including neurodegenerative disorders and cancer.

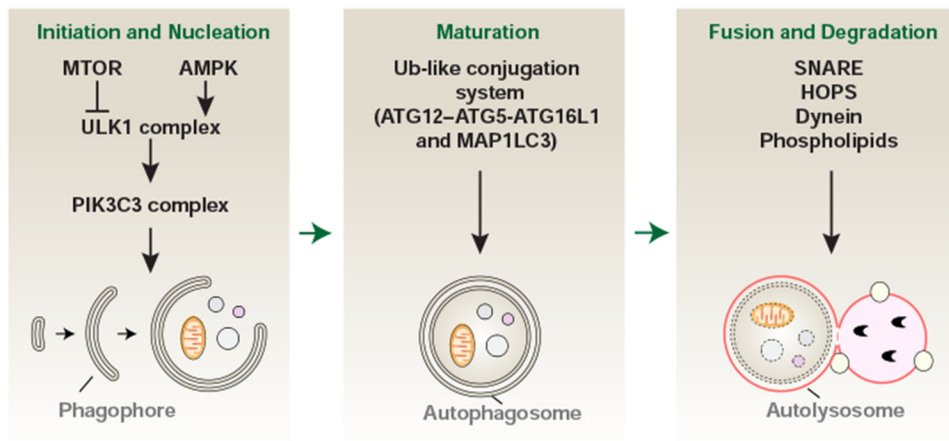


Figure 1. The process of autophagy.

Autophagy is an intracellular degradation process that occurs with the formation and maturation of specific membrane structures, including phagophores, autophagosomes, and autolysosomes, which is controlled by ATG and other proteins. See also Table S1.

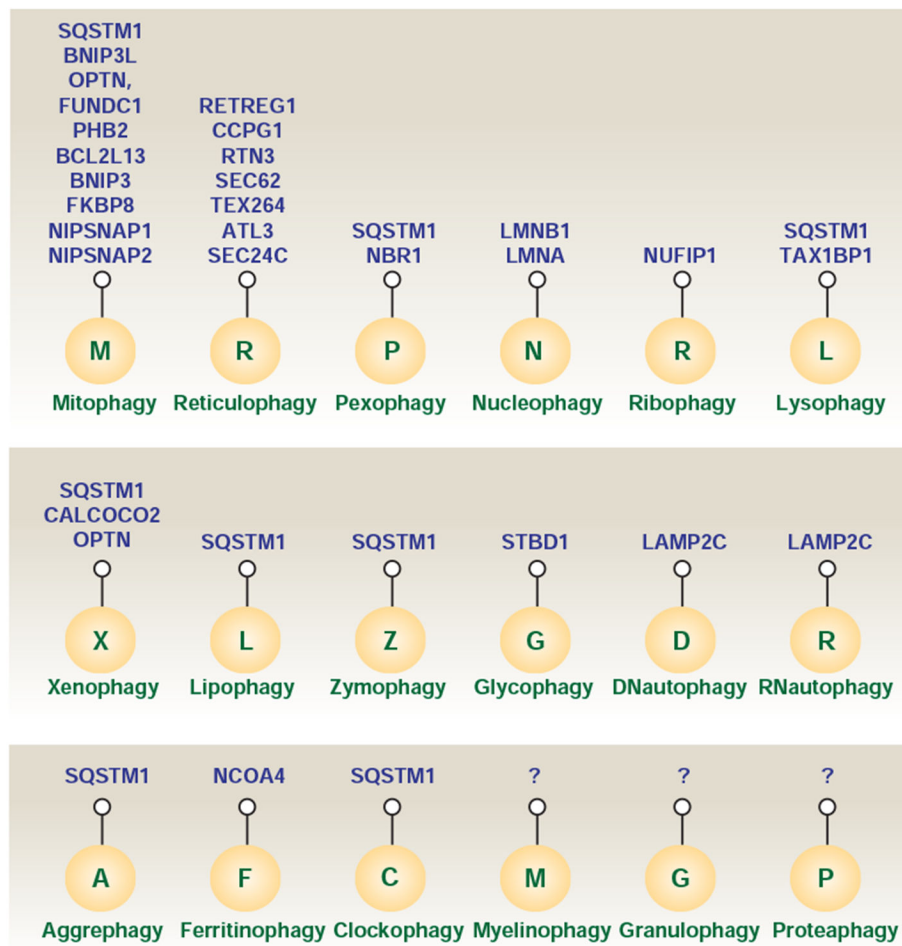


Figure 2. Selective autophagy receptors in mammalian cells.

In addition to bulk degradation in a non-elective manner, selective autophagy serves as an intracellular quality control mechanism to mediate the degradation of specific targets, such as invading pathogens, aggregated proteins, and damaged organelles through specific receptors. Different forms of selective autophagy are usually named by combining a prefix derived from the cargo and the suffix “phagy.” Note the large number of receptors found in the most-studied forms of selective autophagy (mitophagy and reticulophagy). It is unclear whether this correlates with their relative importance as homeostatic mechanisms or whether this reflects more extensive and long-lasting research of these phenomena.

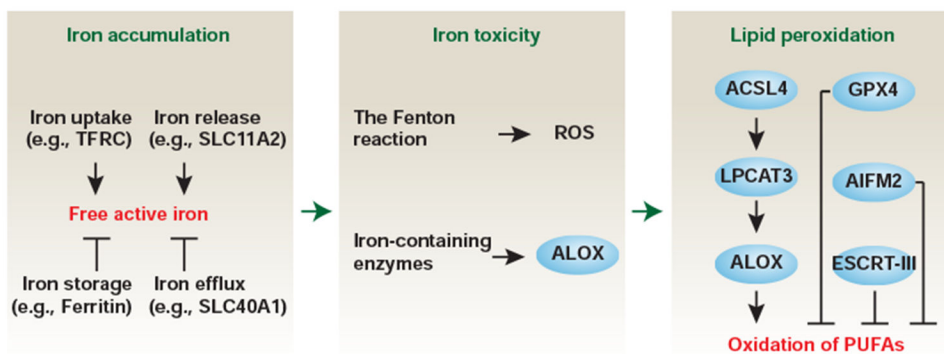


Figure 3. The process of ferroptosis.

The ferroptotic response is characterized by iron accumulation and lipid peroxidation, resulting in the formation of lipid peroxides. Increased iron not only triggers the Fenton reaction to produce ROS, but also increases the activity of iron-containing enzymes (e.g., ALOX), which contributes to lipid peroxidation. The activation of the GPX4, AIFM2, or ESCRT-III pathways can limit or repair the oxidation of PUFAs that result from the activation of the ACSL4-LPCAT3-ALOX pathway. See also Table S1.

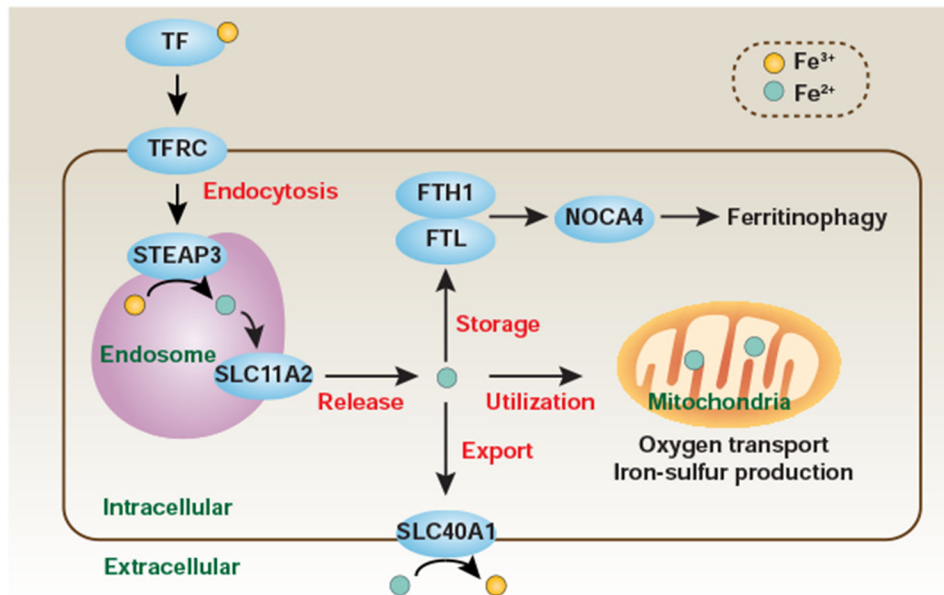


Figure 4. Role of cellular iron metabolism in ferroptosis.

Most cells acquire plasma Fe^{3+} via TFRC-mediated endocytosis of TF-bound iron. In the endosome, Fe^{3+} is reduced to Fe^{2+} by STEAP3 and then released from the endosome to the cytoplasm by SLC11A2. The labile Fe^{2+} can be stored by ferritin, including FTH1 and FTL. Fe^{2+} plays an important function in regulating multiple processes, such as oxygen transport and iron-sulfur production in mitochondria. The export of intracellular iron across the cell membrane requires the iron efflux pump SLC40A1, leading to the production of Fe^{3+} from Fe^{2+} . In general, increased iron uptake (e.g., due to TFRC overexpression), reduced iron storage (e.g., due to the knockdown of ferritin or induction of ferritinophagy), and impaired cellular iron export (e.g., resulting from the knockdown of SLC40A1) enhance the sensitivity to ferroptosis.

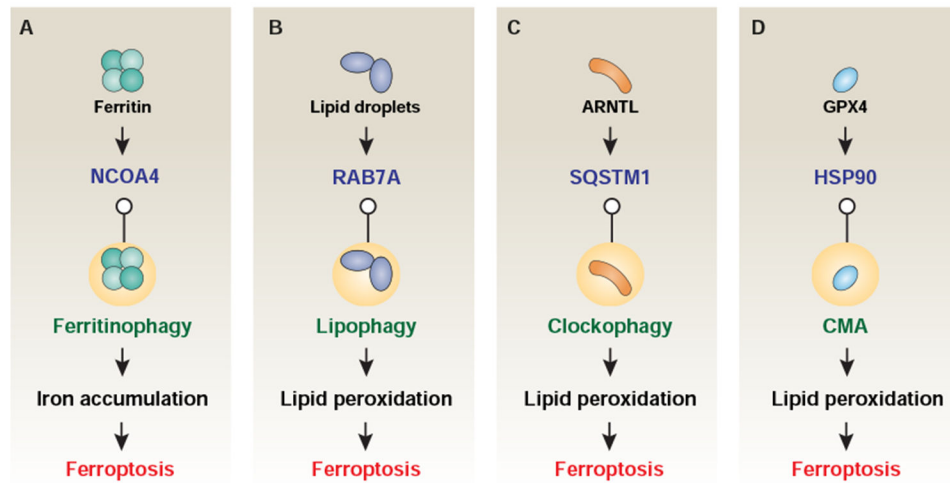


Figure 5. Role of selective autophagy in ferroptosis.

(A) NCOA4-mediated ferritinophagy promotes iron accumulation in ferroptosis. (B) RAB7A-mediated lipophagy, and (C) SQSTM1-mediated clockophagy promote lipid peroxidation in ferroptosis, and (D) HSP90-mediated chaperone-mediated autophagy (CMA) promote lipid peroxidation in ferroptosis.

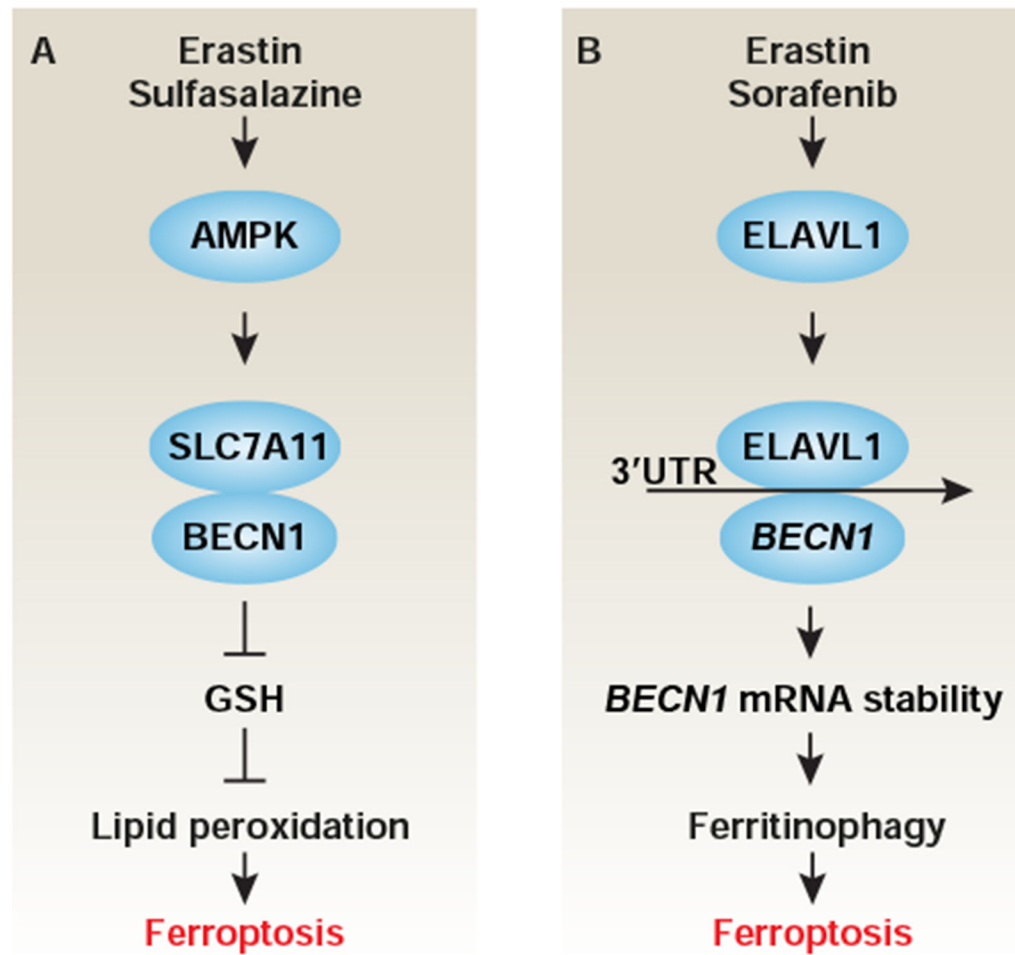


Figure 6. Role of BECN1 in ferroptosis.

(A) AMPK-mediated BECN1 phosphorylation at S90 and S93 is required for the formation of a BECN1-SLC7A11/system x_c^- complex, which leads to lipid peroxidation-dependent ferroptosis via the inhibition of SLC7A11/system x_c^- activity. (B) ELAVL1-mediated *BECN1* mRNA stability increases autophagosome formation and subsequent ferritinophagy-mediated ferroptosis.

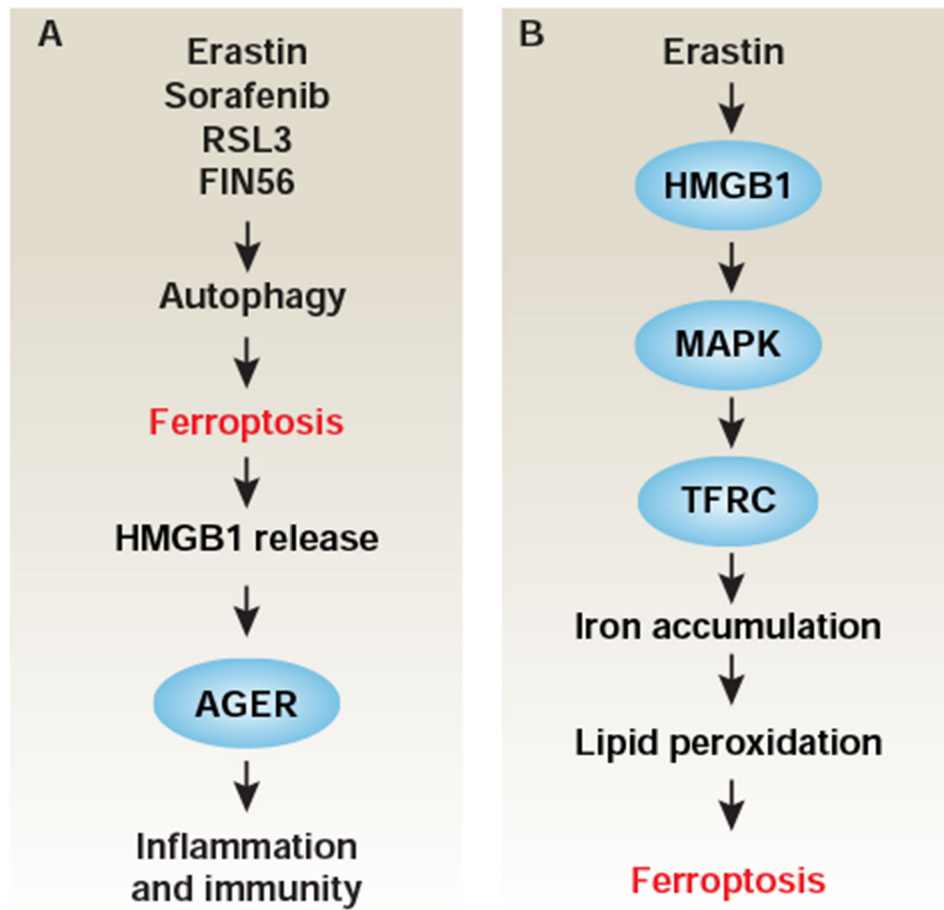


Figure 7. Role of HMGB1 in ferroptosis.

(A) Autophagy-dependent ferroptotic cell death promotes HMGB1 release, which favors inflammation and immunity response through binding its receptor AGER. (B) HMGB1 promotes ferroptosis through upregulation of MAPK-dependent TFRC expression.