Selective protein degradation through chaperone-mediated autophagy: Implications for cellular homeostasis and disease (Review)

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Abstract. Cells rely on autophagy for the degradation and recycling of damaged proteins and organelles. Chaperone-mediated autophagy (CMA) is a selective process targeting proteins for degradation through the coordinated function of molecular chaperones and the lysosome-associated membrane protein-2A receptor (LAMP2A), pivotal in various cellular processes from signal transduction to the modulation of cellular responses under stress. In the present review, the intricate regulatory mechanisms of CMA were elucidated through multiple signaling pathways such as retinoic acid receptor (RAR)α, AMP-activated protein kinase (AMPK), p38-TEEB-NLRP3, calcium signaling-NFAT and PI3K/AKT,

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Abbreviations: CMA, chaperone-mediated autophagy; VTC, vacuolar transporter chaperone; ATG5, autophagy-related 5; ULK1, Unc-51 like autophagy activating kinase; PAS, phagophore assembly site; FIP200, FAK family kinase-interacting protein of 200 kDa; mTOR, mammalian target of rapamycin; ER, endoplasmic reticulum; LAMP2A, lysosome-associated membrane protein 2A; HSC70, heat shock cognate 70; HSP70, heat shock protein 70; cyt-HSC70, cytoplasmic HSC70; PKM2, pyruvate kinase M2; PLIN2, perilipin 2; Htt, huntingtin; RNA, ribonucleic acid; ROS, reactive oxygen species; Nrf2, nuclear factor, erythroid 2; GFAP, glial fibrillary acidic protein; Akt, protein kinase B; IKK, IκB kinase; ATRA, all-trans retinoic acid; AMPK, AMP-activated protein kinase; N-CoR, Nuclear receptor co-repressor; SIRT3, sirtuin 3; MAPK, mitogen-activated protein kinase; NLRP3, NLR family pyrin domain containing 3; TFEB, transcription factor EB, TCR, T cell receptor; NFAT, nuclear factor of activated T cells; PI3K/Akt, phosphoinositide 3-kinases/protein kinase B; MYC, MYC proto-oncogene, BHLH transcription factor; RND3, Rho family GTPase 3; CDKS, cyclin-dependent kinases; EF2, elongation factor 2; CHK1, checkpoint kinase 1; HIF-1α, hypoxia-inducible factor 1 alpha; IFITM3, interferon-induced transmembrane protein 3; C11orf54, chromosome 11 open reading frame 54; TGFβ, transforming growth factor beta; MEF2A, myocyte enhancer factor 2A; GPX4, glutathione peroxidase 4; CDDO, 2-cyano-3,12-dioxooleana-1,9-dien-oic acid; MDA, malondialdehyde; CKB, creatine kinase B; Sb, antimony; ACSL4, acyl-CoA synthetase long-chain family member 4; BCL-2, B-cell lymphoma 2; BBC3, BCL2 binding component 3; TNF, tumor necrosis factor; TCA, tricarboxylic acid cycle; OCT4, octamer-binding transcription factor 4; aKG, alpha-ketoglutarate; IDH1, isocitrate dehydrogenase 1; PARK7/DJ-1, Parkinsonism associated deglycase; MPP, 1-methyl-4-phenylpyridinium; HCC, hepatocellular carcinoma; GB, glioblastoma; PCs, peritumoral cells; EMT, epithelial-mesenchymal transition; p300/CBP, p300/CREB-binding protein; MCL1, myeloid cell leukemia 1; TPD52, tumor protein D52; P73, tumor protein p73; NGFR, nerve growth factor receptor; PRDX1/CRTC1, peroxiredoxin 1/CREB regulated transcription coactivator 1; NSCLC, non-small cell lung cancer; YAP1, yes-associated protein 1; LRRK2, leucine-rich repeat kinase 2; AD, Alzheimer's disease; Aβ, amyloid beta; RCAN1, regulator of calcineurin 1; PD, Parkinson's disease; MEF2D, myocyte enhancer factor 2D; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; TDP-43, TAR DNA-binding protein 43; CVDs, cardiovascular diseases; NO, nitric oxide; VSMCs, vascular smooth muscle cells; IL-1β, interleukin 1 beta; eNOS, endothelial NO synthase; HCV, hepatitis C virus; FFAs, free fatty acids; IFNAR1, interferon alpha and beta receptor subunit 1; IFN, interferon; HNF-1a, hepatocyte nuclear factor 1 alpha; GLUT2, glucose transporter type 2; SNX-10, sorting nexin 10; Pax2, paired box 2; LSD, lysosomal storage disorders; CTNS, cystinosin, lysosomal cystine transporter; COPD, chronic obstructive pulmonary disease; UPR, unfolded protein response; CSE, cigarette smoke extract; DHM, dihydromyricetin; Sal B, salvinorin B; LMP, lysosomal membrane permeabilization

Key words: chaperone-mediated autophagy, cellular homeostasis, LAMP2A, signaling pathways, disease

thereby expanding the current understanding of CMA regulation. A comprehensive exploration of CMA's versatile roles in cellular physiology were further provided, including its involvement in maintaining protein homeostasis, regulating ferroptosis, modulating metabolic diversity and influencing cell cycle and proliferation. Additionally, the impact of CMA on disease progression and therapeutic outcomes were highlighted, encompassing neurodegenerative disorders, cancer and various organ-specific diseases. Therapeutic strategies targeting CMA, such as drug development and gene therapy were also proposed, providing valuable directions for future clinical research. By integrating recent research findings, the present review aimed to enhance the current understanding of cellular homeostasis processes and emphasize the potential of targeting CMA in therapeutic strategies for diseases marked by CMA dysfunction.

Contents

1. Introduction

Autophagy was first conceptualized in the 1960s, with a significant contribution in 1963 when Christian de Duve's team observed the association of lysosomes, which are integral to cellular component degradation, with autophagosomes, leading to the coining of the term 'autophagy' (1). This process, taking place in eukaryotic cells, involves lysosomes-regulated by autophagy-associated genes-degrading cytoplasmic proteins, damaged organelles and intracellular pathogens. Autophagy is induced in response to a spectrum of cellular stresses and is pivotal in maintaining tissue homeostasis. This process involves two principal intermediary systems, namely the proteasome and the lysosome. The proteasome, situated in the cytoplasm and nucleus, degrades damaged or misfolded proteins into smaller peptides (2), thus regulating essential cellular processes such as the cell cycle and DNA repair. In cells, lysosomes degrade not only extracellular substances and membrane proteins, but also intracellular components, including damaged organelles, misfolded proteins and various forms of cellular debris, which are also targeted for lysosomal degradation. This degradation occurs through multiple processes, including macroautophagy (enclosing large cellular components in autophagosomes for lysosomal delivery), microautophagy (engulfing and digesting segments of their own cytoplasm) and chaperone-mediated autophagy (CMA) (direct translocation of specific proteins to lysosomes) (3,4). Lysosomal permeases and transport proteins release amino acids and other degradation by-products into the cytoplasm, facilitating their reuse for macromolecule synthesis and metabolism. Consequently, lysosomes play a pivotal role in degrading both extracellular and intracellular materials, which is essential for maintaining cellular homeostasis. This dual function underscores the importance of lysosomes in cellular recycling processes and their critical contribution to the overall metabolic efficiency of the cell.

Autophagy, a sophisticated self-degradative process, involves phagophore formation, interactions among autophagy-related genes such as autophagy-related 5 (ATG5)-ATG12 and ATG16-like (ATG16L), and is regulated by a variety of signaling pathways. Additionally, autophagy increases energy efficiency through ATP production, manages damage control by eliminating non-functional proteins and organelles, and regulates cellular responses to stress or changes in the extracellular microenvironment (5,6). Autophagy is classified into three types: Macroautophagy, microautophagy and CMA. This classification underscores the diverse mechanisms and functions of autophagy in cellular maintenance and response to environmental cues (Fig. 1).

Macroautophagy. Macroautophagy, extensively studied among autophagy forms, is differentiated from microautophagy by forming a double-membraned vesicle structure. Cytoplasmic components, organelles and various elements are engulfed by a phagophore that then matures into an autophagosome. The outer membrane of the autophagosome fuses with a lysosome, forming an autolysosome that degrades the enclosed cytoplasmic contents (6,7). This biogenesis begins with the activation of the Unc-51 like autophagy activating kinase 1 (ULK1) complex, which identifies the membranes where biogenesis occurs (8). The formation process is orchestrated by ATG proteins, numerous of which were identified in yeast through genetic screenings of autophagy-deficient mutants (9,10). The ATG1 kinase complex, consisting of ATG7, ATG31, ATG29 and ATG13, is essential for this formation (11,12). The majority of ATG proteins are recruited to the phagophore assembly site (PAS), where they form a pre-autophagosomal structure (13). Early in the process, the ULK complex, comprising ULK1 (yeast ATG1 homologue), FAK family kinase-interacting protein of 200 kDa (FIP200; also known as RB1CC1), ATG13 and ATG101, forms a punctate structure near the endoplasmic reticulum (ER) membrane, acting as a scaffold for autophagosome formation (14). In yeast, compartments featuring ATG9 protein clusters, composed of vesicles and tubules, are instrumental in PAS formation (15). In mammals, under conditions of starvation, the ULK1 complex, a pivotal initiator of autophagy, targets and associates with the autophagy isolation membrane. Notably, the induction of autophagy is compromised in the absence of the FIP200 protein, underscoring its critical role in this process (16). Mammalian target of rapamycin (mTOR) serves as a primary negative regulator of autophagy, directly influencing the ULK1-ATG13-FIP200 complex in response to nutrient availability (17). The membrane at the PAS either expands directly or engulfs cytoplasmic components through vesicular expansion. In both yeast and mammals, the ubiquitin-like protein system, comprising ATG8 and ATG12, facilitates phagophore expansion (18).

Macroautophagy is instrumental in maintaining cellular homeostasis by selectively targeting components such as peroxisomes and mitochondria. For instance, macroautophagy

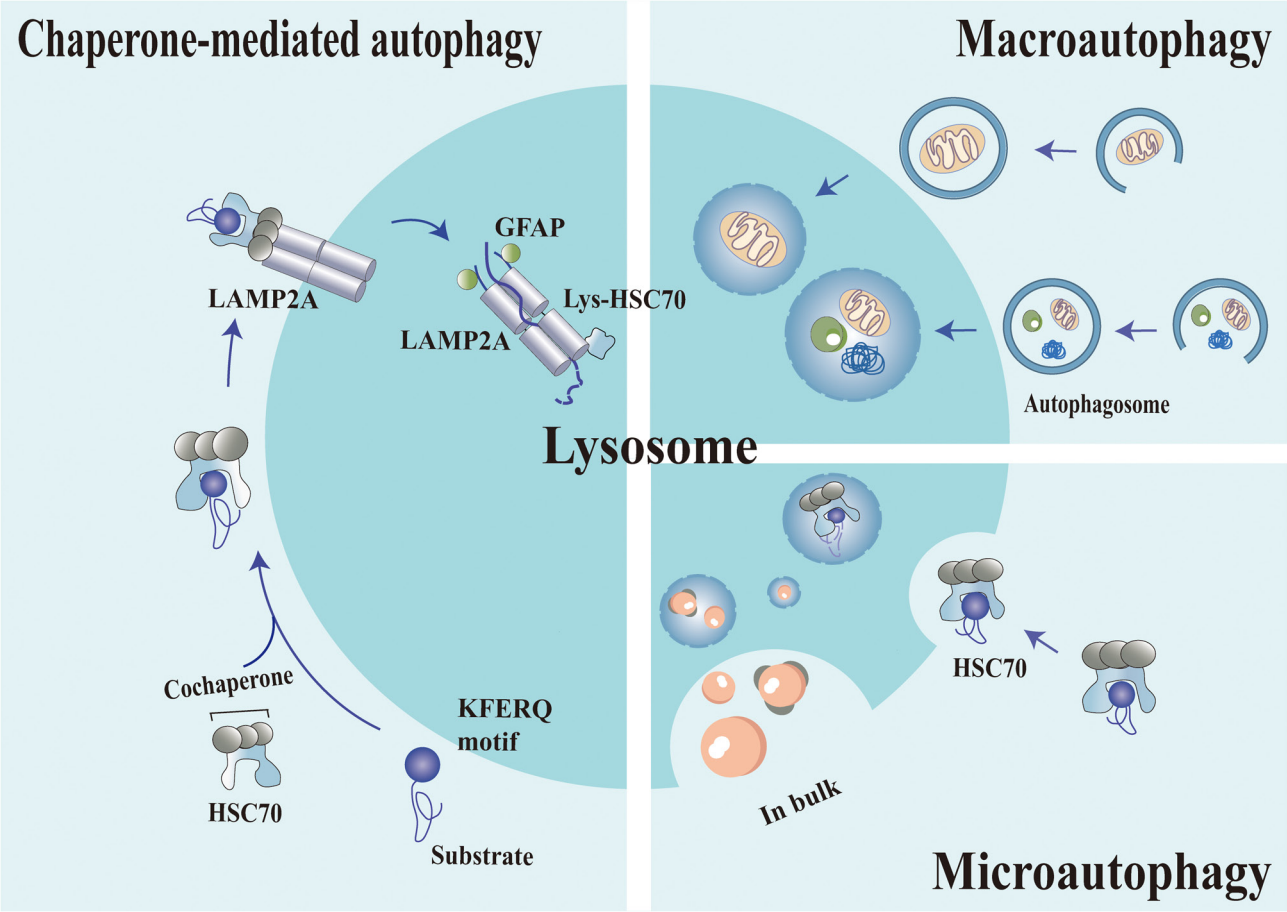

Figure 1. Three different kinds of autophagy. Autophagy manifests in various forms, each distinguished by its unique characteristics and mechanisms. Macroautophagy involves the formation of a double-membraned vesicle (an autophagosome) that envelops cellular material destined for degradation. The autophagosome then fuses with a lysosome, forming an autolysosome wherein the enclosed material is degraded. Microautophagy is characterized by the invagination of the lysosomal membrane, which leads to the formation of a small vesicle. This vesicle then fuses with the lysosome's interior, thereby releasing its contents for degradation within the lysosome. Chaperone-mediated autophagy target proteins are characterized by a distinct KFERQ-like sequence that is recognized by chaperone proteins, notably HSC70. These chaperone-recognized proteins subsequently interact with the LAMP2A receptor on the lysosomal membrane. Upon binding, LAMP2A aggregates to form multimers. Assisted by HSC70, the target protein is gradually translocated across the lysosomal membrane into the lysosomal lumen. Once inside the lysosome, the target protein is rapidly degraded by acidic hydrolases. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. LAMP2A, lysosome-associated membrane protein 2A; GFAP, glial fibrillary acidic protein; HSC70, heat shock cognate 70.

degrades excessive peroxisomes in mouse livers (19), and ULK1, an ATG1 homologue, plays a key role in the targeted degradation of mitochondria and ribosomes in erythrocytes (20). This underlines the specificity and efficiency of macroautophagy in cellular quality control, highlighting its essential function in the removal of redundant or damaged organelles.

Microautophagy. Microautophagy involves lysosomes or vacuoles engulfing and digesting segments of their own cytoplasm, including proteins and organelles. The specialized autophagic tube structure subsequently severs the vesicle membrane, delivering substrates into the vacuolar space. Morphological variations in lysosomes have led to identifying three distinct types of microautophagy in yeast and mammalian cells: Type I, protruding lysosomes or vacuoles; type II, invaginating lysosomes or vacuoles; and type III, endocytosed invaginations (21). During nutrient restriction-induced autophagy, early invaginations form 'invagination tubes' with the vacuolar transporter chaperone (VTC) complex playing a key role in accumulating on the vacuolar membrane (22). Furthermore, this complex potentially serves as an activation site for calmodulin (23). Increased lipid accumulation on the autophagic tube and depletion of integral proteins promote vesicle formation (24). Pre-vesicular structures, formed by enzymes, oscillate within lysosomes and the VTC complex are essential for vesicle scission. Following detachment from the autophagic tube, lipase Aut5p is activated amid autophagic component interactions, compromising vacuolar integrity and facilitating autophagosome degradation (25). Meanwhile, ATG22, an osmolytic enzyme, recycles amino acids, contributing to nutrient and energy cycling (26).

Microautophagy performs crucial biological functions such as maintaining cell size, aiding in membrane formation, and fostering cell proliferation by degrading and recycling nuclei (27). For instance, apical vacuoles in visceral endodermal cells, organelles typical of lysosomes, rely on the endocytosis pathway-regulated CTP-binding protein RAB7. They undergo microautophagy to ensure stability during the early stages of mouse embryogenesis (28). This highlights the

essential role of microautophagy in developmental processes and cellular maintenance, illustrating its relevance in fundamental biological activities.

CMA. Contrary to other autophagy types, CMA does not necessitate the transportation of substrates to the lysosome via vesicles and membrane invagination, but rather through translocation complexes on the lysosomal membrane (29). In 1978, Dice *et al* (30) found that protein degradation in diabetes differs from healthy conditions, revealing that lysosomes in diabetic models facilitate protein degradation and introducing the concept of selective protein targeting by lysosomes. In 1986, Backer and Dice (31) identified a 20-amino-acid sequence in RNase S protein that recognizes and accelerates the degradation of RNase A protein, providing a new perspective on selective degradation. In 1986, further research established that attaching RNase S protein to other proteins enhances degradation metabolism during starvation. RNase S protein, serving as a unique sequence, activates the transport of cytoplasmic proteins to lysosomes in serum-free conditions (30). Subsequently, the KFERQ-like sequence for protein targeting to lysosomes was identified (31), and it was confirmed that heat shock cognate 70 (HSC70) binds to this region, targeting substrates to lysosomes for ATP-dependent degradation, initiating research into chaperone recognition for selective lysosomal degradation (32). In 1996, the identification of the fact that overexpressing LGP96 enhances the selective lysosomal proteolytic pathway in ovarian cells established LGP96's role on the lysosomal membrane as a receptor for substrate protein degradation (33,34). This type of autophagy, characterized by the formation of a protein translocation complex through the recognition of specific substrate proteins by HSC70 and translocation across the lysosomal membrane via lysosome-associated membrane protein 2A (LAMP2A), is formally defined as CMA. The activity of CMA and the levels of HSC70 protein were determined in rat liver-derived lysosomes, showing a decrease in activity and HSC70 levels with aging (35).

2. Molecular composition of CMA

Heat shock protein 70 (HSP70) binds to CMA substrate proteins. The specific binding of substrate proteins to the lysosomal membrane is crucial for CMA. A fundamental requirement for CMA is HSC70's recognition of the KFERQ-like pentapeptide motif in substrate proteins. During serum withdrawal, mutation of this pentapeptide sequence leads to reduced cellular degradation (36). A fluorescence reporting system measuring CMA activity revealed that insertion of an 11-amino-acid sequence containing KFERQ (KFERQ-PS-CFP2) into non-CMA substrate proteins still results in their lysosomal degradation (37). Thus, it is the motif's nature that determines HSC70 binding, not any specific amino acid. The KFERQ motif, essential for all CMA targeting, consists of: i) One or two positively charged residues, namely K and/or R; ii) one or two hydrophobic residues (I, L, V and/or F); iii) one negatively charged residue (D and/or E); and iv) one glutamine (Q) on either side of the pentapeptide (38). While canonical motifs exist in unmodified protein sequences, post-translational modifications such as ubiquitination, acetylation and phosphorylation can create equivalent targeting sequences, thereby enhancing the diversity of CMA substrate proteins (39). However, the degradation rate remains unchanged even when multiple KFEQR sequences exist in a protein (38).

HSC70 participates in cytoplasmic and luminal pathways. Initially, HSC70 attaches to organelle membranes, promoting peptide unfolding through the ATP hydrolysis cycle, thereby facilitating entry into organelles (40). HSC70, operating in an ATP-dependent manner, binds on both sides of the lysosome, thus breaking down polymeric complexes. Concurrently, Hsp90 maintains the conformational stability of LAMP2A complexes (41,42). Luminal HSC70, characterized by a highly acidic isoelectric point, modulates CMA in response to lysosomal acidification (43).

HSC70's targeting function for KFERQ is contingent upon the structural properties of the protein in which the motif is located, and the adaptability of post-translational modifications. The ADP-bound form of HSC70 exhibits the highest affinity for CMA substrate proteins (33). Cytoplasmic HSC70 (cyt-HSC70) recognizes peptide sequences, including the KFERQ motif in CMA substrate proteins, aiding their transport to lysosomal receptors. Cyt-HSC70, when docked on lysosomal membranes, facilitates protein unfolding, a prerequisite for lysosomal entry (33). Other co-chaperones interact with HSC70 and modulate its activity. For instance, Hsp40 activates the ATPase activity of HSC70, which promotes substrate binding, and Hsp70-interacting protein (Hip) stimulates the assembly of HSC70, Hsp40 and protein substrates (44). Hsp90 is also found in the lysosomal lumen, associated with the luminal side of the lysosomal membrane. This protein stabilizes the fundamental components of translocation complexes when organized into oligomeric structures (41). The HSC70-Hsp90 organizing protein acts as an adaptor between HSC70 and Hsp90, identifying unfolded regions in proteins and preventing the aggregation of substrate proteins. Within the HSP family, the ATPase of Hsp90 activates its ATPase activity, thereby stimulating protein binding and release (44). Cell division cycle 48 (Cdc48) enhances the activity of the HSC70-Hsp40 complex. B-cell lymphoma 2 (Bcl-2)-associated athanogene 1 (BAG-1), an Hsp70-related protein, exhibits unique properties as a negative regulator of Hsp70. This protein decouples the ATPase cycle from substrate binding, inducing conformational changes in Hsp70 and enhancing the resistance of its substrate-binding domain to proteolysis (45).

LAMP2A mediates the translocation of HSC70-protein complexes to lysosomes. When the HSC70 chaperone complex translocates to the lysosomal membrane, molecular chaperones within the lysosomal lumen facilitate the translocation of the substrate complex across the membrane, wherein LAMP2A plays a pivotal receptor role. Even though LAMP2A receptors are found in all lysosome types, it is important to note that not every lysosome is competent for CMA. LAMP2A is one of the three splice variants of the LAMP2 gene (46), with LAMP-2B being involved in macroautophagy (47) and LAMP-2C in the uptake and degradation of ribonucleic acid (RNA) molecules by lysosomes (48,49). They share the same luminal region but differ in their transmembrane and cytosolic domains.

LAMP2A is cleaved from the lysosome and subsequently released into the lysosomal matrix for degradation, in a form truncated by metalloproteases and serine proteases (50). Pro-protein convertase A cleaves LAMP2A at its serine active site, which is located between the transmembrane and luminal domains, thus indirectly inducing metalloprotease cleavage at the C-terminal region and thereby accelerating substrate degradation rates (51). Under stress conditions, the degradation of LAMP2A is diminished, resulting in an increased presence of the receptor in the lysosomal membrane. The transport of substrate proteins is associated with the internalization of LAMP2A from the membrane into the lysosomal matrix (50). The interaction of HSP70 chaperones and their co-chaperones with the lysosomal membrane occurs through the four positive residues in the cytosolic tail of LAMP2A. Specific antibodies that inhibit tail binding not only obstruct substrate uptake and degradation in the lysosome (33), but also lead to a significant accumulation of autophagic vesicles in tissues (52).

LAMP2A potentially influences HSC70 function, undergoing rapid cycling to assemble a 700-kDa protein complex on the lysosomal membrane. LAMP2A monomers on this membrane are capable of receiving substrate proteins, and this interaction initiates the formation of the required polymeric complex for substrate translocation. Once the substrate protein reaches the lysosomal lumen, LAMP2A disassembles from the polymeric complex, thereby enabling successive rounds of substrate binding (41). Chaperone proteins located on either side of the lysosomal membrane can regulate LAMP2A lateral mobility. Upon the complex's transmembrane passage, Lys-HSC70 induces the disassembly of LAMP2A from the 700-kDa complex.

It has been demonstrated that LAMP2A mediates the transport of soluble proteins with KFERQ sequence-related amino acid sequences to newly formed exosomes on endocytosed limiting membranes (53). Furthermore, previous research has revealed that specific lipid (containing cholesterol and glycosphingolipids) and protein-containing microstructural regions with LAMP2A are present on the lysosomal membrane. These microstructural regions facilitate the interaction of LAMP2A with proteolytic enzymes, resulting in reduced LAMP2A in lysosomes. This dynamic distribution of LAMP2A, as it enters and exits lysosomal membrane microstructural areas, impacts the CMA process (54).

3. Signaling pathways regulating CMA activity

CMA is a distinct form of autophagy characterized by its precise targeting and degradation of specific cytosolic proteins. This process relies on recognizing specific KFERQ-like motifs in proteins, which, once identified by chaperones, are directly translocated across the lysosomal membrane for degradation (55). The seamless functioning of this mechanism is crucial for maintaining cellular homeostasis. However, it does not operate in isolation. The efficiency and regulation of CMA are closely linked with diverse signaling pathways that either enhance or inhibit its activity (Fig. 2). A deeper exploration of these signaling pathways offers a more comprehensive understanding of CMA's role in health and disease (55).

Retinoic acid receptor (RAR)α. In mammalian systems, RAR signaling is categorized into three distinct types based on gene encoding: RARα, RARβ and RARγ, with RARα being the most ubiquitously expressed (56). Inhibition of RARα significantly upregulates the CMA pathway, leading to an increase in lysosomal degradation. However, when the RARα signaling pathway is blocked and simultaneously supplemented with all-trans retinoic acid (ATRA; an effective activator of RARα signaling), it does not lead to a suppression of autophagic degradation, which indicates that ATRA does not activate macroautophagy via RARα. Consequently, retinoic acid derivatives that modulate by inhibiting RARα's suppression of CMA were studied, without affecting other autophagic pathways or the transcriptional program of RARα (57). CMA activators *in vivo* activate the RARα transcriptional pathway, stabilize the N-CoR1/RARα interaction and ameliorate retinal lesions in a mouse model of retinal pigment degeneration (58). It has been demonstrated that RARα receptor activation attenuates neuroinflammation by promoting phenotypic polarization in microglial cells and modulating the Mafb/Msr1/PI3K-AKT/NF-κB pathway (59). The interaction between CMA and RARα offers potential therapeutic insights for various diseases.

AMP-activated protein kinase (AMPK). In eukaryotic cells, AMPK functions as a critical regulator of autophagy, orchestrating cellular energy balance and systemic energy metabolism (60). The cellular function of AMPK depends on ATP levels; its activation increases the rate of catabolic pathways (ATP production) while reducing that of anabolic pathways (ATP consumption) (61,62). Activation of AMPK prompts CMA to trigger the breakdown of lipid droplets. This mechanism entails either reducing cytoplasmic lipase degradation or initiating lipolysis through macroautophagy. Notably, lipid droplet-associated proteins, perilipin 2 (PLIN2) and PLIN3, act as substrates for CMA degradation and interact with HSC70 during lipid droplet-induced lipolysis (63,64). Sirtuin 3 (SIRT3) regulates the acetylation state and activity of substrates involved in energy metabolism, and activates macroautophagy in lipotoxic hepatocytes via the AMPK-ULK1 pathway (65). Similarly, overexpression of SIRT3 enhances LAMP2A expression, stimulates CMA activity and reduces the accumulation of the lipid-associated protein PLIN2 (65,66).

P38-TEEB-NLRP3. The mitogen-activated protein kinase (MAPK) pathway is involved in numerous cellular regulatory processes. P38 MAPK is a key member of the MAPK family, involved in cell cycle, apoptosis, development, differentiation, senescence and tumorigenesis, and also functions as a specific serine/threonine kinase in modulating inflammatory responses. P38 directly phosphorylates the CMA receptor LAMP2A at threonine residues, resulting in its membrane accumulation and conformational activation, and subsequent activation of the CMA pathway (67). Activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome is important in various pathological processes; the presence of the KFERQ targeting sequence within its amino acid sequence interacts with HSC70, and

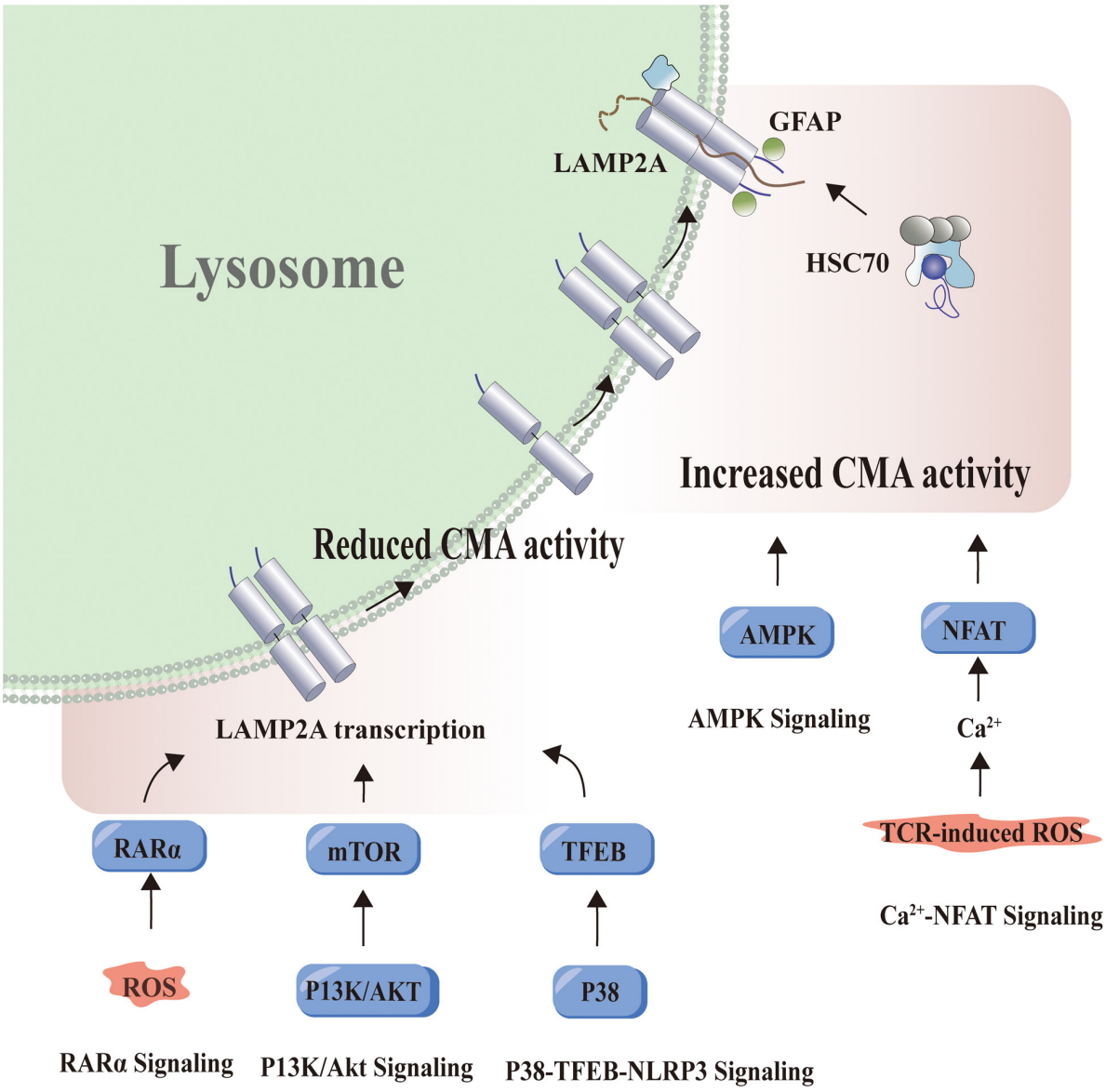

Figure 2. Signaling pathways regulating CMA activity. The calcineurin-NFAT pathway was initially reported to be capable of inducing CMA activation in T cells. Furthermore, this pathway also regulates CMA activity during T cell activation and proliferation. In eukaryotic cells, AMP-activated protein kinase not only regulates autophagy but also induces CMA, which leads to the breakdown of lipid droplets by reducing cytoplasmic lipase activity. In the context of p38-TFEB signaling, p38, which is a serine/threonine kinase of the mitogen-activated protein kinase family, phosphorylates TFEB, thereby inhibiting its activity. This suppression of TFEB activity inhibits NLRP3 inflammasome degradation mediated by CMA, thereby promoting glial cell activation. AKT phosphorylates a downstream substrate of mTOR, which in turn activates mTOR. mTOR is known to negatively regulate autophagic activity. RARα reduces the levels of lysosomal proteins, thereby affecting the interaction between lysosomal proteins Rab11 and Rab, and subsequently limiting CMA activity. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. LAMP2A, lysosome-associated membrane protein 2A; GFAP, glial fibrillary acidic protein; HSC70, heat shock cognate 70; CMA, chaperone-mediated autophagy; AMPK, AMP-activated protein kinase; NFAT, nuclear factor of activated T cells; RARα, retinoic acid receptor alpha; mTOR, mammalian target of rapamycin; TFEB, transcription factor EB; ROS, reactive oxygen species; PI3K/AKT, phosphoinositide 3-kinases/protein kinase B.

the sequence becomes unrecognizable to CMA when these two amino acids mutate to proline. Starvation activates the CMA process in glial cells, leading to the degradation of NLRP3 through S-palmitoylation transferase-mediated palmitoylation of NLRP3 (68). Inhibition of the p38 pathway significantly reduces neuroinflammation caused by the accumulation of α-synuclein, particularly the activation of the NLRP3 inflammasome and increases the levels of the CMA receptor LAMP2A, thereby enhancing the degradation of NLRP3 (69). Transcription factor EB (TFEB) serves as a principal regulator of the autophagosome-lysosome pathway (70), and it downregulates the NLRP2 inflammasome through upregulation of LAMP3A. Inhibiting p38 leads to TFEB nuclear translocation, decreases its phosphorylation level and initiates TFEB-mediated autophagy in Parkinson's models (69). Upon TFEB knockdown, the effects of p38 inhibitors are completely abolished. The p38-TFEB pathway not only inhibits CMA-mediated NLRP3 inflammasome

degradation but also promotes glial cell activation; thus, CMA-mediated processes may represent a novel therapeutic target for Parkinson's disease (PD) (69).

Calcium signaling-NFAT. The calcineurin-NFAT pathway was one of the first mechanisms identified as capable of inducing CMA activation in T-cells. T-cells are found throughout the body's immune organs, performing immune functions within tissues. Activation of the T cell receptor (TCR) leads to upregulation of LAMP2A, thereby initiating the CMA pathway in CD2T cells. In mice with LAMP2A knockout, there is a compromise in both immune tissues and T-cell functionality, resulting in decreased induced proliferation and a significant reduction in cytokine levels (71). Upon T-cell activation, the production of reactive oxygen species (ROS) occurs. TCR activates nuclear factor of activated T cells (NFAT), which in turn modulates LAMP-4A expression in CD2T cells, playing a role in CMA activation. Inhibitors of TCR signaling, including the E3 ubiquitin ligase Itch and the regulator of calcineurin 1 (RCAN1), feature CMA target sequences and are concentrated on the lysosomes of activated T-cells. CMA supports effective T-cell activation by degrading both Itch and Rcan-1 (71). During T-cell activation, the ROS generated prompt the nuclear translocation of NFAT1. NFAT2 directly binds to the proximal promoter region of LAMP2A, including the presumed NFAT1 binding site, thereby promoting LAMP2A mRNA expression and enhancing CMA activation (71). Inhibition of calcineurin through cyclosporin A administration or blockade of ROS production results in the abolishment of CMA activation in these cells. This provides a novel insight into the oxidative stress response linked to CMA activation.

PI3K/AKT. PI3K-AKT pathway mediation occurs through serine or threonine phosphorylation of downstream substrates in response to extracellular signals. This cascade is instrumental in facilitating a variety of cellular processes, including metabolism, proliferation, cell survival, growth and angiogenesis. Central to this pathway are the enzymes PI3K and protein kinase B (AKT), the latter also being referred to as protein kinase B (72). AKT has the capability of phosphorylating substrates downstream of mTOR, thereby activating mTOR. Autophagic activity is negatively modulated by mTOR (73). In models of acute liver failure in rats, or in cells infected with the hepatitis virus, activation of the PI3K/AKT/mTOR pathway is observed, accompanied by significant elevations in HSC70 protein and mRNA levels (74). Molecular inhibitors that target the PI3K/AKT/mTOR axis not only promote cellular proliferation and inhibit apoptosis but also exert protective effects against acute liver failure by activating CMA. Consequently, the PI3K/AKT/mTOR axis may play a pivotal role as an upstream regulator of CMA (74). At the lysosomal membrane, CMA inhibition occurs due to the AKT-dependent phosphorylation of the CMA regulatory factor glial fibrillary acidic protein (GFAP). Lysosomes isolated from mice treated with PI3K inhibitors exhibited enhanced CMA activity and reduced lysosomal GFAP phosphorylation, with no changes observed in macroautophagy (75). Additionally, the PI3K/AKT-dependent CMA mechanism significantly promotes osteoblastic differentiation in rat bone marrow stromal stem cells (74). Vitamin D plays a critical role in stimulating the differentiation of osteoblasts from bone marrow stromal stem cells. Leptin, acting synergistically with the vitamin D metabolite 25-hydroxyvitamin D3, induces osteoblastic differentiation. Leptin upregulates methionine expression by activating the PI3K/AKT signaling pathway, resulting in increased HSC70 expression levels. Furthermore, inhibiting leptin expression can modify its inhibitory effect on CMA. Additionally, leptin suppresses CMA activity through the activation of vitamin D metabolites, thereby further enhancing osteoblastic differentiation (76).

4. CMA in cell physiology: From homeostasis to aging

In the context of CMA, the complex interplay of signaling pathways not only determines its regulatory mechanisms but also highlights its adaptability in cellular processes. Serving as conduits for both external and internal signals, these pathways finely adjust CMA's activity, influencing a wide range of cellular functions. Delving into the role of CMA in cellular senescence and metabolic regulation reveals that this type of autophagy is more than a cellular housekeeper; it is a vital mediator of cellular aging and metabolic adaptation (77). This dual function underscores CMA's significance in preserving cellular vitality and in orchestrating the intricate dynamics of aging and metabolic processes. Additionally, CMA plays a crucial role in cell cycle regulation (78). Alterations in specific signaling pathways can affect CMA activity, thus indirectly modulating different stages of the cell cycle. Metabolically, CMA aids in maintaining cellular equilibrium by ensuring a consistent energy supply within cells. Within the realm of protein homeostasis, CMA guarantees the timely and precise degradation and recycling of cellular proteins, thereby ensuring cellular stability (79,80). In the forthcoming discussion, these functions and their physiological implications associated with CMA are further described (Fig. 3).

CMA regulates ferroptosis. Ferroptosis represents a dependency-driven form of programmed cell death. Under the influence of divalent iron or lipoxygenase, ferroptosis catalyzes the overexpression of polyunsaturated fatty acids on the cell membrane, resulting in lipid peroxidation and subsequent cell death (81). CMA is implicated in the onset of ferroptosis, with glutathione peroxidase 4 (GPX4) degradation being pivotal in inducing lipid peroxidation. The triterpenoid compound 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) inhibits ferroptosis, induced by erastin/glutamate, by blocking GPX4 (82). CDDO acts upstream of lipid peroxidation, suppressing the end-product malondialdehyde without altering GPX4 mRNA levels. Additionally, CDDO suppresses HSP90, a shared regulator of necroptotic death and ferroptosis (82). In ferroptosis, fluorescently tagged CMA substrates translocate from the cytoplasm to the lysosome, indicating CMA activation. Knockdown of LAMP2A and HSC70 significantly alleviates erastin-induced ferroptosis. HSP90 maintains LAMP2A stability, interacts with HSC70 and regulates ferroptosis by modulating CMA. During the ferroptotic activation process, elevated LAMP2A levels and HSP90-dependent CMA induction mediate GPX4 degradation (82). Creatine

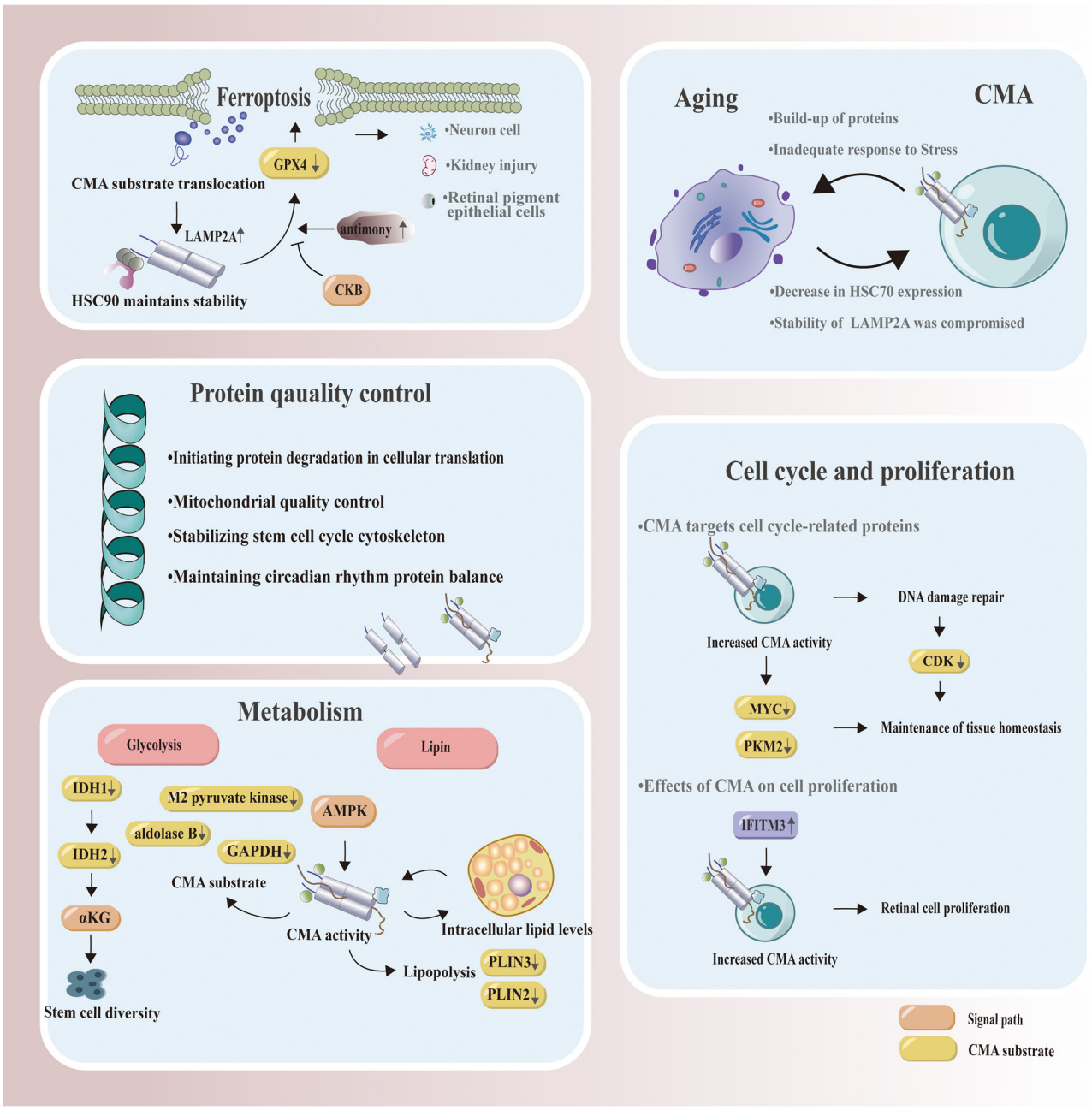

Figure 3. Physiological roles of CMA. In cells affected by ferroptosis, CMA activation not only leads to an increase in LAMP2A levels but also induces CMA in an HSP90-dependent manner, which in turn mediates GPX4 degradation. CKB-mediated GPX4 phosphorylation serves to prevent CMA-mediated degradation of GPX4, while Sb is known to induce neurotoxicity. Upon Sb increase, CMA-related proteins (HSC70 and LAMP2A, which exhibit increased levels) bind to GPX4, thereby forming a chaperone complex that accelerates GPX4 degradation and ultimately leads to ferroptosis in neurons. The complex interplay between ferroptosis and CMA has undergone extensive investigation in various conditions, including renal injury, neuronal injury and impairments of retinal function. CMA plays a crucial role in maintaining metabolic homeostasis, especially in glucose and lipid metabolism. This process influences stem cell diversity by degrading IDH1 and IDH2, subsequently regulating intracellular αKG levels. Furthermore, the activation of AMPK stimulates CMA to initiate the breakdown of lipid droplets, and these intracellular lipid levels in turn influence CMA activity. CMA exhibits a close association with aging, characterized by mutual interaction. As aging progresses, the functionality of CMA becomes impaired and its activity reduces, leading to an accelerated aging process attributable to the accumulation of oxidized or misfolded proteins. CMA plays a crucial role in maintaining cellular homeostasis by regulating key cell cycle proteins such as MYC and PKM2. CMA responds to DNA damage by timely degradation of CDKs, thereby facilitating entry into the cell cycle after DNA repair. The protein IFITM3 promotes retinal cell proliferation through the activation of CMA. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. Sb, antimony; CMA, chaperone-mediated autophagy; GPX4, glutathione peroxidase 4; CKB, creatine kinase B; HSC70, heat shock cognate 70; LAMP2A, lysosome-associated membrane protein 2A; IDH, isocitrate dehydrogenase; αKG, alpha-ketoglutarate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; AMPK, AMP-activated protein kinase; PLIN, perilipin; MYC, MYC proto-oncogene, BHLH transcription factor; PKM2, pyruvate kinase M2; CDK, cyclin-dependent kinase; IFITM3, interferon-induced transmembrane protein 3.

kinase B mediates GPX4 phosphorylation at the serine 104 residue, which prevents CMA-mediated GPX4 degradation (83). Prolonged exposure to antimony (Sb), which is prevalent in agriculture and industry, induces neurotoxicity. Increased levels of Sb enhance the binding of CMA-associated proteins (HSC70 and LAMP2A) to GPX4, forming a chaperone

complex that accelerates GPX4 degradation and neuronal cell ferroptosis (84). In studies examining legumain's nephroprotective role, interactions between HSC70, HSP90 and GPX4 were observed to mediate the lysosomal transport of GPX4, leading to ferroptosis (85). In hyperglycemic environments, excessive secretion of mature neuroglial factor-β in the vitreous body impairs the acidification and activation of retinal pigment epithelial cell lysosomes, thereby hampering autophagolysosomal degradation. Disrupted lysosomal homeostasis leads to elevated levels of the ferroptotic marker acyl-CoA synthetase long-chain family member 4 (ACSL4). ACSL4 has also been identified as a substrate for CMA by the receptor HSC70 (86). CMA, when activated during cellular ferroptosis, maintains autophagolysosomal balance. Both the CMA activator QX77 and ferroptosis inhibitors reduce neuropathy and enhance retinal physiological function (86).

Mitochondrial changes typifying ferroptosis include mitochondrial shrinkage, cristae loss, increased mitochondrial membrane density and outer mitochondrial membrane rupture. Ferroptosis and apoptosis are intertwined processes, with apoptosis potentially transitioning into ferroptosis under specific conditions, thus augmenting cellular sensitivity to apoptosis. CMA, which is possibly activated alongside apoptosis under stress, can result in cell death. BCL2 binding component 3 (BBC3), a pro-apoptotic member of the BCL-2 family, orchestrates various apoptotic responses to cellular insults and is subject to CMA-dependent degradation. In the process of CMA, HSPA8 recruits BBC3 to the lysosome for degradation. Direct interactions between HSPA8 and BBC3 result in the formation of a protein complex, with BBC3 possessing the KFERQ motif (87). Tumor necrosis factor (TNF) elicits a range of cellular responses, including survival, proliferation and apoptosis. Prolonged TNF signaling activates inhibitor of nuclear factor kappa b kinase subunit beta (IKBKB), leading to the phosphorylation of BBC30 at Ser3, which is subsequently suppressed by CMA-mediated degradation (87). Depletion of CMA stabilizes BBC3, thereby compromising cell viability in both the presence and absence of genotoxic insults.

CMA maintains protein homeostasis. CMA plays a crucial role in homeostasis of cellular proteins. Under injury conditions, accumulation of oxidized and aggregated proteins occurs in cells and organs. CMA regulates proteostasis under a variety of stress conditions, thereby preventing protein aggregation. For instance, within oxidized cytoplasmic proteins, ~30% of substrate proteins possessing targeting sequences are degraded through the CMA pathway (88). During toxic exposure, CMA becomes activated, selectively degrading proteins altered by toxic compounds (89). When aging and oxidative stress diminish CMA activity, protein damage intensifies, leading to increased aggregation and elevated oxidation levels (90). Cells regulate the production and degradation of proteins in a coordinated manner to maintain cellular homeostasis and adapt to the constantly changing environmental conditions throughout the day. This equilibrium ensures optimal functioning of fundamental cellular processes even amidst fluctuations in external factors. Diurnal rhythm in cellular proteomics delicately balances protein synthesis with degradation. The CMA sub-proteome varies across different diurnal timepoints. Proteins involved in translation and endocytosis undergo degradation at night, while repair-related proteins degrade during the day. Additionally, Golgi regulatory proteins are subjected to CMA degradation irrespective of diurnal preference (91). The majority of proteins regulated diurnally possess KFERQ targeting sequences, which are recognizable by LAMP2A. Disruption of CMA in mice leads to central and peripheral diurnal rhythm disorders, offering insights into diurnal rhythm abnormalities in the elderly (91).

Proteomic analyses of isolated lysosomes in cancer cells undergoing CMA activation reveal that CMA selectively targets specific cellular processes. Differential gene enrichment analysis comparing CMA activation and LAMP2A knockout indicates significant enrichment of cytoplasmic translation initiation factors during CMA activation (92). Changes in the expression of translation initiation proteins are correlated with malignant transformation in various cancer types. For example, eukaryotic translation initiation factor 4A1 (EIF4A1), EIF4H and DEAD-box helicase 3 X-linked (DDX3X), three translation initiation factors, exhibit elevated expression in primary tumors and undergo degradation through CMA pathways owing to their targeting sequences. Activation of CMA significantly diminishes these translation initiation factor proteins in various cell lines, including ovarian, lung and breast cancer, highlighting CMA's role in protein degradation related to cellular translation initiation (92).

CMA plays a regulatory role in hematopoietic stem cell renewal and metabolic balance. It stimulates the metabolism of long-chain fatty acids, catering to the demands of differentiating hematopoietic stem cells, by timely degrading acetylated marker proteins. Proteomic analysis indicates that a CMA defect results in global proteome remodeling, leading to inability to regulate the cell cycle and skeletal functions, failure to balance protein levels with other protein components, and impaired hematopoietic stem cell function (93).

Regulation of metabolic diversity through CMA. During the early phases of autophagy research, glycolytic enzymes, including those containing the KFERQ motif found in most glycolytic enzymes such as GAPDH, were identified as substrates for CMA This list also includes pyruvate kinase (94) and isoform M2 of aldolase B (95). In LAMP2A knockout mouse livers, enzymes involved in glucose catabolism within lysosomes are not timely degraded. As a result, gluconeogenesis decreases, glycolysis increases, leading to liver glycogen depletion and hepatocyte steatosis (96). Under normal nutritional conditions, enzymes from both glycolysis and the tricarboxylic acid cycle (TCA) cycle are degraded via CMA (96), suggesting a pivotal role for CMA in metabolic adaptation. CMA also plays a role in stem cell self-renewal and differentiation by influencing metabolic products in stem cells, promoting embryonic stem cell self-renewal with high activity, enhancing differentiation and maintaining embryonic stem cell viability through the mediation of pluripotency factors such as octamer-binding transcription factor 4 (OCT4) and SRY-box transcription factor 2 (SOX2) (97). Intracellular α-ketoglutarate (αKG), a product of the TCA cycle and an essential cofactor for demethylation, plays a key role in sustaining undifferentiated pluripotent stem cells (98,99). Following reduced LAMP2A activity, αKG emerges as one of the most abundant metabolites in cells. Conversely, αKG levels

decrease with overexpression of LAMP2A. αKG is produced by various enzymes in the TCA cycle, serine biosynthesis pathway and amino acid metabolism. Sequencing of αKG generation enzymes confirms that both isocitrate dehydrogenase 1 (IDH1) and IDH2 possess CMA-targeting sequences. The expression levels of IDH1 and IDH2 in cells correlate with αKG levels when CMA activity is altered. Thus, by degrading IDH1 and IDH2, CMA regulates intracellular αKG levels, consequently affecting stem cell diversity (97).

CMA, in its role of maintaining metabolic homeostasis, acts as an upstream regulator for lipid autophagy and lipid metabolism. The selective degradation of key enzymes by CMA induces alterations in carbohydrate and lipid metabolism. CMA promotes lipid metabolism through the degradation of lipogenic enzymes and the selective loss of lipid droplet proteins (100). Lipid carriers and protein lipogenic enzymes, both integral to lipid metabolism, have been identified as CMA substrates (63). CMA selectively degrades proteins surrounding lipid droplets, thereby increasing cytoplasmic lipolysis and activating macroautophagy, initiating lipid breakdown (63). Moreover, CMA orchestrates proteins that regulate cell cycle arrest, glycolysis and specific lipid synthesis, thereby driving adipocyte differentiation (101). Rab7, a small GTPase, plays an essential role in the degradation of lipid droplets in starved liver cells. Rab7 modulates the interactions between lipid droplets and vesicles with LAMP1 lysosomes. Depletion of Rab7 leads to significant morphological changes in lysosomes and autophagosomes, suggesting an increased probability of CMA activation (102). Intracellular lipid levels additionally influence CMA activity. Long-term high-fat diets or acute high cholesterol intake reduce CMA's ability to transport lysosomal cytoplasmic substrates into the lumen, decrease LAMP2A receptor levels and disrupt lysosomal membrane structural stability (103).

CMA in cell cycle and proliferation. CMA specifically targets cell cycle-associated proteins, thereby regulating tissue homeostasis. MYC proto-oncogene, BHLH transcription factor (MYC), a member of the helix-loop-helix leucine zipper family of transcription factors, modulates a multitude of target genes both positively and negatively, stimulating the cell cycle through various mechanisms (104). A deficiency in CMA results in the accumulation of MYC protein. Notably, although MYC, containing two CMA targeting sequences, is not directly degraded, its phosphorylated form during cellular transformation is crucial for CMA-mediated indirect regulation (105). Pyruvate kinase M2 (PKM2) stimulates MYC and cyclin D, thereby advancing the G_1 phase of the cell cycle. Acetylated PKM2, undergoing a conformational change, interacts more effectively with HSC70, thus facilitating its CMA-mediated degradation (94). Rho family GTPase 3 (RND3), a regulator of the cell cycle, suppresses MYC expression and its transcriptional activity, thereby affecting several critical genes, including cyclins, cyclin-dependent kinases (CDKs) and the EF2 transcription factor (105). Its recognition by CMA is crucial for the proliferation of gastric cancer cells (106). Checkpoint kinase 1 (CHK1) mediates both normal and DNA damage-induced cell cycle arrest (107). Within cells, CHK1 mediates the S and M phases. During the S phase, CHK1 induces a reduction in CDK2 activity through the phosphorylation and proteasomal degradation of Cdc25A, resulting in slowed or blocked DNA replication. In the presence of DNA damage, CHK1 blocks the G_1/M transition via CHK2 regulatory phosphorylation, leading to CDK1 inactivation (107). In response to DNA damage, upregulated CMA aids in cell cycle re-entry post DNA repair by facilitating the timely degradation of cell cycle CHK1 (108).

Hypoxia-inducible factor 1 (HIF-1), a transcription factor, orchestrates the adaptive response to hypoxia. HIF-1α interacts with CDK1 and CDK2, playing a key role in cellular processes. CDK1 activity hinders lysosomal degradation of HIF-1α, thereby enhancing its protein stability and transcriptional activity. During the G_1/S phase transition, CMA-mediated degradation of HIF-1α is stimulated by CDK2 (109). Within the ubiquitin-proteasome system, substrate recognition is marked by ubiquitination (110). The ubiquitin ligase STIP1 homology and U-box containing protein 1 (STUB1) ubiquitinates lysine 63 (K63), which then binds and degrades HIF1A in the lysosome, in conjunction with CMA proteins (111,112). The interaction of CMA with immune-associated proteins plays a significant regulatory role in cell proliferation. Interferon-induced transmembrane protein 3 (IFITM3), a transmembrane protein, modulates the interferon-mediated innate immune response. IFITM3 regulates viral infections through autophagy, and its knockdown activates CMA, resulting in decreased retinal progenitor cell proliferation, notwithstanding the lack of significant mTOR pathway activation (113). The activation of CMA by IFITM3 plays a pivotal role in cell proliferation, although excessive CMA can lead to cell death (113). Chromosome 11 open reading frame 54 (C11orf54), a cytoplasmic esterase, influences cell proliferation and apoptosis. Transcriptomic analysis of C11orf54-knocked down and control cells demonstrated a marked inhibition of HIF1A signaling, which promoted HSC70 and HIF1A binding (114).

Additionally, CMA plays a crucial role in cell differentiation processes. During the differentiation of mouse mesenchymal stem cells into osteoblasts, LAMP2A levels increase, which promotes the transformation into osteoblasts while concurrently suppressing their adipogenic or chondrogenic potential (115). Vangl2, localized in the lysosomal differentiation-related gene of mesenchymal stem cells, binds to LAMP2A and undergoes degradation in specific domains, thereby interfering with CMA activity and inhibiting osteogenesis (115). Adipocyte differentiation, being a dynamic and multifactorial process, is closely associated with CMA activity both *in vitro* and *in vivo*. Blocking CMA leads to the downregulation of pre-adipocyte differentiation markers PLIN2 and adipocyte proteins, impairing the early stages of adipogenesis and resulting in the accumulation of undifferentiated pre-adipocytes. Persistent cell proliferation, dysregulated glycolysis rate and cell cycle disruption in pre-adipocytes arise from the inability to degrade MYC post-CMA blockage. The use of transforming growth factor beta (TGFβ) molecular inhibitors can partially rescue pre-adipocyte degradation following CMA deficiency, suggesting that CMA plays a pivotal role in adipocyte differentiation by transiently inhibiting TGFβ signaling in pre-adipocytes (101).

CMA's protective mechanism against oxidative stress. Oxidative stress arises from an imbalance between oxidative and antioxidative processes in the body, characterized by elevated intracellular levels of ROS. While ROS play a role in regulating normal cell differentiation and apoptosis, excessive ROS can result in cellular toxicity, a key factor in aging and disease progression. Inhibition of CMA leads to elevated ROS levels and subsequent cell apoptosis. Kiffin *et al* (88) demonstrated that cytoplasmic protein damage, induced by low concentrations of hydrogen peroxide, led to an increase in the number of proteins binding to the lysosomal membrane and being transported into the lysosomal lumen, correlating with the duration of CMA substrate oxidation. CMA responds to oxidative stress by upregulating the activity of its lysosomal translocation complex. Nuclear factor erythroid 2 (Nrf2) is a key antioxidant transcription factor, which is regulated by the Keap1-E3 ligase complex. CMA is critical for the antioxidative response mediated by Nrf2. Upon CMA activation, an increase in both Nrf2 protein levels and its transcriptional activity is observed. LAMP2A stabilizes Nrf2 by reducing the antioxidative levels of Keap2, thereby decreasing Nrf2 ubiquitination, with Keap1 being identified as a CMA substrate (116).

CMA selectively eliminates oxidized and pro-oxidant proteins to mitigate damage. Myocyte enhancer factor 2A (MEF2A), regulated by a critical transcription factor, protects primary neurons from oxidative stress-induced cellular injury. Under mild oxidative stress, MEF2A activity is notably enhanced; however, under excessive conditions of MEF2A activation the cleavage of histone deacetylase 4 in MEF2A is intensified, leading to the release of lysosomal serine proteases from ruptured lysosomes in a protein kinase-independent manner, thus targeting MEF2A for CMA-mediated degradation (117).

Association between CMA and mitochondrial quality control. Mitochondria, crucial for respiration-generated ROS, can experience damage, leading to oxidative and antioxidative imbalances. CMA combats oxidative stress by regulating mitochondrial function. Parkinsonism associated deglycase (PARK7/DJ-1), a crucial redox signaling intermediate, is activated under oxidative stress conditions in the presence of ROS (118). PARK7 is a recognizable CMA protein substrate. When cells undergo changes induced by the mitochondrial toxin 1-methyl-4-phenylpyridinium (MPP), CMA prioritizes the removal of oxidized and non-functional PARK7/DJ-1 by targeting it for lysosomal degradation to maintain the internal balance. Upon LAMP2A knockdown, CMA's ability to mitigate MPP damage diminishes, leading to more pronounced mitochondrial rupture and further ROS increase. Additionally, PARK7 overexpression mitigates cell death and mitochondrial dysfunction induced by MPP, even with concurrent LAMP2A knockdown. The CMA-PARK7 pathway is important for maintaining mitochondrial homeostasis. Dysregulation of this pathway could lead to neuronal stress and death (119). CMA regulates oxidative stress and mitochondrial damage under neuronal stress stimuli such as neurotoxins, thus maintaining mitochondrial quality control and homeostasis. E5 ubiquitin ligase MARCHF3, essential for mitochondrial fission, is a direct CMA substrate, and CMA regulates this ubiquitin ligase to influence mitochondrial positioning factors (120).

CMA impacts aging. It is well-established that, during aging, CMA functionality deteriorates and its activity diminishes, leading to an inefficient stress response and the accumulation of oxidized or misfolded proteins, which is a primary cause of multiple age-related diseases. Lysosomes from elderly rat livers show decreased transport rates of CMA substrates to the lysosomal membrane and reduced cytosolic levels of HSC70 in human fibroblasts (35). While the transcription rate of LAMP2A remains consistent during aging, changes occur in receptor dynamics and stability within the lysosomal lumen, including alterations in proteases responsible for cleavage and specific membrane microstructures. This observation suggests that lysosomal membrane alterations contribute to CMA-associated age-related changes (121). Restoring CMA activity in aging can significantly enhance organ function and the clearance of damaged proteins. Zhang and Cuervo (122) maintained LAMP2A activity in aged transgenic mice using Tet-regulators (responsive to tetracycline or its derivative doxycycline), observing reductions in liver damage markers, apoptotic cells and undegraded damaged products in the lysosomal compartment. Under similar regulatory conditions, aging exacerbates LAMP2A functional defects in mice, leading to decreased hepatocyte vitality, glucose intolerance, dysregulated blood sugar levels and lipid metabolic anomalies (90). With increasing age, there is a rise in cancer-related senile morbidity. Mice with a deficiency in CMA activity exhibit protein homeostasis imbalances in their livers, increased susceptibility to oxidative stress, liver dysfunction and a higher incidence rate of spontaneous liver tumors (90).

As aging progresses, the levels of CMA-related molecules undergo changes. For instance, in 24-month-old rats, HSC70 levels increase in the pons, medulla, striatum and thalamus, thereby inhibiting cytosolic protein denaturation (123). A previous study by Calabrese *et al* (124) found that HSC70 expression increases with age in the hippocampus and substantia nigra, and that augmenting HSP expression can enhance the repair of oxidatively damaged proteins, thereby shielding cells from age-related damage (124). In female rats, the striatal levels of HSC70 and its co-chaperone are lower (125). In age-related macular degeneration, a leading cause of blindness in the elderly, increased concentrations of LAMP2A and HSC70 in the retina are observed, compensating for macroautophagy deficiencies (126). White blood cells from various age groups exhibit distinct LAMP2A expression, with a decline in activity as age progresses (127). In skeletal muscles of aged (27-month-old) versus young (5-month-old) mice, LAMP2A and HSPA8/HSC70 levels decreased. In the myocardium of aged mice, LAMP2A levels increased, while HSPA8/HSC70 levels remained consistent (128).

Cellular senescence, characterized as a primary hallmark of aging, includes cell cycle arrest and impaired DNA damage repair (129). In senescent cells, lysosomal compartment expansion occurs alongside upregulation of LAMP2A transcription. Both macroautophagy and CMA levels show a positive association with aging (130,131). Knocking down LAMP2A significantly induces senescence in primary human fibroblasts, as evidenced by increased senescence-associated β-galactosidase activity, ROS production and lipofuscin accumulation (132). A functional deficiency in CMA can continuously activate CHK1, leading to DNA damage

associated with cellular senescence (108). p21/CDKn1a, a cyclin-dependent kinase inhibitor, and HIF-1α, a primary regulator of oxygen homeostasis, both serve as major targets of CMA (133). In the absence of hypoxic signals, HIF-1α upregulates p21/CDKn1a by displacing MYC at the p21/CDKn1a promoter, inducing cell cycle arrest (134). Thus, during aging, impaired CMA function may induce cellular senescence by degrading HIF-1α, which affects p21/CDKn1a.

5. CMA as a driving force for disease progression

CMA occupies a central role in numerous cellular physiological processes, thus ensuring the balanced functioning of the cell's intricate machinery. Central to this function is the cell cycle, where CMA coordinates the timely degradation and recycling of proteins, thereby aiding in the progression and regulation of various cell cycle stages (103). Furthermore, CMA substantially impacts cellular metabolism by maintaining a balance between energy production and consumption, and by ensuring vital substrates are promptly available for essential cellular reactions. However, the significance of CMA extends beyond mere normal physiological functions. Disturbances or malfunctions in CMA can result in pathological implications and a myriad of diseases. In oncology, aberrations in CMA activities are known to contribute to tumor progression and resistance to therapies (135,136). Similarly, disruptions in CMA affecting organs such as the lungs (137), heart (138), liver (139) and kidneys (140) can lead to debilitating conditions, underscoring its crucial role not only in maintaining cellular health but also in the onset and progression of diseases. A comprehensive knowledge of CMA is pivotal, both for understanding normal cell functions and for unraveling the complexities of various diseases.

Cancer. Consistent upregulation of CMA has been observed in various cancer types and cell lines, including melanoma, lung, gastric, colorectal and uterine cancer (141). This *in vivo* dependency on CMA has been confirmed using human lung cancer xenograft models (141). The ability of CMA to promote limitless proliferation of tumor cells, a hallmark of cancer, is remarkable. Changes in cell cycle control are central to malignancy, and interfering with CMA can drive tumorigenesis. Following malignant transformation, increased CMA activity has been shown to promote tumorigenesis (141). CMA modulates several molecules involved in cell cycle regulation, including the tumor suppressor protein p73 (p73), which can induce cell cycle arrest and apoptosis. Neural growth factor receptor (NGFR) is upregulated in various cancer types, including glioblastoma (GB) and breast carcinoma, enhancing transformed cell survival. NGFR directly binds to p73, inhibiting its transcriptional activity and promoting its degradation through CMA (142). Tumor cells exploit the LAMP2A-PRDX1/CRTC1 axis to regulate the activation of tumor-associated macrophages, thereby promoting tumor growth (143). Tumor cells require a higher glucose energy supply than normal cells, and through glycolytic metabolism, the embryonic M2 isoform of PKM2 in these cells facilitates aerobic glycolysis. Acetylated PKM2, degraded by CMA, promotes tumor cell proliferation when ectopically expressed (94).

Activation of CMA in cancer cells contributes to cancer development. Malignant cells in hepatocellular carcinoma (HCC) commonly demonstrate defects in autophagy and are associated with CMA activation. In patients with liver cancer, a significant correlation has been found between LAMP2A expression and tumor size and recurrence (144). Research by Ding *et al* (144) suggested that the growth and recurrence of HCC require CMA activation, as demonstrated using a murine tumor xenograft model. The dysregulated proteins yes-associated protein 1 (YAP1) and interleukin 6 cytokine family signal transducer (IL6ST) in HCC are recognized for promoting tumor growth. These proteins demonstrate dependency on binding with the CMA chaperone HSP8A and accumulate in isolated lysosomes following CMA induction (145). Immunohistochemical staining of LAMP2A and p62 in hepatitis C virus (HCV)-infected liver tissue samples validated the autophagy compensation mechanism between HCC and cirrhotic areas (144). Beclin 1 plays a crucial role in the initiation of autophagy and the fusion of autophagosomes with lysosomes. Reduced expression of beclin 1 leads to impaired autophagy in human HCC, and downregulation of beclin 1 in HCC is associated with poor prognosis (146,147). Mutations in beclin 1 accelerate the development of pre-cancerous lesions induced by hepatitis B virus (148). Research by Aydin *et al* (149) identified that cellular responses to HCV-induced stress inhibited autophagy due to the activation of stress-adaptive CMA, resulting in beclin 1 degradation and autophagy-related p62 accumulation. Activation of CMA hinders the endocytosis and degradation of epidermal growth factor receptor (EGFR), thereby activating the EGFR signaling pathway. This results in impaired autophagosome-lysosome fusion, consequently activating downstream oncogenic signaling pathways such as RAS/RAF/MEK/ERK, which further drives the progression of cirrhosis-associated HCC (149). In lung cancer cells, LAMP2A modulates their malignant phenotype by regulating apoptosis through CMA. Knockdown of LAMP2A alters the cancer phenotype and enhances drug sensitivity (150). CMA-mediated stabilization of myeloid cell leukemia 1 (MCL1), a pro-survival protein, has been confirmed to facilitate the survival of non-small cell lung cancer (NSCLC) cell lines (151). Nuclear receptor co-repressor (N-CoR) plays a pivotal role in transcriptional control mediated by tumor suppressor proteins and possesses a CMA targeting sequence. CMA induces the degradation of misfolded N-CoR, thereby neutralizing ER stress in NSCLC and indirectly promoting NSCLC cell survival (152).

Knockdown of LAMP2A in gastric cancer cells impedes cell proliferation, resulting in increased expression of the cell cycle-related protein RND3 and its interaction with HSP8A, leading to degradation via CMA, thereby supporting rapid proliferation of gastric cancer cells (106). CMA-mediated degradation of acetyltransferase p300/CREB-binding protein (p300/CBP) can increase the chemoresistance of colorectal cancer to 5-fluorouracil (153). ALL1 fused gene from chromosome 1q (AF1Q), a biomarker for myeloid leukemia and a target substrate for CMA, undergoes lysosomal degradation upon CMA activator treatment (154). The prostate-specific and androgen-responsive gene, tumor protein d52 (TPD52) subtype 1, amplified in prostate cancer, is closely associated with poor prognosis. TPD52 activates CMA physiologically

by enhancing the transfer of CMA protein substrates in prostate cancer cells through complex formation with HSPA8. Acetylation of TPD52 at lysine 52 plays a key role in modulating the interaction between TPD52 and HSPA8, thereby regulating CMA activity (155). In histopathological samples from patients with lung cancer, high expression of the CMA marker LAMP2A is associated with the prognosis of both adenocarcinoma and squamous cell carcinoma (156).

CMA in the tumor microenvironment is closely related to tumor progression, being particularly activated under conditions such as nutrient deficiency, hypoxia and elevated ROS (88,157,158). GB is a highly lethal malignant brain tumor for which no effective therapeutics are currently available. Interaction between GB and peritumoral cells (PCs) facilitates an increase in ROS, resulting in upregulation of CMA in PCs via LAMP2A expression. This anomalous induction of CMA in GB impacts the proteome and transcriptome of PCs, influencing the expression of immune-suppressive and inflammatory genes (159). When the crosstalk between CMA-deficient PCs and mouse GB cells is compromised, reduced tumor cell viability and impaired tumorigenesis occur (159).

Although CMA activity is positively correlated with increased expression in various cancer cells, it exhibits antitumoral effects in non-transformed cells (160). Inhibition of CMA in fibroblasts enhances MYC-mediated cell transformation, intensifying MYC-driven oxygen consumption and extracellular acidification, and shifting metabolism from oxidative phosphorylation to aerobic glycolysis, which is closely associated with tumorigenesis (105). Although MYC degradation primarily occurs through the proteasome, CMA indirectly mediates this process. CMA participates in DNA damage repair, aiding in the prevention of malignant transformation by maintaining genomic stability and reducing oncoprotein expression to counteract cancer. Translationally controlled tumor protein (Tpt1/TCTP) is subject to both transcriptional and translational regulation. Acetylated TCTP, recognized and degraded by CMA, helps to maintain cellular homeostasis. The degradation of oncoprotein mouse double minute 2 homolog (MDM2) via CMA contributes to the inhibition of tumorigenesis (161,162). Breast cancer represents the most prevalent malignancy. Microrchidia family CW-type zinc finger 2, an oncoprotein upregulated in various cancer types, interacts with HSP8A and LAMP2A, and undergoes phosphorylation modifications to prevent CMA-lysosomal degradation (163). TFEB and trehalose, a disaccharide that induces TFEB activation, enhance the expression of OCT4 in stem cells and LAMP2A. In ovarian cancer stem cells, fructose treatment reduces spheroid formation while elevating LAMP2A expression. CMA influences cancer stem cells through fructose metabolism (164). Aberrant activation of NF-κB can promote tumorigenesis. The p65 protein, which is central to this pathway, interacts with HSC70 targeting a basic sequence and is degraded through the CMA pathway. Additionally, in various epithelial-mesenchymal transition (EMT) models, diminished CMA activity is observed, and overexpression of LAMP2A can rescue EMT impairment by degrading p65 (165). CMA offers a novel avenue for cancer therapy; however, the underlying mechanisms of its antitumoral function in relation to cancer cells require further exploration (Fig. 4).

Neurodegenerative disorders. Post-mitotic neurons encounter challenges with misfolded protein aggregates, which, undiluted by cell division, necessitate clearance via the proteolytic system (166). Within the central nervous system, multifactorial injuries result in neuronal dysfunction. CMA controls the selective degradation of cytosolic proteins, potentially protecting neurons from degeneration. Inhibition of CMA leads to destabilization of proteins within neurons (166). Numerous proteins related to neurodegeneration possess CMA targeting sequences, including leucine-rich repeat kinase 2 (LRRK2) (167), ubiquitin C-terminal hydrolase isozyme L1 (UCH-L1) (168), α-synuclein and microtubule-associated protein t (TAU). These proteins directly interact with LAMP2A and are degraded via CMA (169). However, post-translational modifications of α-synuclein affect its degradation by CMA, since phosphorylated and dopamine-modified forms of α-synuclein are resistant to CMA degradation (170). The persistent association of α-synuclein with the lysosomal membrane further impairs CMA, resulting in a vicious cycle. Vascular protein sorting 35 (VPS35), crucial for endosome-to-Golgi retrieval, exhibits defects that alter lysosomal morphology, reduce LAMP2A vesicles and contribute to the elimination of α-synuclein accumulation (171).

When pathogenic proteins directly compromise CMA components, thereby hindering their degradation capability, the toxic effects of CMA dysregulation may act as a primary etiological factor or as a secondary consequence when protein homeostasis is indirectly affected (172). Factors such as genetics, aging and the microenvironment contribute to protein aggregation and neurodegenerative changes that result from CMA dysregulation (Fig. 5).

Alzheimer's disease (AD) is the most prevalent form of dementia in the elderly (173), primarily characterized by extracellular amyloid-β (Aβ) deposition and intracellular neurofibrillary tangles of TAU. CHIP, a ubiquitin ligase that interacts directly with Hsp70/90, exhibits neuroprotective properties (174,175), and can inhibit toxicities linked to abnormal protein accumulation in *Drosophila* and mouse disease models (176). Under normal circumstances, TAU is eliminated by macroautophagy, and acetylated TAU is degraded via CMA. Pathogenic TAU mutants that are resistant to CMA associate with lysosomal membranes, thereby interfering with the degradation of other proteins (177). Effective lysosomal translocation of TAU requires its binding with HSC70 in acidic environments. However, continuous accumulation of acetylated TAU affects the pH sensitivity of HSC70 binding, subsequently slowing down CMA (178). While TAU's affinity for isolated lysosomes may not be perfectly tailored for CMA substrate interaction, truncated TAU mutants do not completely translocate into the lysosomal lumen for degradation, despite the existence of two CMA targeting motifs in TAU C-terminal region (179). Acetylation of soluble TAU represents an early event in neurodegeneration, and blockade of CMA exacerbates the proliferation of pathogenic TAU in mice, worsening the disease state (178). RCAN1 plays a vital role in the pathogenesis of AD, with its expression being significantly increased in AD brains. In addition to degradation through the ubiquitin-proteasome pathway, RCAN1, which possesses two CMA recognition motifs, undergoes lysosomal degradation via CMA (180).

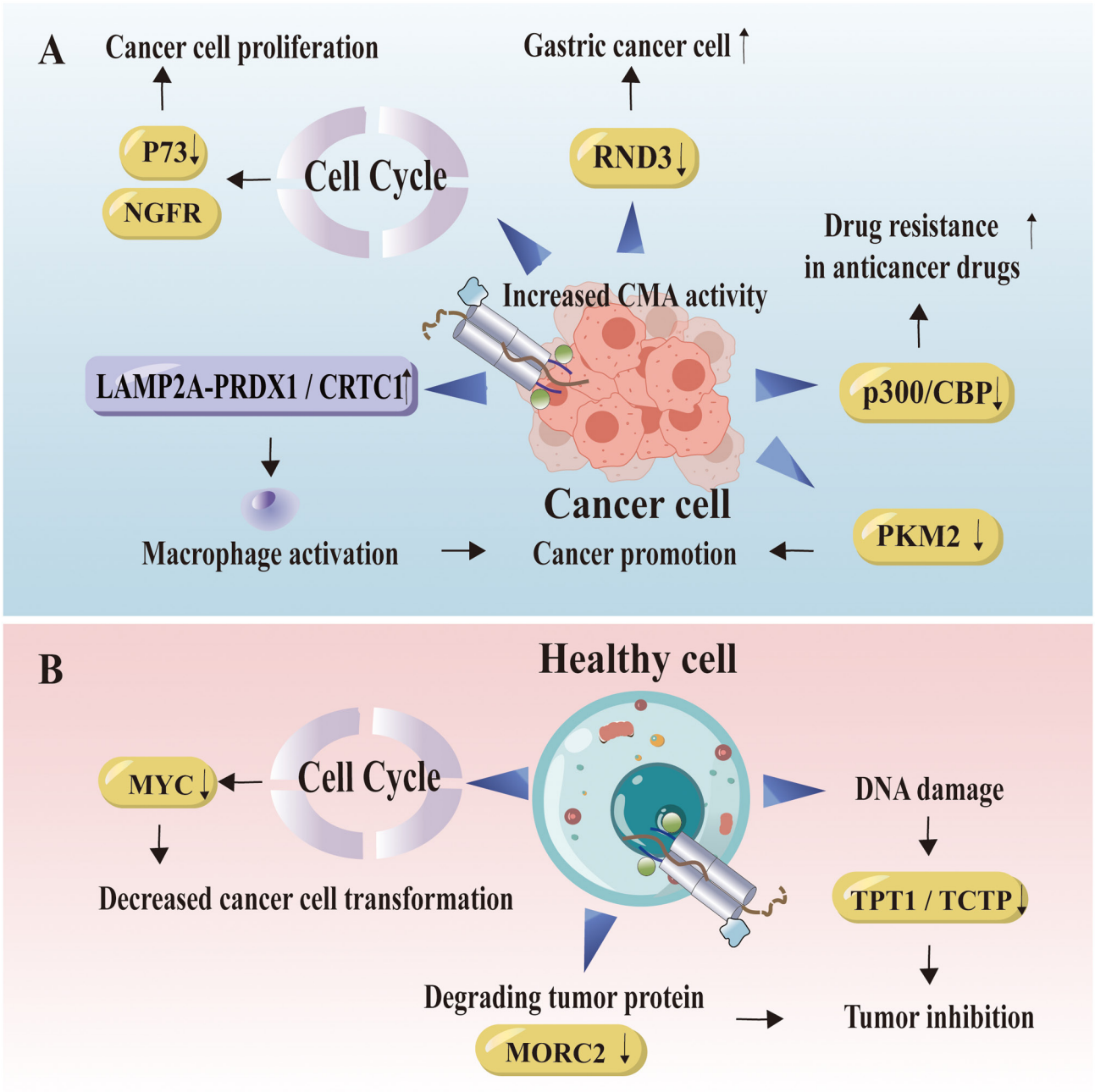

Figure 4. CMA is associated with cancer. (A) Activation of CMA in tumor cells is implicated in the promotion of cancer development. Increased CMA activity influences cell cycle alterations, downregulates the tumor suppressor factor P73, leads to the degradation of RND3 and facilitates the rapid proliferation of cancer cells. Tumor cells promote their own proliferation by regulating the LAMP2A-PRDX1/CRTC1 axis. Proteins related to degradation promote tumor development, which is manifested as increased resistance to drugs for treating colorectal cancer. (B) In non-carcinogenic cells, CMA exhibits antitumor effect. In normal cells, CMA regulates the cell cycle, contributes to DNA damage prevention to inhibit malignant tumor transformation and degrades pro-oncogenic proteins. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. NGFR, nerve growth factor receptor; RND3, Rho family GTPase 3; CMA, chaperone-mediated autophagy; LAMP2A, lysosome-associated membrane protein 2A; PRDX1/CRTC1, peroxiredoxin 1/CREB regulated transcription coactivator 1; p300/CBP, p300/CREB-binding protein; PKM2, pyruvate kinase M2; MYC, MYC proto-oncogene, BHLH transcription factor; MORC2, microrchidia family cw-type zinc finger 2; TPT1/TCTP, translationally controlled tumor protein.

Inhibiting the intracellular degradation of RCAN1 further diminishes calcineurin-NFAT activity.

PD ranks as the second most prevalent neurodegenerative disorder worldwide (181). In the substantia nigra of individuals affected by PD, LAMP2A and HSC70 levels are significantly reduced (182), highlighting CMA's role in the disease's etiology. Both α-synuclein and LRRK2 have been identified as major pathogenic proteins in PD and as substrates for CMA (167,183). Mutations in LRRK2 are among the leading causes of hereditary neurodegenerative disorders. The most common pathogenic mutation in LRRK2, G2019S, exhibits slowed degradation and can inhibit CMA by disrupting the formation of translocation complexes at the lysosomal membrane, leading to impaired degradation of LRRK2 within lysosomes (167). Furthermore, other proteins associated with PD also engage in CMA, reinforcing the relevance of CMA dysregulation in the progression of PD. Myocyte enhancer factor 2D (MEF2D), critical for neuronal survival, relocates

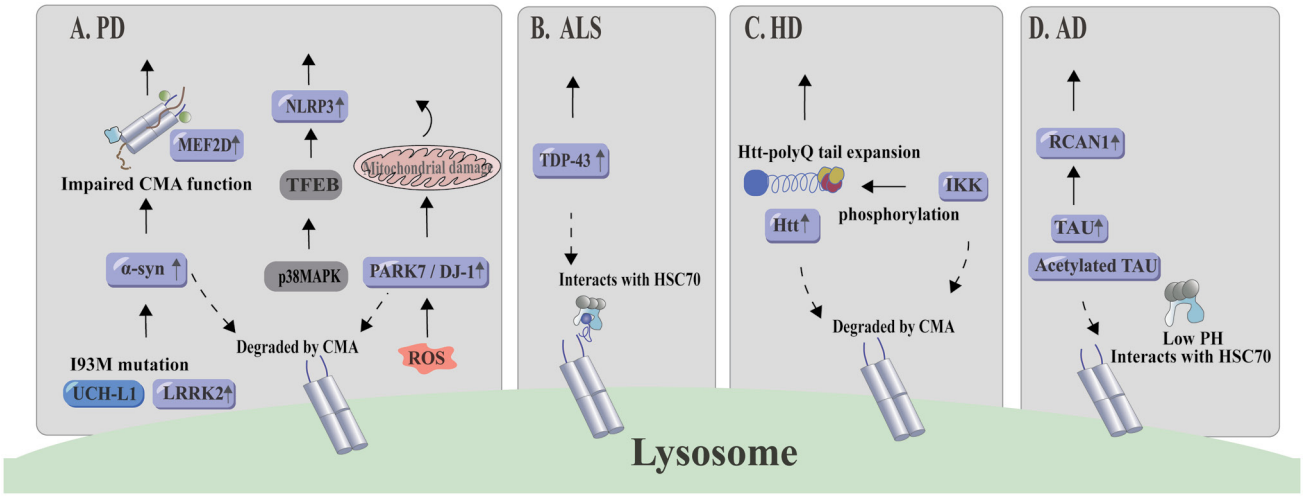

Figure 5. CMA and neurodegenerative diseases. (A) CMA involves proteins associated with PD. The primary pathogenic proteins in PD, α-synuclein and LRRK2, are degraded via CMA. Mutations in ubiquitin C-terminal hydrolase-L1 impair CMA functionality, leading to α-synuclein accumulation. Upon ROS activation, neuronal cells exposed to mitochondrial toxins show a CMA-driven inclination to degrade PARK7/DJ-1. This may exacerbate PD progression if CMA functionality declines. Additionally, the P38-TFEB signaling pathway limits CMA-mediated NLRP3 inflammasome degradation, thereby stimulating glial cell activity. (B) TDP-43, a key protein in amyotrophic lateral sclerosis, undergoes degradation through CMA. (C) After phosphorylation by inflammatory kinase IKK, Huntington's protein becomes a target for CMA-mediated degradation. (D) In an acidic environment, acetylated intraneuronal microtubule-binding protein TAU forms a stable complex with HSC70, which facilitates its CMA-mediated degradation. Concurrently, calmodulin phosphatase 1 regulator undergoes degradation through the CMA lysosomal pathway. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. PD, Parkinson's disease; NLRP3, NLR family pyrin domain containing 3; MEF2D, myocyte enhancer factor 2D; CMA, chaperone-mediated autophagy; TFEB, transcription factor EB; PARK7/DJ-1, Parkinsonism associated deglycase; UCH-L1, ubiquitin C-terminal hydrolase L1; LRRK2, leucine-rich repeat kinase 2; ROS, reactive oxygen species; ALS, amyotrophic lateral sclerosis; TDP-43, TAR DNA-binding protein 43; HSC70, heat shock cognate 70; HD, Huntington's disease; IKK, IκB kinase; Htt, huntingtin; AD, Alzheimer's disease; RCAN1, regulator of calcineurin 1.

from the nucleus to the cytosol for degradation in lysosomes. Elevated levels of MEF2D in α-synuclein transgenic mice and the brains of patients with PD indicate cytosolic degradation of MEF2D, which interacts with HSC70 via CMA. However, this degradation process is hindered by α-synuclein, resulting in its cytosolic accumulation (184,185). Mutations in ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have been linked to familial PD, with observed changes in dopaminergic neurons in mice carrying UCH-L1 mutations (186). UCH-L1, functioning in the CMA pathway, interacts with LAMP2A and HSC70. The I93M mutation causes structural instability, leading to abnormally enhanced interactions, thus impairing CMA function and facilitating the accumulation of α-synuclein proteins (155,187).

Amyotrophic lateral sclerosis (ALS) is a motor neuron disorder characterized by the accumulation of misfolded proteins within motor neurons, resulting in progressive paralysis and eventual mortality. TAR DNA-binding protein 43 (TDP-43; also known as TARDBP) is a pathological hallmark of neurodegenerative diseases, with the majority of mutation-driven amyotrophic lateral sclerosis cases leading to increased intrinsic aggregation of TDP-43. Previously, it has been shown that TDP-43 is associated with human HSC70 (HSPA8) in mammalian cells (188). Under chronic stress, the *Drosophila* adult HSC70-4 protein forms complexes with TDP-43 in motor neurons (189). Both naturally occurring and overexpressed TDP-43 are degraded through macroautophagy and the proteasomal system, whereas ubiquitinated TDP-43 undergoes degradation via the CMA pathway, interacting with HSC70 (190). Ormeño *et al* (191) demonstrated that TDP-43 could be degraded both *in vivo* and *in vitro* via CMA, with the inhibition of CMA significantly inducing TDP-43 aggregation. However, in later stages of aggregation, TDP-43 affects LAMP2A peri-nuclear localization, leading to CMA-related lysosomal dysfunction (191). In peripheral blood mononuclear cells of patients with ALS, reduced HSC70 mRNA and protein levels, decreased LAMP2A, and stable TDP-43 mRNA were observed, suggesting an elevation in TDP-43 protein levels attributed to diminished protein clearance rates (192).

Huntington's disease is an autosomal dominant disorder resulting from polyQ tail expansion due to mutations in the Huntingtin (Htt) gene. Despite Htt not directly containing the 'KFERQ' HSC70 binding motif within its sequence, it can be phosphorylated by the inflammatory IκB kinase (IKK). Following phosphorylation, the N-terminal fragment of Htt may acquire a KFERQ-like motif, affecting the levels of phosphorylated Htt through CMA degradation (193). In conditions where LAMP2A expression is high, mutated Htt can be degraded prior to inducing toxicity; however, with advancing age and reduced LAMP2A, mutated Htt accumulates, exacerbating the onset of neurodegenerative diseases (193). One mechanism to mitigate the toxicity of mutated Htt protein involves HSP40 and HSP70 chaperones, which capture it through hydrophobic interactions and redirect it to lysosomes for degradation (194). Increased expression of HSP70 significantly improves motor function in transgenic motor neuron disease mice by offering protection against polyQ (195). Hsp70s prevent the oligomerization of Htt protein, and the expression of Hsp70s progressively inhibits the development of Huntington's disease in mouse models by counteracting Htt conformational effects (196). Consequently, upregulation of Hsp70 can mitigate the toxicity of mutated Htt and restore various physiological protein functions. In addition to the macroautophagy degradation pathway for Htt, CMA-related

proteins interact with Htt fragments (Htt-552). Htt-552 can be recognized by HSC70 and transported to lysosomes through LAMP2A, and alterations in CMA-related proteins also influence Htt accumulation (197).

Cardiovascular diseases (CVDs). CVDs continue to be the primary cause of global mortality, representing 1/3 of all mortalities (198). Atherosclerosis is the main risk factor. Areas abundant in lipids, necrosis and macrophages, along with increased cytokine secretion and production of nitric oxide (NO) and ROS, establish a pro-inflammatory and oxidative environment. This environment induces the dedifferentiation of vascular smooth muscle cells (VSMCs) from a contractile phenotype to an activated, secretory and migratory phenotype. Activated VSMCs migrate to the intima, further exacerbating inflammation, oxidative stress, and the deposition of collagen and elastin proteins in the fibrous cap (198). In mouse models, aortic plaque associated with necrosis significantly enlarged following the knockout of LAMP2A; however, increased CMA activity markedly ameliorated these symptoms (199). Similarly, defects in CMA led to reduced lipid metabolism, impaired clotting function, decreased VSMC resistance to lipid toxicity and increased dedifferentiation processes in mice, thereby further elevating the risk of atherosclerosis (199).

As atherosclerosis progresses, CMA activity becomes compromised. Macrophages are the primary cell type expressing CMA biomarkers, with observed co-localization of LAMP2A and macrophages in murine atherosclerotic lesions and colitis models (138). The NLRP3 inflammasome maintains balance through CMA-mediated degradation. Inactivation of CMA in macrophages leads to NLRP3 inflammasome overactivation, promoting interleukin 1 beta (IL-1β) secretion, vascular inflammation and the progression of atherosclerosis (138). Enhancing CMA activity may improve systemic metabolic parameters and vascular function, and inhibit pathological changes in VSMCs and macrophages (199). These strategies show promise as innovative therapeutic approaches for preventing CVDs (200). NO, produced by endothelial NO synthase (eNOS), helps prevent myocardial ischemia-reperfusion injury. Mutations in NOS residues inhibit CMA, leading to further damage. Under hypoxia-reoxygenation conditions, glutathionylated eNOS, targeted by the HSC90 chaperone, reveals specific sequences, leading to its transport to LAMP2A vesicles for degradation. Prompt deglutathionylation can prevent CMA, thus ameliorating myocardial ischemia (201). Hypoxia is a major factor in myocardial infarction injuries. In samples from patients with ischemic heart failure, LAMP2A expression levels were found to be elevated. Overexpression of LAMP2A has been shown to reduce hypoxia-induced cardiomyocyte apoptosis by 50%, indicating the potential cardioprotective effects of CMA activation in ischemic heart diseases (202) (Fig. 6).

Liver diseases. HCV is a major cause of chronic hepatitis, liver cirrhosis and HCC. Characterized as an enveloped, positive-sense, single-stranded RNA virus, HCV belongs to the *Flaviviridae* family (203). In total, ~20% of infected adults spontaneously clear the HCV infection, while the remaining 80% develop chronic hepatitis, with increased risks of cirrhosis, HCC and end-stage liver disease (204). The HCV genome consists of 3,010 kb RNA, encoding a polyprotein of 96 amino acids. This polyprotein undergoes cleavage by viral proteases and host signal peptidases, resulting in three structural proteins essential for viral particle formation (205), and seven non-structural proteins involved in viral replication (206). Chronic HCV infection significantly increases the risk of hepatic steatosis. *In vitro* studies demonstrated a considerably higher accumulation of lipid droplets in HCV-infected hepatoma cells compared with uninfected cells. Co-culturing HCV with free fatty acids (FFAs) induces macrovesicular steatosis by downregulating the expression of interferon (IFN)-α receptors, thereby inhibiting the antiviral activity of IFN-α. The CMA-targeting amino acid sequence is found in IFN alpha and beta receptor subunit 1 (IFNAR1) on the IFN-λ receptor, and autophagy responses induced by HCV and FFA result in decreased IFNAR1 expression and its selective degradation through CMA. Activation induced by HCV and FFA leads to increased LAMP2A protein expression, resulting in the formation of a protein complex that includes HSC70, LAMP2A and IFNAR1 (207). HCV infection leads to the downregulation of hepatocyte nuclear factor-1α (HNF-1α) expression, both post-transcriptionally and translationally, and enhances its interaction with HSC70. Importantly, the HNF-1α protein includes a CMA targeting sequence within its POU domain. The HCV NS5A protein aids in recruiting HSC70 to HNF-1α, directing the lysosomal membrane-cytosolic LAMP2A recognition protein complex to the lysosome for degradation (208). It has been confirmed that HCV-induced degradation of HNF-1α via CMA suppresses glucose transporter type 2 (GLUT2) gene expression, leading to reduced surface GLUT2 expression and disrupted glucose uptake into cells, culminating in glucose metabolic disorders (209). During cellular responses induced by microbial stress, autophagy regulation under pathological conditions becomes essential, and the continual replication of HCV in hepatocytes promotes autophagy regulation through CMA activation, thus enhancing cell survival. Sorting nexin 10 (SNX-10), which plays a role in endo-lysosomal transport and stabilization, regulates CMA activity by enhancing LAMP2A stability. It does this by inhibiting the interaction with tissue plasminogen activator in SNX-10 knockout mice, thereby mitigating alcoholic liver injury and steatosis (210) (Fig. 6).

Renal disease. Elevated levels of lys-HSC70 have been reported in various nephropathies, yet the significance of these alterations remains to be elucidated. A previous study showed an upregulation of CMA in renal tubular epithelial cells in response to toxic modifications of lipid-binding proteins prevalent in the kidney such as α-2-microglobulin. Enhanced CMA activity facilitates the removal of these toxic protein forms prior to intracellular accumulation (89).

Renal hypertrophy is a potential pathological condition in the kidney where altered selective protein degradation by CMA may play a role. This hypertrophic cell growth may originate from an imbalance between protein synthesis and degradation. Hypertrophy is a characteristic feature of diabetic kidneys, characterized by reduced levels of LAMP2A. The nuclear transcription factor paired box 2 (Pax2), crucial for renal cell proliferation regulation, is overexpressed due to decreased CMA activity, promoting tubular epithelial cell

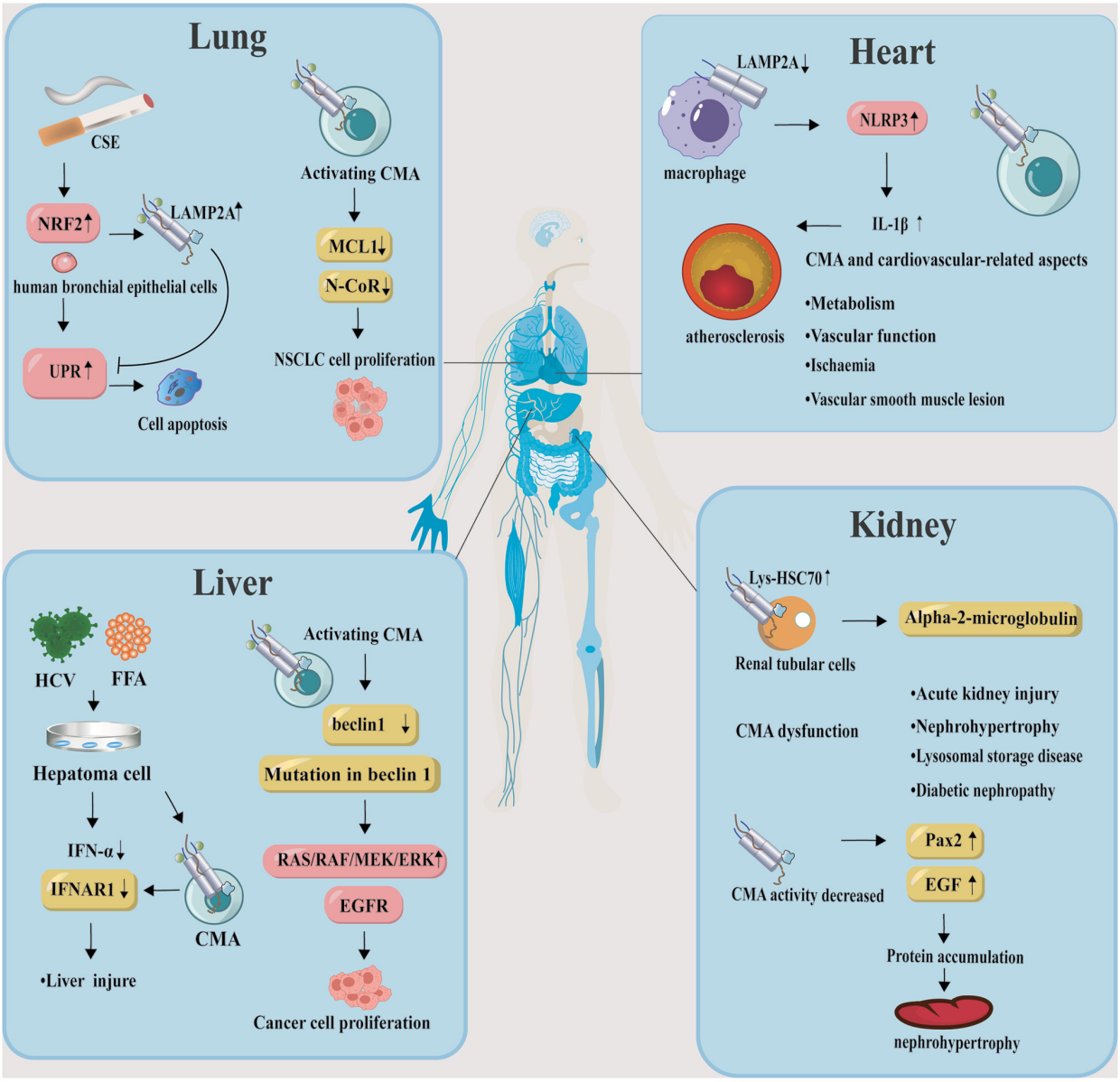

Figure 6. CMA dysfunction contributes to multiple diseases. In lung diseases, exposure to cigarette smoke extract is known to induce an increase in the UPR in human bronchial epithelial cells, thereby promoting apoptosis and ultimately contributing to the development of chronic obstructive pulmonary disease. Overexpression of LAMP2A has been shown to mitigate this UPR and can potentially reverse apoptosis. CMA enables the degradation of N-CoR, a factor that is associated with MCL1 errors, thereby promoting the survival of NSCLC cells. Macrophages are presumed to be the primary cell type expressing CMA markers in cardiovascular diseases. A reduction in CMA activity leads to the activation of the NLRP3 inflammasome in macrophages, subsequently promoting IL-1β secretion, vascular inflammation and atherosclerosis progression. Furthermore, CMA is instrumental in the functionality and metabolism of new blood vessels. Chronic HCV infection markedly increases the risk of hepatic steatosis. Co-culturing HCV with free fatty acids leads to downregulation of IFNAR1, subsequently inhibiting the antiviral activity of IFN-α and inducing hepatic steatosis. IFNAR1 is capable of colocalizing with LAMP2A for degradation through CMA. Activation of CMA results in the degradation of beclin 1, thereby inhibiting the endocytosis of EGFR and subsequently activating EGFR signaling. This causes autophagosome-lysosome damage and the activation of downstream carcinogenic signaling pathways such as RAS/RAF/MEK/ERK, eventually leading to the growth of cirrhotic hepatocellular carcinoma. Various forms of nephropathy are associated with abnormally high levels of lys-HSC70, and CMA upregulates toxic modifications in renal tubular cells in response to α2-microglobulin. Decreased activity of LAMP2A leads to increased expression of the nuclear transcription factor Pax2, thereby upregulating the proliferation of renal tubular cells and promoting hypertrophy. Macrophages are presumed to be the primary cell type expressing CMA markers in cardiovascular diseases. A reduction in CMA activity leads to the activation of the NLRP3 inflammasome in macrophages, subsequently promoting IL-1β secretion, vascular inflammation and atherosclerosis progression. Furthermore, CMA is instrumental in the functionality and metabolism of new blood vessels. Chronic HCV infection markedly increases the risk of hepatic steatosis. Co-culturing HCV with free fatty acids leads to downregulation of IFNAR1, subsequently inhibiting the antiviral activity of IFN-α and inducing hepatic steatosis. IFNAR1 is capable of colocalizing with LAMP2A for degradation through CMA. Activation of CMA results in the degradation of beclin 1, thereby inhibiting the endocytosis of EGFR and subsequently activating EGFR signaling. This causes autophagosome-lysosome damage and the activation of downstream carcinogenic signaling pathways such as RAS/RAF/MEK/ERK, eventually leading to the growth of cirrhotic hepatocellular carcinoma. Various forms of nephropathy are associated with abnormally high levels of lys-HSC70, and CMA upregulates toxic modifications in renal tubular cells in response to α2-microglobulin. Decreased activity of LAMP2A leads to increased expression of the nuclear transcription factor Pax2, thereby upregulating the proliferation of renal tubular cells and promoting hypertrophy. The figure was generated using Adobe Illustrator 2021, version 25.0, by Adobe Inc. CSE, cigarette smoke extract; CMA, chaperone-mediated autophagy; NRF2, nuclear factor, erythroid 2; LAMP2A, lysosome-associated membrane protein 2A; UPR, unfolded protein response; MCL1, myeloid cell leukemia 1; N-CoR, nuclear receptor co-repressor; NSCLC, non-small cell lung cancer; NLRP3, NLR family pyrin domain containing 3; IL-1β, interleukin 1 beta; HCV, hepatitis C virus; FFA, free fatty acid; IFN-α, interferon alpha; IFNAR1, interferon alpha and beta receptor subunit 1; EGFR, epidermal growth factor receptor; HSC70, heat shock cognate 70; Pax2, paired box 2.

growth and maintaining the hypertrophic state (211). In conditions such as diabetes or acidosis, protein degradation in the kidney is inhibited. The growth factor in diabetic nephropathy, epidermal growth factor, does not affect the proteasomal degradation pathway, implying that 30% of kidney proteins possess the CMA targeting sequence KFERQ (212). In acute rat diabetic models, streptozotocin-induced reductions in renal cortical protein degradation were observed, along with increases in the quantity of KFERQ-containing proteins. *In vivo*, CMA protein degradation levels were inhibited (211), suggesting that a reduction in CMA may primarily underlie diabetic renal hypertrophy.

In lysosomal storage disorders (LSDs), the accumulation of metabolites leads to cellular dysfunction. Cystinosis, an LSD, is caused by defects in the cystine transporter protein cystinosin (CTNS), primarily affecting the kidney. In cystinosis, Rab11 and RILP are significantly downregulated, resulting in decreased stability of LAMP2A and its mislocalization, which disrupts CMA autophagic balance and directly impacts organ function in patients. The application of CMA activators in CTNS-deficient mice led to an increase in Rab11 expression and transport, suggesting that upregulating CMA could be a potential therapeutic strategy for cystinosis (213). Oxidative stress, production of ROS and accumulation of lipid peroxidation are key factors in acute kidney injury. CMA activation serves as a primary response mechanism to oxidative stress and is closely linked to ferroptosis. However, additional research is required to elucidate the interplay between CMA and renal diseases (Fig. 6).

Pulmonary diseases. Chronic obstructive pulmonary disease (COPD) is primarily caused by smoke exposure, which amplifies both macroautophagy and CMA in mice (214). Exposure to smoke leads to oxidative modifications in pulmonary proteins, resulting in the accumulation of misfolded proteins and intensified unfolded protein response (UPR). This process is closely associated with the development of COPD and pulmonary inflammation (215). Therefore, CMA may play a crucial role in the pathogenesis of COPD. Cigarette smoke extract (CSE) induces LAMP2A expression and CMA activation in human bronchial epithelial cells via an Nrf2-dependent mechanism. Knockdown of LAMP2A intensifies the UPR due to CMA inhibition and is coupled with enhanced cell apoptosis during CSE exposure. Overexpressing LAMP2A reverses this apoptosis. This suggests a potential causal association between Nrf2-regulated impairment of CMA and lung epithelial cell apoptosis mediated by an enhanced UPR, contributing to the development of COPD. Consequently, activating CMA may represent a therapeutic approach for COPD (216). The etiological mechanisms of COPD are diverse. Reduced autophagic function leads to bronchial epithelial cell senescence. Both macroautophagy and CMA are reported to have compensatory functions. Following CSE stimulation, macroautophagy activity peaks at 24 h, whereas LAMP2A activity continues for 72 h, suggesting a potential role for CMA in cell autophagy during disease progression (217). CMA is involved in the mediation of the onset of ferroptosis. The triterpenoid compound CDDO and HSP90 obstruct a key complex involved in necrotic apoptosis, thus blocking ferroptosis via CMA inhibition (82). However, the exact role of CMA in regulating ferroptosis in relation to COPD pathogenesis is yet to be fully understood (Fig. 6).

6. Therapeutic strategies targeting CMA

Increasing evidence has linked CMA with the pathogenesis of various organ systems, making CMA modulation a potential therapeutic target. Therapies based on the mechanism of CMA may have therapeutic value for age-related diseases through CMA intervention. In neurodegenerative diseases, the decline of CMA has been closely associated with the abnormal aggregation of pathogenic proteins in neurons. CMA can regulate amyloid proteins, potentially serving as a therapeutic approach for AD. A previous study revealed that resveratrol, a polyphenol found in red wine, can accelerate the degradation of toxic Aβ through a CMA-dependent pathway. In a transgenic *Caenorhabditis elegans* model expressing Aβ1-42, resveratrol significantly inhibited paralysis by suppressing the expression of LAMP2A (218). Additionally, bioactive compounds derived from medicinal plants, such as dihydromyricetin and salvianolic acid B, suppress the accumulation of α-synuclein, enhance the CMA pathway, and exert anti-inflammatory effects (219). Chronic caffeine treatment counteracts pathologies induced by mutant α-synuclein, such as dopaminergic neuronal cell death, while preserving normal autophagic processes in the striatum, thereby correcting defects in macroautophagy and CMA of α-synuclein (220). MA activators such as CA77.1 and QX77 have demonstrated significant therapeutic potential by enhancing LAMP2A expression, aiding in the degradation of pathogenic proteins, and showing efficacy in models of neurodegenerative diseases (55).

CMA exhibits a dual role in cancer, making it an interesting therapeutic target. On one hand, overactivation of CMA can promote tumor cell survival and metastasis. Inhibiting CMA, for example by reducing LAMP2A levels, can suppress tumor growth and spread. Conversely, activating CMA can lead to the degradation of specific oncogenic mutants, thereby inhibiting tumor growth. For instance, ManA promotes the interaction between CMA substrates and LAMP2A, slowing tumor growth and intensifying the anti-tumorigenic action of activated CMA under hypoxia. Additionally, compounds such as chloroquine, which neutralize lysosomal pH, regulate lysosomal activity and have been applied in clinical trials for cancer treatment (221,222). However, CMA activation requires a combination of nutritional stress and autophagy blockade, which may lead to side effects.

The role of CMA in autoimmune diseases has also garnered widespread attention. In lupus erythematosus mouse models, increased levels of LAMP2A and HSC70 result in overactivation of CMA. The immunomodulatory agent phosphorylated peptide P140 binds to HSPA8, targeting CMA, and demonstrates potential as an inhibitor for lupus treatment. P140 has shown promising results in phase II and III clinical trials, indicating its efficacy and safety in reducing lupus disease activity and maintaining remission (223,224).

CMA is crucial for the self-renewal and differentiation of stem cells. Studies on embryonic and adult stem cells may lay the foundation for potential new treatment methods and applications in clinical cell therapy. In metabolic diseases,

the regulation of CMA also holds potential therapeutic value. Metformin, a commonly used type 2 diabetes medication, is a novel activator of CMA, promoting the phosphorylation of the key CMA mediator HSC70 via the TAK1-IKKα/β signaling cascade. Enhancing CMA activity can improve metabolic disorders, reduce fat accumulation, and thereby benefit diabetes and related metabolic diseases (225,226).

As the understanding of the CMA mechanism deepens, more innovative CMA modulators are being developed. For example, the natural compound trehalose modulates autophagy via transient lysosomal enlargement and membrane permeabilization, enhancing LAMP2A expression (225). These modulators reveal potential not only in laboratory studies but also for future clinical applications.

Overall, research on CMA as a therapeutic target continues to deepen, and there is hope that precise regulation of the CMA pathway will provide new therapeutic strategies for various diseases. However, translating these research findings into clinical treatments still presents significant challenges, requiring more clinical trials and studies to verify their safety and efficacy. By continuously exploring the biological functions of CMA and its roles in different diseases, researchers can aim to open new therapeutic avenues and improve patient outcomes.

7. Discussion

Backer and Dice (30) were the first to elucidate the selective autophagy process of CMA based on the KFERQ motif. Subsequently, the work of Cuervo and Dice (35) highlighted the crucial roles of the chaperone-substrate complex and LAMP2A in the CMA pathway. Recent advancements have shed light on the molecular mechanisms and pathophysiological implications of CMA. Protein quality control is fundamental to CMA, orchestrating various cellular functions by regulating the quality and abundance of target proteins. At the molecular level, CMA plays a crucial role in maintaining organismal homeostasis, cellular energy balance and protein degradation mechanisms. Alterations in CMA activity can predispose individuals to disease pathogenesis. Although current therapeutic targets for autophagy in human diseases are limited, methodologies for CMA research are undergoing refinement. Kaushik and Cuervo (55) provided detailed assays for assessing CMA functionality in cells and tissues. Assessing LAMP2A levels in cultured cells or tissues is one of the most effective and reliable methods for measuring CMA activity. Enhanced CMA activity positively correlates with elevated levels of lysosomal LAMP2A and HSC70. Another diagnostic technique involves measuring the translocation and degradation of CMA substrates in purified CMA-rich lysosomes from target cells or tissues (227). Substrate turnover can be detected by incubating intact lysosomes with CMA substrates in the absence of exogenous lysosomal protease inhibitors. Subsequent immunoblotting and quantification enable the identification of lysosomes specific to CMA. The use of fluorescent CMA reporter genes aids in assessing CMA substrate delivery and degradation (37). Dong *et al* (228), using a transgenic mouse model expressing a CMA reporter, validated its effectiveness for spatiotemporal monitoring of CMA at the cellular and tissue levels. This image-based approach enables the differentiation of CMA activity disparities, even in organs where hepatocytes predominate by 80%, as shown in the KFERQ-Dendra mouse model.

CMA is a specialized type of autophagy, differing significantly from other autophagic pathways in substrate selectivity and mechanism. The past decade has significantly enhanced the present understanding of the intricate molecular underpinnings of CMA and its wide-ranging physiological and pathophysiological implications. At the core of CMA is its unique mechanism of substrate recognition. Understanding the precise determinants of this specificity can inform therapeutic strategies targeting CMA activity manipulation. The dynamic regulation of LAMP2A, including its rapid turnover and factors affecting its stability, has been a focus of intense study, shedding light on how CMA activity is modulated under various physiological and stress conditions. In conclusion, although the understanding of molecular CMA has greatly expanded, several aspects remain unexplored. This includes identifying the full range of CMA substrates, understanding the detailed regulation of LAMP2A, and developing targeted therapeutics that precisely modulate CMA activity. As research in this field progresses, it promises to yield novel insights into cellular homeostasis mechanisms and potential therapeutic strategies for a diverse array of pathologies.

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Availability of data and materials

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Authors' contributions

JW conceptualized the study, conducted the project administration and acquired the funding. HJ wrote the original draft, reviewed and edited the manuscript, performed the visualization and supervised the study. Both authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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