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REVIEW

LAMP2A, LAMP2B and LAMP2C: similar structures, divergent roles

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ABSTRACT

LAMP2 (lysosomal associated membrane protein 2) is one of the major protein components of the lysosomal membrane. There currently exist three LAMP2 isoforms, LAMP2A, LAMP2B and LAMP2C, and they vary in distribution and function. LAMP2A serves as a receptor and channel for transporting cytosolic proteins in a process called chaperone-mediated autophagy (CMA). LAMP2B is required for autophagosome-lysosome fusion in cardiomyocytes and is one of the components of exosome membranes. LAMP2C is primarily implicated in a novel type of autophagy in which nucleic acids are taken up into lysosomes for degradation. In this review, the current evidence for the function of each LAMP2 isoform in various pathophysiological processes and human diseases, as well as their possible mechanisms, are comprehensively summarized. We discuss the evolutionary patterns of the three isoforms in vertebrates and provide technical guidance on investigating these isoforms. We are also concerned with the newly arising questions in this particular research area that remain unanswered. Advances in the functions of the three LAMP2 isoforms will uncover new links between lysosomal dysfunction, autophagy and human diseases.

Abbreviation: ACSL4: acyl-CoA synthetase long-chain family member 4; AD: Alzheimer disease; Ag: antigens; APP: amyloid beta precursor protein; ATG14: autophagy related 14; AVSF: autophagic vacuoles with unique sarcolemmal features; BBC3/PUMA: BCL2 binding component 3; CCD: C-terminal coiled coil domain; CMA: chaperone-mediated autophagy; CVDs: cardiovascular diseases; DDIT4/REDD1: DNA damage inducible transcript 4; ECs: endothelial cells; ER: endoplasmic reticulum; ESCs: embryonic stem cells; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GBA/β-glucocerebrosidase: glucosylceramidase beta; GSCs: glioblastoma stem cells; HCC: hepatocellular carcinoma; HD: Huntington disease; HSCs: hematopoietic stem cells; HSPA8/HSC70: heat shock protein family A (Hsp70) member 8; IL3: interleukin 3; IR: ischemia-reperfusion; LAMP2: lysosomal associated membrane protein 2; LDs: lipid droplets; LRRK2: leucine rich repeat kinase 2; MA: macroautophagy; MHC: major histocompatibility complex; MST1: macrophage stimulating 1; NAFLD: nonalcoholic fatty liver disease; NFE2L2/NRF2: NFE2 like bZIP transcription factor 2; NLRP3: NLR family pyrin domain containing 3; PARK7: Parkinsonism associated deglycase; PD: Parkinson disease; PEA15/PED: proliferation and apoptosis adaptor protein 15; PKM/PKM2: pyruvate kinase M1/2; RA: rheumatoid arthritis; RARA: retinoic acid receptor alpha; RCAN1: regulator of calcineurin 1; RCC: renal cell carcinoma; RDA: RNautophagy and DNautophagy; RNAi: RNA interference; RND3: Rho Family GTPase 3; SG-NOS3/eNOS: deleterious glutathionylated NOS3; SLE: systemic lupus erythematosus; TAMs: tumor-associated macrophages; TME: tumor microenvironment; UCHL1: ubiquitin C-terminal hydrolase L1; VAMP8: vesicle associated membrane protein 8

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Introduction

LAMP2 (lysosomal associated membrane protein 2) is one of the important components of the lysosomal membrane, together with LAMP1, accounting for approximately 50% of all proteins of the lysosome membrane [1]. LAMP2 seems to have more specific functions because LAMP2 single deficiency has more severe consequences than LAMP1 single deficiency [1]. In humans, LAMP2 undergoes alternative splicing of exon 9, leading to the isoforms LAMP2A, LAMP2B, and LAMP2C, which differ primarily in the sequence of their transmembrane and cytosolic tail (Figure 1). The three isoforms are distributed in a cell- and tissue-specific pattern [2,3]. Although both LAMP1 and LAMP2 are abundant and ubiquitous, the latter may play

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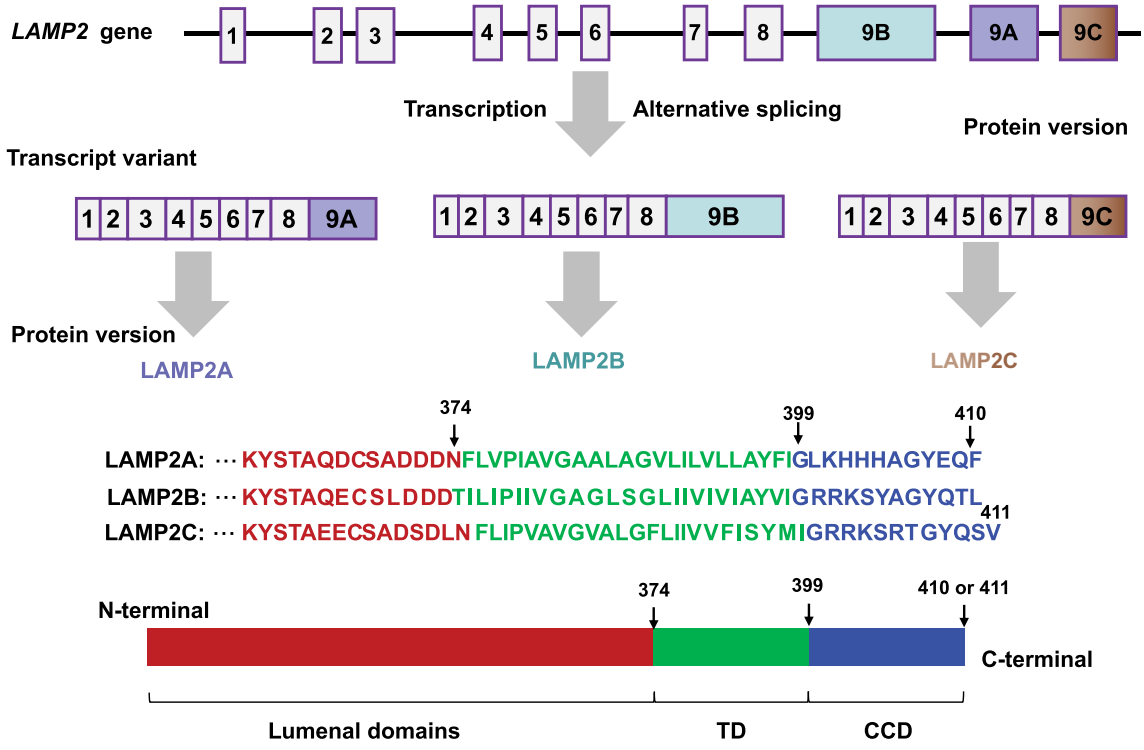

Figure 1. Schematic drawing of the structure of human LAMP2. The human LAMP2 gene has nine exons. Alternative splicing of pre-*LAMP2* mRNA produces three isoforms: LAMP2A, LAMP2B and LAMP2C. The three isoforms have identical lumenal domains (aa 1 to aa 374, red) at the N terminus but distinct transmembrane (TD, aa 375 to aa 398, green) and C-terminal cytoplasmic domains (CCD, aa 399 or aa 340 to the C terminus for humans, blue).

more specific roles in certain cellular events due to the distinct distribution and function of the three LAMP2 isoforms [4].

LAMP2 was originally associated with Danon disease, an X chromosome-linked genetic disorder clinically characterized by the triad of hypertrophic cardiomyopathy, myopathy, and intellectual disability [5,6]. This theory is further substantiated by the finding that *lamp2*-deficient mice manifest similar features to Danon patients [5,6]. Recently, a dramatic increase in studies has focused on a single isoform of LAMP2, especially LAMP2A. In most cases, each of these isoforms fulfills specialized functions. Interestingly, they were all involved in autophagy (Figure 2). LAMP2A serves as a receptor and a protein channel on lysosomes for chaperone-mediated autophagy (CMA), a highly selective form of autophagy that imports cytosolic proteins bearing a specific CMA motif to lysosomes for degradation [7–9]. LAMP2B mediates a unique autophagosome-lysosome fusion mechanism in cardiomyocytes, which has important implications in regulating mitochondrial and contractile function [10,11]. As an exosomal transmembrane protein, LAMP2B has been widely used to manufacture engineered exosomes for targeted drug delivery [12]. Another isoform, LAMP2C, has been shown to play a role in RNautophagy and DNautophagy (RDA), in which RNA and DNA are directly taken up by lysosomes in an ATP-dependent manner and degraded [13–15]. Understanding how LAMP2 works in organisms provides insight into the pathogenesis of some human diseases and indicates new therapeutic targets and treatment strategies.

Lamp2a

LAMP2A is identified as the rate-limiting component of CMA and, in fact, is exclusive for CMA [7,8]. CMA is a special form of autophagy that is distinct from macroautophagy. The function of CMA is more specific, as its substrates are only special proteins containing KFERQ-like motifs, which are specifically recognized and bound by HSPA8/HSC70 (heat shock protein family A (Hsp70) member 8) to form HSPA8-substrate complexes [16]. The 12-amino-acid cytosolic tail of LAMP2A is required for lysosomal docking of HSPA8-substrate complexes [7,17]. Blockage of LAMP2A remains, to date, the most specific way to inhibit CMA [17]. LAMP2A-mediated CMA plays an important role in maintaining the homeostasis of amino acids in cells because approximately 30% of proteins in mammalian cells are CMA substrates [17]. Although LAMP2A is detectable in almost all mammalian cells, it varies among cell types and tissues, suggesting possible differences in the role of CMA in these cells. LAMP2A is highly expressed in tissues or organs, including the placenta, lung and liver; expressed at low levels in the kidney, pancreas, cardiac muscle and skeletal muscle; and expressed at high levels in macrophages, T cells, hepatocytes, hematopoietic stem cells (HSCs), embryonic stem cells (ESCs), tumor cells, nerve cells and fibroblasts [17]. CMA dysfunction has been linked with the pathogenesis of certain diseases, such as cardiovascular diseases, aging, neurodegenerative diseases, cancer and metabolic syndrome, *in vivo* using *Lamp2a* knockout mice or medaka (Table 1).

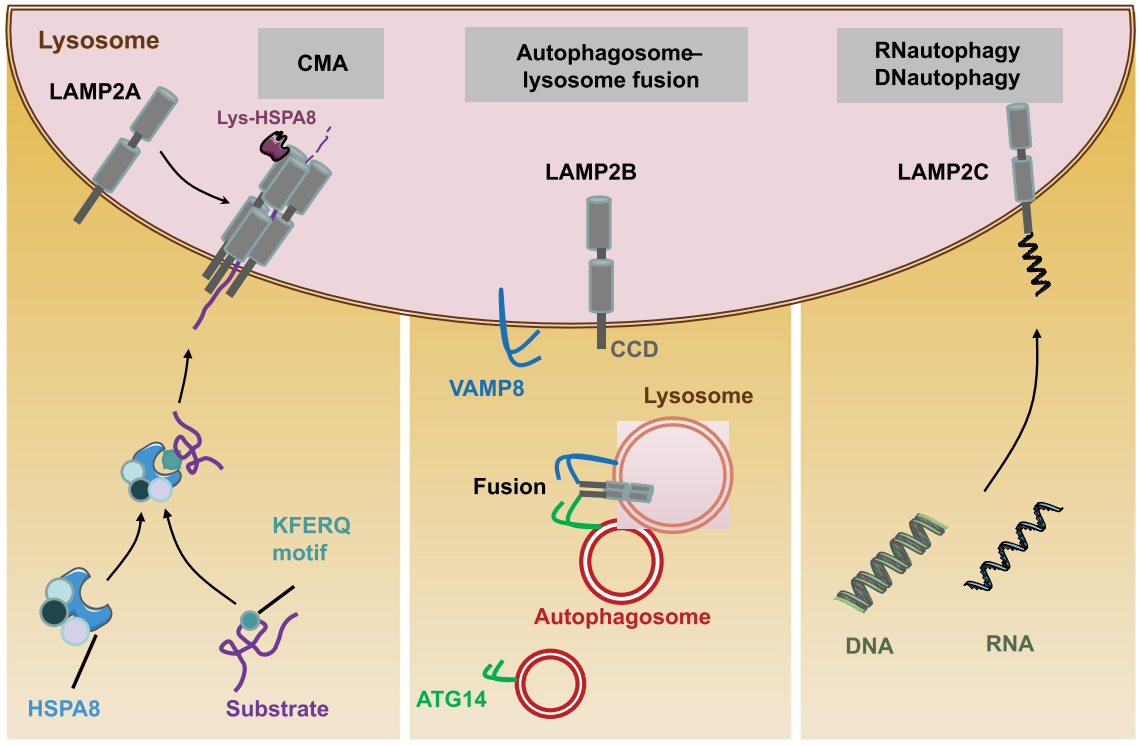

Figure 2. The role of three LAMP2 isoforms in autophagy. LAMP2A serves as a receptor and channel for transporting cytosolic proteins in a process called chaperone-mediated autophagy (CMA). LAMP2B regulates human cardiomyocyte function by mediating autophagosome-lysosome fusion by interacting with ATG14 and VAMP8 through its C-terminal coiled coil domain (CCD). LAMP2C mediates a novel type of autophagy targeting RNA or DNA for lysosomal degradation, termed RNautophagy and DNautophagy, respectively.

LAMP2A and cardiovascular diseases

Although studies have shown a strong link between macroautophagy and cardiovascular diseases (CVDs) for more than a decade, the role of CMA in cardiovascular disease has only been studied in the last two years [18,19,33]. LAMP2A was highly expressed in macrophages in atherosclerotic plaques but less expressed in other types of cells, including smooth muscle cells and endothelial cells. The protein level of LAMP2A gradually decreased in atherosclerotic lesions during atherosclerosis progression [18,19]. A decline in LAMP2A was also observed in advanced lesions in humans [18], indicating that CMA function was impaired. Importantly, macrophage LAMP2A-deficient mice have accelerated atherosclerosis progression, probably by increased NOD-like receptor (NLR) family, pyrin domain-containing protein 3 (NLRP3) inflammasome activation [18] and lipid accumulation [33]. LAMP2A deficiency also promotes the dedifferentiation of vascular smooth muscle cells, which increases plaque vulnerability to proatherosclerotic challenges [19].

In addition, two recent studies have also revealed the involvement of CMA in myocardial ischemia-reperfusion (IR) injury [34,35]. Subramani et al reported that CMA in endothelial cells (ECs) may serve as a protective mechanism to remove deleterious glutathionylated NOS3/eNOS (SG-NOS3) to prevent further IR damage, although this process contributes to an unwanted side effect that is the irreversible loss of NOS3 [34]. Another group showed that LAMP2A was significantly increased in ischemic heart failure patient samples and that overexpression of LAMP2A in primary rat neonatal cardiomyocytes reduced hypoxia-induced cardiomyocyte injury [35]. These studies showed a strong link between CMA and CVDs. Future studies are needed to elucidate the mechanisms that lead to alterations in CMA activity in CVDs.

LAMP2A and aging

Aging is the main risk factor associated with neurodegenerative diseases, cancer and CVDs. Anti-aging now becomes the eternal goal of people. In addition to intrinsic genetic timing mechanisms, aging is also related to an increased half-life of numerous proteins and the accumulation of damaged or abnormal proteins inside cells [36]. Failure in protein homeostasis has been proposed to lead to common age-related human disorders, such as Parkinson disease (PD) and Alzheimer disease (AD) [37]. As CMA is responsible for the selective degradation of cytosolic proteins and maintenance of protein homeostasis, it is reasonable to establish a link between CMA and aging.

The first direct evidence of CMA decline during aging was presented by Cuervo et al. [38]. They found a progressive decrease in the levels of LAMP2A and CMA activity in lysosomes isolated from aged rat liver as well as from cultured late-passage human fibroblasts [38]. They also demonstrated that the reduced LAMP2A was due to the altered dynamics and stability of LAMP2A in the lysosomal compartment, resulting in its increased mobilization to microdomains and subsequent degradation in the lysosome [39]. Age-related decline in CMA was also verified in mouse skeletal and

Table 1. Genetic models deciphering the role of CMA in animal models.

Genetic animal model (cell specific)	CMA activity	Disease models	Effects	Mechanisms	References
lamp2a KO mice(macrophage) (macrophage)	↓	Atherosclerosis	Promotes atherosclerosis progression	Promotes NLRP3 inflammasome activation	[18]
lamp2a KO mice (macrophages and VSMCs) (macrophages and VSMCs)	↓	Atherosclerosis	Worsens atherosclerotic pathology	Promotes dedifferentiation of VSMC; Promotes a proinflammatory state in macrophages	[19]
HsLAMP2A overexpression (embryonic stem cell) (embryonic stem cell)	↑		Reduces plaque vulnerability	Systemic and vascular beneficial effects	
lamp2afl/fl Lck-Cre mice(T cells) (T cells)	↓	Immunization/ aging	Diminishes adaptive immune responses; Diminishes T cell responses	Targeted the ubiquitin ligase Itch and the calcineurin inhibitor RCAN1 for degradation	[20]
lamp2afl/fl/Alb-Cre mice(hepatocytes) (hepatocytes)	↓	Aging/ liver disease	Enhances susceptibility to oxidative stress and hepatic dysfunction	Accelerates proteostasis failure in aging	[21]
Alb-Tet-off-Lamp2a micehepatocytes) hepatocytes)	↑	Aging/ liver disease	Improves cellular maintenance and hepatic function	Improves ability to handle protein damage	[22]
lamp2afl/fl/Vav-iCre mice (hematopoietic stem cells, HSCs) (hematopoietic stem cells, HSCs)	↓	Aging/stem-cell transplantation	Impairs HSC activation	Impairs lipid and glucose metabolism	[23]
hLAMP2A ER-Cre overexpression	↑		Restore the functionality of old mouse and human HSCs	Promotes proteostasis and glucose and fatty acid metabolism in HSCs	
Nigral injection of AAV6-Lamp2a in rats	↑	Parkinson disease (PD)	Mitigates SNCA (synuclein alpha)-induced neurodegeneration in mice	Decreases total SNCA levels and diminishes aberrant SNCA species	[24]
Intrastriatal delivery of a virus expressing the fusion of mutant HTT and HSPA8 in mice	↑	Huntington Disease (HD)	Increase the life span of HD model mice	Degradation of mutant HTT (huntingtin) protein	[25]
lamp2afl/fl/Camk2a-Cre mice(neuronal cells) (neuronal cells)	↓	Alzheimer disease (AD)	Accelerates pathology in a mouse model of AD-related proteotoxicity	Regulates a subset of the proteome at risk of aggregation	[26]
Intratumor injection of shRNA against Lamp2a (lung cancers) (lung cancers)	↓	Human lung cancer xenografts in mice	Delays xenograft tumor growth; Reduces the number of cancer metastases	Reduces the metastatic capacity of lung cancer cells	[27]
lamp2afl/fl/Alb-Cre mice(hepatocytes) (hepatocytes)	↓	Hepatic steatosis	Leads to liver damage and reduces liver function; Causes hepatosteatosis	Modulates liver carbohydrate and lipid metabolism through regulated enzyme degradation	[28]
			Decreases lipid droplets breakdown	Impaired degradation of PLIN2 (perilipin 2) and PLIN3	[29]
			Increases lipid droplets in liver	Impaired degradation of PLIN5	[30]
lamp2afl/fl/PDGFR-directed-Cre mice(fibro/adipogenic progenitor cells) (fibro/adipogenic progenitor cells)	↓	Adipogenesis/ adipocyte differentiation	Impairs adipocyte differentiation at the early commitment steps; Reduces fat mass; Increases inflammation and fibrosis.	Through degradation of key regulatory signaling proteins and transcription factors that dictate proliferation, energetic adaptation, and signaling changes required for adipogenesis	[31]
lamp2a KO medaka	↓	Subjected to fasting	Exhibits significantly enlarged livers, higher levels of lipid droplets and reduced glycogen content in the liver	Impaired degradation of several enzymes related to carbohydrate and lipid metabolisms	[32]

cardiac muscle and in T cells [20,40]. Because LAMP2A is the rate-limiting component of CMA, CMA was blocked to better illuminate the relationship between aging and CMA dysfunction by selectively downregulating LAMP2A in vitro [41]. CMA-defective cells became more sensitive to different oxidative stresses (H_2O_2, paraquat, and cadmium), although protein turnover is compensated by activation of macroautophagy (MA), suggesting that CMA is essential for mammalian cells to guarantee cellular survival during cell injury in aging [41]. Two *in vivo* studies utilizing conditional knockout mice to selectively block CMA in T cells [20]and hepatocytes [21] provided the most definitive proof of the importance of CMA in aging. Deficient CMA with aging diminishes T-cell responses [20]. Loss of hepatic CMA was also reported to accelerate proteostasis failure in aging [21]. To determine the functional relevance of CMA decline with aging, they also analyzed the consequences of restoring the expression of LAMP2A throughout the lifespan. Restoration of LAMP2A in T cells from old mice resulted in enhancement of activation-induced responses [20]. The preservation of CMA activity in aging livers in transgenic mice improved cellular maintenance and hepatic function [22]. A recent

study by Dong et al demonstrated that CMA activity in hematopoietic stem cells (HSCs) decreased with age and that genetic or pharmacological activation of CMA can restore the functionality of old mouse and human HSCs [23]. All of these findings reveal the direct link between CMA and aging and suggest that restoration of CMA activity may represent a therapeutic tool to improve age-related diseases.

LAMP2A and neurodegenerative diseases

Intracellular accumulation of aberrant and misfolded proteins in the form of toxic multimeric complexes is the basis of most neurodegenerative disorders [42]. Over the past years, CMA malfunction has been implicated in the pathogenesis of PD [43–45], AD [46] and Huntington disease (HD) [47,48]. A series of pathogenic proteins in neurodegenerative diseases have been validated as CMA substrates, including SNCA (synuclein alpha), PARK7 (Parkinsonism associated deglycase), LRRK2 (leucine rich repeat kinase 2), UCHL1 (ubiquitin C-terminal hydrolase L1) (in PD); MAPT/Tau (microtubule associated protein tau), RCAN1 (regulator of calcineurin 1) and APP (amyloid beta precursor protein) (in AD); and HTT (huntingtin; in HD) [17,20,49–51]. The inability of dysfunctional CMA to degrade these pathogenic proteins is a common pathological basis of neurodegenerative diseases.

PD was the first human disease found to be associated with CMA malfunctioning in 2004 [44]. Lysosomal clustering and accumulation of CMA-specific LAMP2A and HSPA8 proteins were observed in the aged mutant striatum along with increased GAPDH (a CMA substrate) by immunohistochemistry of the dorsal striatum and flow cytometry of ventral midbrain cells [49]. Subsequent postmortem analysis also verified that PD brains were associated with decreased LAMP2A levels in dopaminergic cell lines and increased half-life of SNCA compared with age-matched control brain samples [43]. Another autopsy study suggested that dysregulation of CMA-mediated protein degradation occurs before substantial SNCA aggregation in PD, indicating that CMA failure is an early event in PD pathogenesis [45]. Similar to PD, CMA malfunction was also found in AD and HD, with similar failure of pathogenic protein degradation via CMA.

There is a complex tangle between these proteins and CMA in neurodegenerative diseases. For example, while wild-type SNCA was selectively translocated into lysosomes for degradation by CMA, its pathogenic variants were less obedient to this autophagic degradation [44]. This variant was poorly degraded by CMA because of its obliterated entry into the lysosomal lumen resulting from its organization into oligomeric complexes at the lysosomal surface, inhibiting both its degradation and that of other substrates [17,44,52]. Mutant MT alleles of the *GBA* gene glucosylceramidae beta) are the most common genetic risk factors for PD. A recent study showed that MT GBA at the lysosomal surface inhibits CMA, causing accumulation of CMA substrates, including SNCA [53]. Acetylation of soluble MAPT/tau is an early pathological event in neurodegeneration [54]. Under normal conditions, tau is degraded by CMA, whereas upon acetylation, it has an inhibitory effect on CMA activity and leads to tau pathology propagation in mice [54]. In summary, wild-type and mutant or modified proteins seem to have different fates for degradation by the CMA pathway. How to promote the degradation of these neurotoxic proteins through CMA represents a potential treatment for neurodegenerative diseases.

In light of the implication of CMA in pathogenic protein degradation, successful attempts have been made to upregulate CMA levels in both *in vitro* and *in vivo* studies [24–26,55,56]. Overexpression of LAMP2A through a recombinant adeno-associated virus in the rat substantia nigra activated CMA, decreased SNCA turnover, and protected against neurodegeneration in a PD rat model [24]. Another study succeeded in artificially enhancing the targeting of mutant huntingtin for CMA degradation and ameliorated the disease phenotype in the R6/2 mouse model of HD [25]. A recent study by Cuervo et al. demonstrated that chemical activation of CMA with CA77.1 ameliorated proteotoxicity-driven neurodegeneration and improved neuronal function [26]. Another study showed that metformin, a hypoglycemic drug with multiple benefits, could improve disease pathologies in an AD mouse model by activating CMA [55]. In addition to degrading neurotoxic proteins, CMA also has a protective effect on the PD model by promoting CMA-mediated NLRP3 degradation to relieve neuroinflammation [56].

Therefore, upregulation of CMA represents a promising therapeutic intervention against neurodegenerative diseases. Future efforts should be directed to elucidate the mechanisms behind the failed lysosomal translocation of pathogenic SNCA and MAPT/tau, as well as how to stabilize LAMP2A in age-related neurodegenerative disorders.

LAMP2A and cancer

In recent years, increasing numbers of connections have been established between LAMP2A and cancer biology. However, the precise role of LAMP2A in cancer is still unclear. Most studies have shown that LAMP2A is increased in tumors and is required for tumor growth [27,57–61]. LAMP2A is elevated in different types of cancer cells, as well as in human cancers of different types and origins [27,60]. For instance, LAMP2A expression was significantly increased in colorectal cancer/CRC patients and mouse models [62]. Inhibition of CMA by knocking down LAMP2A expression in CT26 colon carcinoma cells facilitated apoptosis and impeded the proliferation of CT26 cells [62]. LAMP2A knockdown hindered cell proliferation in both human lung cancer cell lines and gastric cancer cells [27,60] and reduced the size of preexisting lung tumors in mice [27]. Auzmendi-Iriarte et al. found that LAMP2A expression was enriched in patient-derived glioblastoma stem cells (GSCs), and its depletion diminished GSC-mediated tumorigenic activities [63]. Clinical sample results showed that LAMP2A correlates with poor overall survival in glioblastoma [63]. At the same time, a study by Desideri et al. have reached the opposite conclusion [64]. They showed that LAMP2A expression was downregulated in human hepatocellular carcinoma (HCC) biopsies and that LAMP2A

downregulation promotes the proliferation and migration of HCC cells [64].

Although the mechanism of CMA in tumor progression is not explicit, it probably results from the combination of several mechanisms. Tumors are hypermetabolic tissues that are highly dependent on glycolysis [17,58]. At the same time, it needs to combat hypoxia, oxidative stress and DNA damage in the tumor microenvironment [17,58]. Accordingly, the role of CMA in protein regulation provides almost all conditions needed for tumor growth [17,58]. First, the acetylated embryonic M2 isoform of pyruvate kinase (PKM2) undergoes lysosomal-dependent degradation via CMA, contributing to aerobic glycolysis and the production of glycolytic intermediates that promote tumorigenesis [65]. Second, CMA alleviated endoplasmic reticulum (ER) stress by degrading misfolded NCOR and protected cancer cells against ER stress-induced apoptosis [66]. Third, anti-proliferation proteins (such as RND3) [60], tumor suppressor proteins (such as MST1 and PEA15/PED) [67,68] and pro-apoptotic proteins (such as BBC3/PUMA) [69] have been identified as CMA substrates and can be degraded by CMA, which directly contributes to cancer cell survival [17]. This was verified in breast cancer in which inhibition of LAMP2A led to accumulation of GAPDH, AKT1 phosphorylation, generation of ROS, and induction of cellular apoptosis, as well as increased sensitivity to doxorubicin [67]. Fourth, LAMP2A may be related to cells in the tumor microenvironment (TME), such as tumor-associated macrophages (TAMs). LAMP2A is expressed in TAMs but not in tumor cells and upregulated in TAMs by tumor cells, which is associated with tumor progression and poor prognosis in breast cancer [70]. Thus, inhibiting CMA activity can be exploited as a potential therapeutic application for cancer treatment.

LAMP2A and metabolic diseases

As CMA can only target proteins for lysosomal degradation, it is supposed not to be implicated in cellular metabolism, except providing free amino acids resulting from protein breakdown. However, recent studies have demonstrated that CMA is closely associated with metabolic diseases [28–30,71–74]. CMA plays a vital role in glucose and lipid metabolism by degrading key proteins in these processes, and its dysfunction leads to the occurrence of metabolic diseases such as nonalcoholic fatty liver disease (NAFLD) and diabetes complications.

In 2014, Cuervo et al [28] first uncovered a previously unknown function of CMA in the pathogenesis of common metabolic diseases. They generated a mouse with a conditional knockout for *Lamp2a* in the liver and found that loss of CMA led to pronounced abnormalities in hepatic carbohydrate and lipid metabolism, resulting in a negative impact on the overall energetic balance of the organism [28]. Using lysosomal proteomics of both control and *lamp2a* KO mice, they further demonstrated that key enzymes in carbohydrate and lipid metabolism were degraded by CMA and that impairment of their regulated degradation caused metabolic abnormalities in CMA-defective mice [28]. In their later work, they found another mechanism that underlies the favorable effect of CMA on lipid metabolism [29]. They provide evidence that the lipid droplet (LD)-associated proteins PLIN2 (perilipin 2) and PLIN3 are CMA substrates and that their degradation through CMA promotes lipolysis [29]. In another recent study, Ma et al demonstrated that CMA activity was reduced in liver tissues from NAFLD patients and high-fat diet/HFD-fed mice. Mechanistically, PLIN5 is a CMA substrate, and its degradation through CMA is required for LD breakdown [30,71]. Thus, CMA serves as a critical upstream regulator of LD catabolism, and enhancing CMA activity is a potential target for the therapeutic development of NAFLD.

CMA is also involved in diabetes and/or diabetes complications. As early as 2004, studies by Sooparb et al. revealed a relationship between CMA and diabetic nephropathy [72]. They found that CMA activity was inhibited in diabetic nephropathy, leading to excessive protein accumulation and kidney damage [72]. Two recent studies also reported the role of CMA in diabetic retinopathy. The CMA activator Q×77was effective in preventing early diabetic retinopathy by degrading the ACSL4 protein and resisting ferroptosis [73]. CMA can also degrade the stress response protein regulated in development and DDIT4/REDD1 (DNA damage inducible transcript 4) protein, a retinal protein implicated in visual deficits in diabetic patients [74]. However, more in vivo studies are needed to confirm the relationship between CMA and diabetes or diabetes complications, especially in *lamp2a* knockout mice.

In conclusion, CMA can affect metabolic processes by degrading key enzymes in glucose and lipid metabolism. CMA may serve as a potential therapeutic target for metabolic diseases. However, CMA is mainly intervened by *lamp2a* knockout in current research due to few specific CMA agonists or inhibitors. Future studies are needed to explore CMA agonists, which may have important implications for human metabolic diseases.

LAMP2A and other diseases

CMA plays important roles in promoting immunological recognition and antigen presentation [75,76], and abnormalities in this process have been implicated in autoimmune diseases. CMA regulates T-cell activation through the targeted degradation of negative regulators of T-cell activation, and modulation of CMA activity in T cells improves T-cell function with age [20]. A recent study revealed that IgG antibodies binding to the C-terminal residues of *LAMP2A* are present in serum from patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), suggesting the involvement of CMA in autoimmune disease [77]. In addition to the abovementioned role of CMA in the maintenance of HSC function, another similar study has shown that CMA can regulate the function of ESCs [78], suggesting that CMA may be a promising therapeutic target for promoting HSC or ESC function in conditions such as aging or stem cell transplantation. CMA contributes to the rhythmic removal of clock machinery proteins and to the circadian remodeling of a subset of the cellular proteome

[79]. CMA plays an important role in adipogenesis through timely degradation of key regulatory signaling proteins and transcription factors that dictate proliferation, energetic adaptation, and signaling changes required for adipogenesis [31].

Lamp2b

Similar to LAMP2A, LAMP2B is also constitutively expressed in most tissues and cells and is especially highly expressed in skeletal muscle, cardiac muscle, and brain [80,81]. At present, there are few studies on LAMP2B. LAMP2B has been associated with *Danon* disease in earlier studies [5,6,11]. In recent years, studies have suggested that LAMP2B may be involved in cardiovascular disease and cancers and as a component of exosome membranes.

LAMP2B and Danon disease

LAMP2B is primarily associated with Danon disease, an X chromosome-linked genetic disorder, characterized by hypertrophic cardiomyopathy and myopathy [5,6,82]. In 2000, *lamp2*-deficient mice were generated, which displayed comparable vacuolar cardioskeletal myopathy, verifying that primary LAMP2 deficiency is the cause of Danon disease [6]. Although most Danon patients carry mutations that result in deficiency of all three LAMP2 isoforms, some Danon patients carry mutations that only lead to LAMP2B deficiency [83], supporting the notion that LAMP2B deficiency is both necessary and sufficient for Danon pathogenesis [11]. It is perplexing that although primary LAMP2 deficiency, more precisely, the LAMP2B isoform, was identified as the cause of Danon disease approximately 20 years ago, it is only recently that the mechanism of cardiomyopathy and autophagy dysregulation in Danon patients was found.

In the very early stages of Danon disease research, people have observed the extensive accumulation of autophagic vacuoles with unique sarcolemmal features (AVSF) in many tissues, including skeletal and cardiac muscle, liver, pancreas, spleen and kidney [6]. In 2019, Chi et al [11] provided overwhelming evidence that LAMP2B was required for autophagosome-lysosome fusion in human cardiomyocytes [11]. LAMP2B interacts with ATG14 (autophagy related 14) and VAMP8 (vesicle associated membrane protein 8) through its C-terminal coiled-coil domain (CCD) to promote autophagosome-lysosome fusion [11]. In addition, they further showed that LAMP2B deficiency in human cardiomyocytes caused mitochondrial and contractile abnormalities, verifying that LAMP2B was specifically responsible for metabolic defects in cardiomyocytes [11]. Finally, gene correction of LAMP2 mutation rescued the Danon phenotype with increased contractile force generation [11]. Another similar study by Manso et al. [10] reported that *lamp2* KO mice receiving AAV9-LAMP2B treatment had improved autophagic flux and cardiac function.

Although this study investigated the role of LAMP2B in a mouse model of Danon disease, it is reasonable to assume that LAMP2B may play an important role in other heart diseases, as the restoration of LAMP2B improves mitochondrial function and contractile function of the myocardium [10]. Future studies are needed to further explore the role of LAMP2B in heart failure, myocardial infarction, cardiomyopathy and other cardiovascular diseases.

LAMP2B and cancers

An earlier study in 2001 showed that the expression of *LAMP2B* mRNA in human colorectal tumors is significantly higher than that in normal counterparts [84], indicating that LAMP2B is related to neoplastic progression, but no further studies have confirmed a causal relationship between them. Another study reported that LAMP2B knockdown using siRNA abolished the fusion of autophagosomes with lysosomes and hindered resveratrol-induced apoptosis of human colorectal cancer DLD1 cells, suggesting that LAMP2B-mediated autophagy plays an important role in inducing cancer cell death [85]. In a recent study, Nishikawa et al [86] revealed an association between shorter survival and high expression of both LAMP2A and LAMP2B in patients with renal cell carcinoma (RCC), possibly due to the high expression of LAMP2A and LAMP2B causing tumor cells to acquire sunitinib resistance.

LAMP2B and exosomes

Although LAMP2B was first linked to Danon disease, it has attracted much attention as one of the components of exosome membranes in recent years. Exosomes are natural transport nanovesicles that can transfer signal molecules, such as proteins, DNA, mRNAs, and microRNAs, from original cells to recipient cells to facilitate cell-to-cell communication [87]. Attempts to exploit exosomes to deliver exogenous cargoes for therapeutic applications have been considered. However, targeting specific tissues or cell types while avoiding nonspecific delivery, especially to the liver, remains challenging. Currently, restructuring the transmembrane proteins of exosomes to fuse with ligands or homing peptides has been widely used to enable exosomes to target tissues or organs carrying the corresponding receptors [88,89].

Among the reported exosomal transmembrane proteins, LAMP2B has been widely used to manufacture engineered exosomes. LAMP2B was identified as an abundant protein in exosomal membranes in 2008 [90]. Alvarez-Erviti et al. [91] were the first to engineer exosomes by fusing neuron-targeting peptides to the extraexosomal N-terminus of murine LAMP2B. These targeted exosomes were able to deliver therapeutic siRNA to the mouse brain, while nonspecific uptake in other tissues was not observed [91]. Tian Y et al. [92] established a doxorubicin delivery platform to tumors by engineering immature dendritic cells/imDCs to express exosomal membrane protein LAMP2B fused to αv integrin-specific iRGD peptide. Purified exosomes from immature dendritic cells were then loaded with the chemotherapeutic drug doxorubicin. These engineered exosomes delivered doxorubicin specifically to tumor tissues and led to inhibition of tumor growth without overt toxicity [92]. In another study, Bellavia et al. [93] engineered HEK293T cells to express the exosomal protein LAMP2B fused to a fragment of IL3

(interleukin 3). IL3-LAMP2B fused exosomes loaded with imatinib were able to target chronic myeloid leukemia/CML cells and inhibit cancer cell growth [93]. Similar engineered exosomes are also used in the treatment of other diseases, such as glioblastoma [94], anaplastic thyroid carcinoma [95], stroke [96,97] and brain infections [98], myocardial infarction [88,99], myocarditis [100] and cardiac hypertrophy [101], muscle atrophy and chronic kidney disease [102–104], acute lung injury [105] and pulmonary fibrosis [106], osteoarthritis [107] and spinal cord injury [108].

Lamp2c

Compared with the ubiquitous expression of LAMP2A and LAMP2B in human tissues, LAMP2C has a more restricted tissue distribution. LAMP2C only showed notable levels in the small intestine, heart, brain and skeletal muscle but was almost undetectable in other human tissues [81]. LAMP2C has been implicated in nucleic acid degradation, presentation of cytoplasmic antigens and melanoma growth (Figure 1).

LAMP2C and nucleic acid degradation

LAMP2C was primarily implicated in a novel type of autophagy, termed "RNautophagy" [13] and "DNautophagy" [14] in 2013, in which RNA and DNA were taken up directly into lysosomes for degradation, respectively. LAMP2C serves as a receptor for this pathway, with its cytosolic tail specifically binding to RNA and DNA [13,14]. This process, ATP-dependent and unlike CMA, was independent of HSPA8 [11]. The other two isoforms, LAMP2A and LAMP2B, were not involved in RNautophagy or DNautophagy. It is well known that mRNA can be degraded by exonucleases in the cytoplasm and nucleus [109]; thus, this study is very groundbreaking because it is the first pathway identified to selectively deliver nucleic acids into lysosomes [13]. They also determined that RNutophagy contributed to at least 10% to 20% of the total amount of RNA degradation in living cells [13]. However, RNautophagy and DNautophagy were not completely abolished in lysosomes derived from *lamp2*-deficient mice, indicating that there are LAMP2-independent pathway(s) operating in RNautophagy and DNautophagy [13,14]. Further studies are needed to clarify the physiological significance of RNautophagy and DNautophagy.

LAMP2C and presentation of cytoplasmic antigens

As both macroautophagy and CMA (LAMP2A) play vital roles in surveillance of the cytoplasm that facilitates antigen (Ag) capture and MHCII presentation [110], the function of LAMP2C in B lymphocyte MHCII Ag presentation was also explored by Pérez et al [81] in a recent study. In this study, transcripts for the three LAMP2 isoforms increased with human B-cell activation via BCR cross-linking and TLR ligand exposure [81]. Among them, the relative increase in LAMP2C expression was highest in B cells during TLR activation. LAMP2C expression impacted cytoplasmic Ag presentation via MHCII, while MHCII presentation of exogenous or membrane-derived Ag was not altered [81]. In addition, MHCII presentation of cytoplasmic Ag via the CMA pathway was significantly reduced, indicating that LAMP2C may function as an endogenic negative regulator of CMA in B lymphocytes [81]. Although much needs to be learned, there is considerable knowledge of the regulation of CMA. Together with the findings elaborating LAMP2A and CMA in T cells [20,75], this research suggests a novel role for LAMP2 in lymphocyte functions and the immune system.

LAMP2C and tumor growth

Compared to the increased evidence suggesting that LAMP2A and CMA are implicated in the promotion of tumor growth, LAMP2B and LAMP2C are rarely reported in the field of cancer. Recently, the same group that illuminated that LAMP2C inhibited MHC class II presentation of cytoplasmic antigens [81] showed that LAMP2C negatively regulated melanoma growth and survival [111]. Human melanoma cells exposed to IFNG/IFN-γ (interferon gamma) exhibited a twofold to threefold induction of *LAMP2C* mRNA, with modest changes in LAMP2A and no induction of LAMP2B [111]. Melanoma cells transfected with a plasmid encoding LAMP2C displayed an increased cell cycle, greater apoptosis and necrosis [111]. Moreover, melanoma cell xenografts with increased LAMP2C expression display reduced growth in immune-compromised murine hosts [111]. Intriguingly, ectopic LAMP2C expression in melanoma cells reduced the cellular levels of LAMP2A and LAMP2B proteins and disrupted CMA and MA, as assessed by increased cellular protein levels of CMA substrates and reduced autophagic flux, respectively [111]. Therefore, the complex physical association between LAMP2A and LAMP2C in living cells may influence tumor growth by regulating autophagy. However, further studies are needed.

The relationship between the three isoforms: can they replace each other?

Based on the distinct distribution of the three isoforms, we wondered what are the benefits and whether they function independent from each other or can they be replaced reciprocally in distribution and function? For example, LAMP2A is highly expressed in the placenta, lung and liver and expressed at low levels in cardiac muscle and skeletal muscle [17]. Correspondingly, LAMP2B is highly expressed in skeletal muscle and cardiac muscle [80,81] but expressed at very low levels in the liver and colon [62]. In particular, LAMP2C has a more restricted tissue distribution. They seem to complement each other in tissue distribution. As 30% of proteins in mammalian cells are degraded by LAMP2A-mediated CMA [17], how do cells and tissues with low CMA activity handle this portion of proteins? In this case, will the other two isoforms, LAMP2B or LAMP2C, compensate for LAMP2A? and vice versa. In tissues with low LAMP2B or LAMP2C, will LAMP2A work in compensation? It seems reasonable to make such a speculation. However, some current evidence does not support this view. For example, LAMP2A is the only one of the three isoforms required for CMA [7,8].

LAMP2B is specifically responsible for metabolic defects in the cardiomyocytes of Danon patients [11]. LAMP2C is exclusive for nucleic acid degradation among the three isoforms [13,14]. The three isoforms seem to function independently from each other. In addition, the mechanism underlying the differences and functions between the three LAMP2 isoforms also needs to be explored.

In the meantime, some evidence supports the existence of cross-talk between these isoforms that allows one pathway to compensate for the other. LAMP2B showed a considerable increase in *lamp2a* knockout hepatocytes, while the levels of other LAMPs in *lamp2a*-KO mice were comparable [28]. However, it is not clear whether the increased LAMP2B can compensate for the function of LAMP2A. In addition, decreased LAMP2A and LAMP2B protein levels were observed in melanoma cells with ectopic LAMP2C expression [111]. It is difficult to clarify how LAMP2C affects the LAMP2A protein because the levels of *LAMP2A* mRNA were unchanged in melanoma cells with ectopic LAMP2C, suggesting alterations in the posttranscriptional regulation of LAMP2A molecules. Another study by Pérez L et al [81] also showed that increased LAMP2C expression in B cells perturbed CMA. Therefore, there may be an endogenous regulatory mechanism among these three subtypes. A great deal of research is needed in the future to elucidate the relationship between the three isoforms and how they function independently and, in some cases, are related to each other.

The evolutionary patterns of the three isoforms

LAMP2 is an integral membrane protein with two lumenal domains (constituting 90% of the entire protein) at the N-terminal region, located inside the lysosome, a single transmembrane domain (TD; approximately 24 amino acids) to anchor LAMP2 to the lysosomal membrane, and a short (12 or more amino acids) C-terminal cytoplasmic tail domain (CCD) to recognize the target protein [1,2]. Jalali et al. [112] conducted a bioinformatics study of the molecular evolution of LAMP2 from nine major placental mammalian orders. Sequence analysis of the complete genomes of multiple species has revealed varying degrees of conservation in the *LAMP2* gene across mammals, with moderate conservation in the lumenal domains and high conservation in the TD and CCD [112]. The least conserved region is the region between amino acids 100 and 150 (numbered according to the *H. sapiens LAMP2* coding sequence), with a hotspot in the 135–144 interval in the N-domain lumenal domain of LAMP2 [112]. This variational region is possibly involved in the functional flexibility of LAMP2 and could suggest a regulatory function for this domain. In contrast, the TD (375–398) and CCD (401–410 *H. Sapiens*) of all three LAMP2 isoforms are highly conserved across mammals [112]. Thus, alterations in the TD and CCD are supposed to yield more severe functional implications compared to those in other domains. Specifically, the GYXXX sequences located at the CCD are required for targeting LAMP2 to the lysosomal membrane [113,114] and are highly conserved among tetrapod clades, with GYEQF, GYQTL and GYQSV present at the C-terminus of LAMP2A, LAMP2B and LAMP2C, respectively. A consensus motif of GXXXXXXG is situated within the CCD, which encompasses positively charged residues R, K, and H flanked by two glycine (G) residues. Positively charged residues within this motif play crucial roles in binding to the target protein through electronic interactions with negatively charged residues on the target sequence [8]. In LAMP2A, these residues consist of lysine (K) and arginine (R) followed by two histidines (HH) in most mammalian species, denoted as KRHH. In a small number of other mammals (such as *H. sapiens*) and some bats, it is KHHH. In LAMP2B and LAMP2C, the positively charged residues are RRK, which has remained completely conserved in mammals.

The TDs display a high frequency of hydrophobic amino acids necessary for lysosomal membrane anchorage [115]. Inactive LAMP2A forms homotrimers, while upon CMA activation, higher-order oligomers of LAMP2A are formed in the lysosomal membrane, which are necessary for substrate protein translocation across the lysosomal membrane [116]. The motif GXXXXG (382–387 *H. sapiens*), known as the dimerization motif, is essential for the multimerization of LAMP2A [117]. The two glycines (G) are completely conserved across mammals, while only one G is found in some fish species, including *Oryzias latipes*. In most mammals, the motif is GAALAG, which consists of four hydrophobic amino acids located between two glycines (G). In some other mammalian species, including *Mus musculus* and *Rattus norvegicus*, and in *Gallus gallus*, the hydrophobic amino acids are AAL. In LAMP2B and LAMP2C, the similar GXXXXG motifs are GAGLSG and GVALG, respectively. Although it remains unclear whether the two motifs exert similar functions in LAMP2B and LAMP2C, they exhibit a high degree of conservation across mammalian species.

It is now clearly established that macroautophagy is an evolutionarily conserved mechanism responsible for degrading cellular proteins and organelles in eukaryotes [118]. Macroautophagy appeared "early" during evolution, as it has been identified across a wide range of eukaryotic groups, including plants, amoebozoa, yeasts and metazoa [118]. In contrast, CMA appears "late" in evolution [119]. CMA was once believed to be restricted to mammals and birds due to the lack of LAMP2A or any discernible LAMP2A protein beyond the tetrapod clade [120]. Recently, Lescat et al. [32] opened up new vistas in this direction. The authors first demonstrated that the LAMP2 gene and its structure containing the three alternatively spliced exons are not restricted to mammals or birds but also exist in the genome of various fish species, suggesting that LAMP2 emerged concomitantly with the origin of the vertebrate lineage [32]. They identified expressed sequences in several fish species that displayed high homology to the TD and CCD of mammalian LAMP2A [32]. In addition, using a photoactivable (PA) KFERQ-PA-mCherry1 fluorescent reporter, they further proved that a fibroblast cell line from medaka fish (*Oryzias latipes*) displayed CMA activity [32]. More importantly, *lamp2a* knockout medaka display severe alterations in carbohydrate and fat metabolism, consistent with what has been observed in the liver of mice deficient for CMA [32,68]. These findings suggest that CMA emerged at the root of the

LAMP2A

	Lumenal domains	Transmembrane domain	Cytoplasmic tail domain
H. Sapiens	...YSTAQDCSADDDN	FLVPIAVGAALAGVLILVLLAYFI	GLKHHHAGYEQF
G. gorilla	... YSTAQDCSADDDN	FLVPIAVGAALAGVLILVLLAYFI	GLKHHHAGYEQF
M. mulatta	...YSTAQDCSADDDN	FLVPIAVGAALAGVLILVLLAYFI	GLKRHHAGYEQF
M. musculus	...YSTAQDCSADEDN	FLVPIAVGAALGGVLILVLLAYFI	GLKRHHTGYEQF
G. gallus	...KFSIAEDCSPEVDY	FIVPIAVGAALGGLVVLVIMAYFL	GHKKHHNTGYEQF
X. tropicalis	...ETFATAEECFAEQN	FIVPIVVGAALGALVILVTVAYFI	GRRKQHSAGYEQM
O. latipes	...FSTAEECQGDAES	FLVPIAVGVALLVLIAVVVVAFFI	GRRRNMATGYESF

LAMP2B

	Lumenal domains	Transmembrane domain	Cytoplasmic tail domain
H. Sapiens	... YSTAQECSLDDDT	ILIPIIVGAGLSGLIIVIVIAYVI	GRRKSYAGYQTL
G. gorilla	... YSTAQDCSADDDN	ILIPIIVGAGLSGLIIVIVIAYVI	GRRKSYAGYQTL
M. mulatta	...YSTAQDCSADDDN	ILIPIIVGAGLSGLIIVIVIAYVI	GRRKSYAGYQTL
M. musculus	... YSTAQECSLDDDT	ILIPIIVGAGLSGLIIVIVIAYVI	GRRKSYAGYQTL
G. gallus	...FSIAQECSLDDDT	ILIPIVVGAALAGLIVIIVIAYII	GRRKSYAGYQTL
X. tropicalis	...FATAQQCSLDDDS	ILIPIVVGAALAGLIVIIVIAYII	GRRKGYSGYQTL
O. latipes	...FSTAHECSLDDTS	ILIPIIVGAALAVLILIVVIAYVI	GRRKTYVGYQTL

LAMP2C

	Lumenal domains	Transmembrane domain	Cytoplasmic tail domain
H. Sapiens	... YSTAEECSADSDLN	FLIPVAVGVALGFLIIVVFISYMI	GRRKSRTGYQSV
G. gorilla	... YSTAEECSADSDLN	FLIPVAVGVALGFLIVVVFISYMI	GRRKSRTGYQSV
M. mulatta	... YSTAEECSADSDLN	FLIPVAVGVALGFLIIVVFISYMI	GRRKSRTGYQSV
M. musculus	... YSTAEECAADSDLN	FLIPVAVGVALGFLIIAVFISYMI	GRRKSRTGYQSV
G. gallus	... FSIAEECFADSDLN	FLIPVAVGMALGFLIILVFISYII	GRRKSRTGYQSV
X. tropicalis	... FATAEECLADSDLS	FLIPIAVGAVLVFLIILVLVSYLI	GRRKSRTGYQSV
O. latipes	... FSTAEECFLDSDLS	FLVPIAVGVALSFLIILVLISYLI	GRRKSRTGYQSV

Figure 3. Sequence variability within different functional domains of the three LAMP2 isoforms in vertebrate from six species. The amino acid sequence of the lumenal domains, transmembrane domain (TD) and C-terminal cytoplasmic domains (CCD) of LAMP2A, LAMP2B and LAMP2C. The GYXXX motif (green) located at the CCD are required for targeting LAMP2 to the lysosomal membrane, with GYEQF, GYQTL and GYQSV present at the C terminus of LAMP2A, LAMP2B and LAMP2C, respectively. The GYXXX motif (green) located at the CCD are required for targeting LAMP2 to the lysosomal membrane. Another motif GXXXXXG, situated within the CCD, encompasses positively charged residues (red) which play crucial roles in binding to the target protein. The TDs display a high frequency of hydrophobic amino acids necessary for lysosomal membrane anchorage. The motif GXXXXG (purple) located at TD, known as the dimerization motif, is essential for the multimerization of LAMP2A. The TD and CCD domains, as well as the aforementioned critical motifs, exhibit a high degree of conservation across vertebrate species for all three LAMP2 isoforms.

vertebrate lineage, much earlier in evolution than previously thought. In contrast, no homologous sequence was found in invertebrate species, suggesting that CMA is indeed restricted to vertebrates [32].

In general, the TD and CCD gradually resemble those of humans as species complexity increases in the course of vertebrate evolution from fish to amphibians, reptiles, birds and mammals (Figure 3). Taking LAMP2A as an example, the amino acid sequences of TD and CCD in *Oryzias latipes*, *Xenopus tropicalis*, *Gallus gallus*, *Mus musculus*, *Macaca mulatta* and *Gorilla gorilla* exhibited homology of 47%, 58%, 61%, 91%, 97% and 100% with *Homo sapiens* respectively (Figure 3). In mammals, LAMP2 contains three alternative splicing forms for its last exon [4]. In relatively lower vertebrates, including fish, amphibians and reptiles, LAMP2B and/or LAMP2C are present in almost all species, and their C-termini conform to the conserved GYQTL or GYQSV motif observed in mammals, while LAMP2A has only been observed in a limited number of species with distinct C-terminal sequences of GYQQF, GYEQL, GYEQM, GYESF

or GYEQFN rather than the typical sequence of GYEQF in mammals [32,121]. The distinct emergence times of the three isoforms during vertebrate evolution suggest that LAMP2B and LAMP2C may have arisen earlier than LAMP2A in vertebrates.

Methods to study the three isoforms

As the three isoforms have identical lumenal domains but distinct transmembrane and cytoplasmic domains, it is crucial to carefully choose a highly selective primary antibody that recognizes only one specific isoform. Unfortunately, there are few such highly selective primary antibodies on the market at present. Special attention should be given to the description of the immunogen provided on the company's website when choosing an antibody. Only antibodies specific to the cytosolic tail of individual LAMP2 isoforms can be distinguished. Notably, the majority of LAMP2 isoform antibodies are not knockout (KO)-validated in all species. Therefore, it is advisable to generate *lamp2a, lamp2b* and *lamp2c* knockout cells or mice separately, or use RNA interference to individually knockdown the three isoforms. Subsequently, the antibody specificity should be verified using the knockout/knockdown cells prior to conducting formal experiments. We urgently call for the development of antibodies against the cytosolic tail of each LAMP2 isoform.

How to activate or inhibit the expression of the three isoforms is another point. Currently, there are no viable chemical approaches to selectively inhibit or activate the expression of a single isoform. Despite recent advancements in the development of selective CMA activators [17], research on both CMA activators and inhibitors still has a long way to go. The most selective and efficient approach to modulate the protein expression of a specific isoform is through LAMP2 gene knockdown or knockout, which encodes for cytosolic and transmembrane domains of LAMP2A, LAMP2B or LAMP2C proteins. LAMP2A, LAMP2B, and LAMP2C are produced by alternative splicing of the last exon [8]. Genome editing of the LAMP2 splice variant can be achieved by mean of the CRISPR-Cas9 method [32,122]. For example, the human LAMP2 gene contains 9 exons; exons 1–8 and part of exon 9 encode the lumenal domain; the rest of exon 9 encodes the transmembrane and cytoplasmic domains [8]; CRISPR/Cas9 gene-editing technology was used to accurately knock out *LAMP2A* by deleting the whole exon 9a that is specific for the LAMP2A isoform from the genome in human cells [122]. In medaka, which has 10 exons, the sgRNAs were designed to target intronic sites spanning the *lamp2a* exon 10a in order to create *lamp2a* knockout medaka using CRISPR-Cas9 method [32]. *Lamp2a* conditional knockout mice are usually generated using the Cre-loxP system to conditionally disrupt exon 9 of the LAMP2 gene, which encodes the cytosolic and transmembrane domains of the LAMP2A protein [20,28]. Mice in which the exon 9a of LAMP2 is flanked by loxP sites (called $Lamp2a^{f/f}$ here) are crossed with mice expressing Cre recombinase to generate a cell-specific *lamp2a* knockout mouse [20,28]. In addition, small interference techniques can also be employed to knockdown cellular levels of LAMP2A. Similarly, the designed interference sequences should also specifically target exon 9a in human and mice cells [41].

Specifically, there are confusions in the nomenclature of LAMP2B and LAMP2C in chickens and mice. Currently, chicken and mouse LAMP2B correspond to human LAMP2C, while chicken and mouse LAMP2C correspond to human LAMP2B [4]. Given that the nomenclature of LAMP2B and LAMP2C isoforms in chicken and mouse differs from that of humans and other vertebrates, participants at the 2005 Gordon Research Conference on "Autophagy in stress, development and disease" held in Il Ciocco recommended unifying the nomenclature of these isoforms with current human nomenclature [4]. To avoid confusion with the already published papers, we once again call on follow-up researchers to pay attention to this issue.

Limitations

Collectively, unveiling the delicacy of organisms in the design of the three isoforms of LAMP2 seems quite challenging. Currently, LAMP2A has been extensively studied, whereas the other two isoforms, LAMP2B and LAMP2C, have received limited attention. Some elaborate studies have compared the effects of certain isoforms in both animal and human samples (Table 2). However, most of the research has been limited to cellular or animal experiments, with a dearth of studies on human samples, particularly concerning LAMP2B and LAMP2C, which is likely attributed to the absence of primary antibodies. The role of LAMP2A as a transport receptor for CMA is far from being molecularly understood, and there are some justified doubts about the role of CMA in general and how this process should be handled by the LAMP2A isoform. In addition, there are also reports that cast some doubt on the unique role of LAMP2 (LAMP2A) in selective protein degradation [123,124]. The classical view equates LAMP2A and CMA. Although LAMP2A is the most critical component of CMA, it is uncertain whether LAMP2A is unique to CMA. For example, protein degradation via CMA was apparently normal in LAMP1 LAMP2 double-deficient fibroblasts even under conditions where CMA was supposed to be more active (prolonged starvation in confluent cells) [123]. Another study reported that LAMP2 deficiency in the murine brain did not affect the normal steady state level of SNCA, a neuronal substrate of CMA under physiological and prolonged starvation conditions [124]. These two studies, although by knocking out *Lamp2* instead of *Lamp2a*, seemed to hint that LAMP2 is not the only receptor in CMA and there may exist alternative receptors or mechanisms for CMA besides LAMP2A.

It should also be noted that although LAMP2 deficiency caused the accumulation of autophagic vacuoles in mouse embryonic fibroblasts/MEFs and brains, possibly as a result of impaired autophagosome-lysosome fusion due to LAMP2B deficiency [11], the protein degradation rates by macroautophagy in basal or inducible conditions were not changed [123,124]. The increased accumulation of autophagic vacuoles could be attributed to impaired degradation of organelles such as mitochondria or other macromolecules such as lipids or possibly to defective export of degradation products from

Table 2. Comparison of the three LAMP2 isoforms in animal and human studies.

Diseases	Human Samples or Cells	Animal Samples or Cells	References
Atherosclerosis	LAMP2A was reduced in advanced atherosclerotic lesions of humans	LAMP2A gradually descended during atherosclerosis progression;LAMP2A knockout in macrophages accelerated atherosclerosis *LAMP2A* knockout in macrophages accelerated atherosclerosis	[18]
Ischemic heart failure	LAMP2A protein levels were significantly elevated in the heart lysates from ischemic heart failure patients	Hypoxia enhanced LAMP2A levels in rat Cardiomyocytes	[35]
Aging	LAMP2A was decreased in late PDLs (population doubling levels) of human fibroblasts	LAMP2A was decreased in lysosomes isolated from aged rat liver	[38]
Immune disease	Silencing of LAMP2A expression in human TH1 cells impaired T cell responses	Adaptive immune responses were impaired in T cells-specific lamp2a knockout mice	[20]
Parkinson disease (PD)	LAMP2A reduced in the brains of PD patients	Overexpression of LAMP2A in mice through the nigral injection mitigates SNCA (synuclein alpha)-induced neurodegeneration	[43] [45] [24]
Alzheimer disease (AD)	CMA is inhibited in early AD stages	Knockout of lamp2a in neurons in mice accelerated pathology in a mouse model of AD	[26]
Cancers	LAMP2A was upregulated in cancer cell lines and in human tumors of different types and origins;LAMP2A knockdown hindered cell proliferation both in human lung cancer cell lines and gastric cancer cells LAMP2A knockdown hindered cell proliferation both in human lung cancer cell lines and gastric cancer cells	Downregulation of LAMP2A reduced the size of lung tumors in mice;knocking down of LAMP2A facilitated apoptosis and impeded the proliferation of mice CT26 colon carcinoma cells knocking down of LAMP2A facilitated apoptosis and impeded the proliferation of mice CT26 colon carcinoma cells	[27] [60] [62]
Nonalcoholic fatty liver disease (NAFLD)	LAMP2A protein level was reduced in liver tissues from NAFLD patients	LAMP2A protein level was reduced in liver tissues from high-fat diet-fed mice;liver-specific lamp2a knockout mouse exhibited metabolic dysregulation; lamp2a-deficient fish exhibited severe alterations in carbohydrate and fat metabolisms liver-specific *lamp2a* knockout mouse exhibited metabolic dysregulation; *lamp2a*-deficient fish exhibited severe alterations in carbohydrate and fat metabolisms	[28] [30] [32]
Danon disease	Mutations in the LAMP2 gene and a deficiency of the LAMP2 protein were the cause of human Danon disease;LAMP2B deficiency in human cardiomyocytes caused mitochondrial and contractile abnormalities LAMP2B deficiency in human cardiomyocytes caused mitochondrial and contractile abnormalities	Systemic AAV9.LAMP2B injection reversed metabolic and physiologic multiorgan dysfunction in a mice model of Danon disease;LAMP2-deficient mice displayed vacuolar cardioskeletal myopathy LAMP2-deficient mice displayed vacuolar cardioskeletal myopathy	[10] [11] [6]
Cancers	LAMP2C mRNA expression was increased in human melanoma cell lines	Ectopic expression of LAMP2C decreased tumor growth in a xenograft mouse model	[111]

autophagic vacuoles to the cytoplasm. It is also possible that retarded protein degradation per autophagic vacuole was compensated by the increased number of vacuoles, and therefore, the difference in protein degradation rates between control cells and LAMP2-deficient cells was not observed [123].

As the three isoforms of LAMP2 differ primarily in the sequence of their transmembrane and cytosolic tail, more effort is required to elaborate on the nature of the interactome of the different cytoplasmic tails of the LAMP2 isoforms and how they should contribute to the different functions. What is the role of LAMP2 glycosylation (*N*- and O-glycosylation), and how does this affect isoform-specific functions? How is lysosomal docking of proteins (CMA) or nucleic acids (DNAphagy and RNAphagy) mediated by the only 12 aa long C-tail of LAMP2A and LAMP2C? In fact, it is mysterious to consider that such short and presumably unstructured domains could exert such specific functions. In addition, how far the transmembrane region(s) of LAMP2 (isoform) is/are involved in these processes is also unclear. How are different alternative splice isoforms of LAMP2 conserved among different species?

Currently, there is also a lack of information on the upstream signaling mechanisms that modulate the three isoforms, especially for LAMP2B and LAMP2C. This, on the other hand, hinders the study of the 3 isoforms. Early studies showed that lysosomal LAMP2A levels do not depend on its transcriptional level but on its degradation by CTSA (cathepsin A) in lysosomes [7,8]. However, the trial went cold because there was no follow-up research. Encouragingly, recent studies have suggested that lysosomal levels of LAMP2A can be regulated by transcription factor NFE2L2/NRF2 signaling [9] and RARA (retinoic acid receptor alpha) signaling [125]. The former signaling was reported to directly regulate the mRNA and protein levels of LAMP2A and consequently CMA activity [125], and the latter can only present evidence that LAMP2A is one of the downstream targets of this pathway [9]. Future studies should focus on the effects of these signaling pathways on the protein degradation of CMA substrates to further reinforce their regulatory effect on CMA. In the case of LAMP2B and LAMP2C, it may take a long time to reach the wealth of knowledge accumulated on LAMP2A.

Conclusions

Macroautophagy and CMA are two of the best-studied autophagic pathways that coexist in mammalian cells, both of which are vital for cellular quality control under physiological and pathological conditions [126]. Recent studies support the existence of cross-talk between these two autophagic pathways during the autophagy-lysosomal degradation process

[41,126,127]. Interestingly, LAMP2 is involved in both macroautophagy and CMA. LAMP2A serves as a receptor of CMA [17]; LAMP2B regulates autophagosome-lysosome fusion in cardiomyocytes [11]. LAMP2C mediates nucleic acid degradation via the RNautophagy-DNautophagy-lysosomal pathway [13,14]. Therefore, LAMP2 May link macroautophagy and CMA and play a critical role in human diseases.

Currently, there exists an impressive array of connections between the three isoforms and human disease, particularly with regards to LAMP2A [17]. Restoration of LAMP2A or LAMP2B levels genetically has been proven beneficial for several diseases in mouse models, such as age-related disease [22], neurodegenerative diseases [24] and Danon disease [11]. Highly selective activators for LAMP2A are needed to further investigate the role of CMA in these diseases and other metabolic disorders, such as fatty liver and atherosclerosis, which has important implications for the development of new drugs targeting CMA. Although it is not clear how far the studies regarding the 3 isoforms could go, advances in the functions of the three LAMP2 isoforms will uncover new links between lysosomal dysfunction and human diseases, which will open new avenues for basic and clinical research.

Disclosure statement

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References

[1] Eskelinen EL. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. Mol Aspects Med. 2006;27(5–6):495–502. doi: 10.1016/j.mam.2006.08.005

[2] Gough NR, Fambrough DM. Different steady state subcellular distributions of the three splice variants of lysosome-associated membrane protein LAMP-2 are determined largely by the COOH-terminal amino acid residue. J Cell Bio. 1997;137 (5):1161–1169. doi: 10.1083/jcb.137.5.1161

[3] Lichter-Konecki U, Moter SE, Krawisz BR, et al. Expression patterns of murine lysosome-associated membrane protein 2 (Lamp-2) transcripts during morphogenesis. Differentiation. 1999;65(1):43–58. doi: 10.1046/j.1432-0436.1999.6510043.x

[4] Eskelinen EL, Cuervo AM, Taylor MR, et al. Unifying nomenclature for the isoforms of the lysosomal membrane protein LAMP-2. Traffic. 2005;6(11):1058–1061. doi: 10.1111/j.1600-0854.2005.00337.x

[5] Endo Y, Furuta A, Nishino I. Danon disease: a phenotypic expression of LAMP-2 deficiency. Acta Neuropathol. 2015;129 (3):391–398. doi: 10.1007/s00401-015-1385-4

[6] Tanaka Y, Guhde G, Suter A, et al. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature. 2000;406(6798):902–906. doi: 10.1038/35022595

[7] Cuervo AM, Dice JF. A receptor for the selective uptake and degradation of proteins by lysosomes. Science. 1996;273 (5274):501–503. doi: 10.1126/science.273.5274.501

[8] Cuervo AM, Dice JF. Unique properties of lamp2a compared to other lamp2 isoforms. J Cell Sci. 2000;113(**Pt** 24):4441–4450. doi: 10.1242/jcs.113.24.4441

[9] Pajares M, Rojo AI, Arias E, et al. Transcription factor NFE2L2/NRF2 modulates chaperone-mediated autophagy through the regulation of LAMP2A. Autophagy. 2018;14(8):1310–1322. doi: 10.1080/15548627.2018.1474992

[10] Manso AM, Hashem SI, Nelson BC, et al. Systemic AAV9. LAMP2B injection reverses metabolic and physiologic multiorgan dysfunction in a murine model of Danon disease. Sci Transl Med. 2020;12(535):12(535. doi: 10.1126/scitranslmed.aax1744

[11] Chi C, Leonard A, Knight WE, et al. LAMP-2B regulates human cardiomyocyte function by mediating autophagosome–lysosome fusion. Proc Natl Acad Sci U S A. 2019;116(2):556–565. doi: 10.1073/pnas.1808618116

[12] Salunkhe S, Chitkara D, Basak M, et al. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J Control Release. 2020;326:599–614. doi: 10.1016/j.jconrel.2020.07.042

[13] Fujiwara Y, Furuta A, Kikuchi H, et al. Discovery of a novel type of autophagy targeting RNA. Autophagy. 2013;9(3):403–409. doi: 10.4161/auto.23002

[14] Fujiwara Y, Kikuchi H, Aizawa S, et al. Direct uptake and degradation of DNA by lysosomes. Autophagy. 2013;9(8):1167–1171. doi: 10.4161/auto.24880

[15] Hase K, Fujiwara Y, Kikuchi H, et al. Rnautophagy/Dnautophagy possesses selectivity for RNA/DNA substrates. Nucleic Acids Res. 2015;43(13):6439–6449. doi: 10.1093/nar/gkv579

[16] Chiang HL, Terlecky SR, Plant CP, Dice JF. A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science. 1989;246(4928):382–385. doi: 10.1126/science.2799391

[17] Kaushik S, Cuervo AM. The coming of age of chaperone-mediated autophagy. Nat Rev Mol Cell Biol. 2018;19 (6):365–381. doi: 10.1038/s41580-018-0001-6

[18] Qiao L, Ma J, Zhang Z, et al. Deficient chaperone-mediated autophagy promotes inflammation and atherosclerosis. Circ Res. 2021;129(12):1141–1157. doi: 10.1161/CIRCRESAHA.121.318908

[19] Madrigal-Matute J, de Bruijn J, van Kuijk K, et al. Protective role of chaperone-mediated autophagy against atherosclerosis. Proc Natl Acad Sci U S A. 2022;119(14):e2121133119. doi: 10.1073/pnas.2121133119

[20] Valdor R, Mocholi E, Botbol Y, et al. Chaperone-mediated autophagy regulates T cell responses through targeted degradation of negative regulators of T cell activation. Nat Immunol. 2014;15 (11):1046–1054. doi: 10.1038/ni.3003

[21] Schneider JL, Villarroya J, Diaz-Carretero A, et al. Loss of hepatic chaperone-mediated autophagy accelerates proteostasis failure in aging. Aging Cell. 2015;14(2):249–264. doi: 10.1111/acel.12310

[22] Zhang C, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. Nat Med. 2008;14(9):959–965. doi: 10.1038/nm.1851

[23] Dong S, Wang Q, Kao Y-R, et al. Chaperone-mediated autophagy sustains haematopoietic stem-cell function. Nature. 2021;591 (7848):117–123. doi: 10.1038/s41586-020-03129-z

[24] Xilouri M, Brekk OR, Landeck N, et al. Boosting chaperone-mediated autophagy in vivo mitigates α-synuclein-induced neurodegeneration. Brain. 2013;136(7):2130–2146. doi: 10.1093/brain/awt131

[25] Bauer PO, Goswami A, Wong HK, et al. Harnessing chaperone-mediated autophagy for the selective degradation of mutant huntingtin protein. Nat Biotechnol. 2010;28(3):256–263. doi: 10.1038/nbt.1608

[26] Bourdenx M, Martín-Segura A, Scrivo A, et al. Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome. Cell. 2021;184(10):2696–2714 e25. doi: 10.1016/j.cell.2021.03.048

[27] Kon M, Kiffin R, Koga H, et al. Chaperone-mediated autophagy is required for tumor growth. Sci Transl Med. 2011;3(109):109ra117. doi: 10.1126/scitranslmed.3003182
[28] Schneider JL, Suh Y, Cuervo AM. Deficient chaperone-mediated autophagy in liver leads to metabolic dysregulation. Cell Metab. 2014;20(3):417–432. doi: 10.1016/j.cmet.2014.06.009
[29] Kaushik S, Cuervo AM. Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis. Nat Cell Biol. 2015;17(6):759–770. doi: 10.1038/ncb3166
[30] Ma SY, Sun KS, Zhang M, et al. Disruption of Plin5 degradation by CMA causes lipid homeostasis imbalance in NAFLD. Liver Int. 2020;40(10):2427–2438. doi: 10.1111/liv.14492
[31] Kaushik S, Juste YR, Lindenau K, et al. Chaperone-mediated autophagy regulates adipocyte differentiation. Sci Adv. 2022;8 (46):eabq2733. doi: 10.1126/sciadv.abq2733
[32] Lescat L, Véron V, Mourot B, et al. Chaperone-mediated autophagy in the light of evolution: insight from fish. Mol Biol Evol. 2020;37(10):2887–2899. doi: 10.1093/molbev/msaa127
[33] Qiao L, Wang H-F, Xiang L, et al. Deficient chaperone-mediated autophagy promotes lipid accumulation in macrophage. J Cardiovasc Transl Res. 2021;14(4):661–669. doi: 10.1007/s12265-020-09986-3
[34] Subramani J, Kundumani-Sridharan V, Das KC. Chaperone-mediated autophagy of eNOS in myocardial ischemia-reperfusion injury. Circ Res. 2021;129(10):930–945. doi: 10.1161/CIRCRESAHA.120.317921
[35] Ghosh R, Gillaspie JJ, Campbell KS, et al. Chaperone-mediated autophagy protects cardiomyocytes against hypoxic-cell death. Am J Physiol Cell Physiol. 2022;323(5):C1555–C1575. doi: 10.1152/ajpcell.00369.2021
[36] Cuervo AM. Autophagy and aging: keeping that old broom working. Trends Genet. 2008;24(12):604–612. doi: 10.1016/j.tig.2008.10.002
[37] Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med. 2015;47(3):e147. doi: 10.1038/emm.2014.117
[38] Cuervo AM, Dice JF. Age-related decline in chaperone-mediated autophagy. J Biol Chem. 2000;275(40):31505–31513. doi: 10.1074/jbc.M002102200
[39] Kiffin R, Kaushik S, Zeng M, et al. Altered dynamics of the lysosomal receptor for chaperone-mediated autophagy with age. J Cell Sci. 2007;120(5):782–791. doi: 10.1242/jcs.001073
[40] Zhou J, Chong SY, Lim A, et al. *Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging*. Aging (Albany NY. Aging. 2017;9(2):583–599. doi: 10.18632/aging.101181
[41] Massey AC, Kaushik S, Sovak G, et al. Consequences of the selective blockage of chaperone-mediated autophagy. Proc Natl Acad Sci U S A. 2006;103(15):5805–5810. doi: 10.1073/pnas.0507436103
[42] Martinez-Vicente M, Cuervo AM. Autophagy and neurodegeneration: when the cleaning crew goes on strike. Lancet Neurol. 2007;6(4):352–361. doi: 10.1016/S1474-4422(07)70076-5
[43] Alvarez-Erviti L, Rodriguez-Oroz MC, Cooper JM, et al. Chaperone-mediated autophagy markers in Parkinson disease brains. Arch Neurol. 2010;67(12):1464–1472. doi: 10.1001/archneurol.2010.198
[44] Cuervo AM, Stefanis L, Fredenburg R, et al. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science. 2004;305(5688):1292–1295. doi: 10.1126/science.1101738
[45] Murphy KE, Gysbers AM, Abbott SK, et al. Lysosomal-associated membrane protein 2 isoforms are differentially affected in early Parkinson's disease. Mov Discord. 2015;30(12):1639–1647. doi: 10.1002/mds.26141
[46] Caballero B, Wang Y, Diaz A, et al. Interplay of pathogenic forms of human tau with different autophagic pathways. Aging Cell. 2018;17(1):17(1. doi: 10.1111/acel.12692
[47] Koga H, Martinez-Vicente M, Arias E, et al. Constitutive upregulation of chaperone-mediated autophagy in Huntington's disease. J Neurosci. 2011;31(50):18492–18505. doi: 10.1523/JNEUROSCI.3219-11.2011
[48] Qi L, Zhang X-D, Wu J-C, et al. The role of chaperone-mediated autophagy in huntingtin degradation. PLoS One. 2012;7(10):e46834. doi: 10.1371/journal.pone.0046834
[49] Ho PW, Leung C-T, Liu H, et al. Age-dependent accumulation of oligomeric SNCA/α-synuclein from impaired degradation in mutant LRRK2 knockin mouse model of Parkinson disease: role for therapeutic activation of chaperone-mediated autophagy (CMA). Autophagy. 2020;16(2):347–370. doi: 10.1080/15548627.2019.1603545
[50] Issa AR, Sun J, Petitgas C, et al. The lysosomal membrane protein LAMP2A promotes autophagic flux and prevents SNCA-induced Parkinson disease-like symptoms in the Drosophila brain. Autophagy. 2018;14(11):1898–1910. doi: 10.1080/15548627.2018.1491489
[51] Sala G, Marinig D, Arosio A, et al. Role of chaperone-mediated autophagy dysfunctions in the pathogenesis of parkinson's disease. Front Mol Neurosci. 2016;9:157. doi: 10.3389/fnmol.2016.00157
[52] Martinez-Vicente M, Talloczy Z, Kaushik S, et al. Dopamine-modified α-synuclein blocks chaperone-mediated autophagy. J Clin Invest. 2008;118(2):777–788. doi: 10.1172/JCI32806
[53] Kuo SH, Tasset I, Cheng MM, et al. Mutant glucocerebrosidase impairs α-synuclein degradation by blockade of chaperone-mediated autophagy. Sci Adv. 2022;8(6):eabm6393. doi: 10.1126/sciadv.abm6393
[54] Caballero B, Bourdenx M, Luengo E, et al. Acetylated tau inhibits chaperone-mediated autophagy and promotes tau pathology propagation in mice. Nat Commun. 2021;12(1):2238. doi: 10.1038/s41467-021-22501-9
[55] Xu X, Y Sun, Xufeng Cen, et al. Metformin activates chaperone-mediated autophagy and improves disease pathologies in an Alzheimer disease mouse model. Protein Cell. 2021;12 (10):769–787. doi: 10.1007/s13238-021-00858-3
[56] Chen J, Mao K, Yu H, et al. P38-TFEB pathways promote microglia activation through inhibiting CMA-mediated NLRP3 degradation in Parkinson's disease. J Neuroinflammation. 2021;18 (1):295. doi: 10.1186/s12974-021-02349-y
[57] Kon M, Cuervo AM. Chaperone-mediated autophagy in health and disease. FEBS Lett. 2010;584(7):1399–1404. doi: 10.1016/j.febslet.2009.12.025
[58] Kaushik S, Cuervo AM. Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends Cell Biol. 2012;22 (8):407–417. doi: 10.1016/j.tcb.2012.05.006
[59] Li W, Nie T, Xu H, et al. Chaperone-mediated autophagy: Advances from bench to bedside. Neurobiol Dis. 2019;122:41–48. doi: 10.1016/j.nbd.2018.05.010
[60] Zhou J, Yang J, Fan X, et al. Chaperone-mediated autophagy regulates proliferation by targeting RND3 in gastric cancer. Autophagy. 2016;12(3):515–528. doi: 10.1080/15548627.2015.1136770
[61] Saha T. LAMP2A overexpression in breast tumors promotes cancer cell survival via chaperone-mediated autophagy. Autophagy. 2012;8(11):1643–1656. doi: 10.4161/auto.21654
[62] Peng JQ, Han S-M, Chen Z-H, et al. Chaperone-mediated autophagy regulates apoptosis and the proliferation of colon carcinoma cells. Biochem Biophys Res Commun. 2020;522(2):348–354. doi: 10.1016/j.bbrc.2019.11.081
[63] Auzmendi-Iriarte J, Otaegi-Ugartemendia M, Carrasco-Garcia E, et al. Chaperone-mediated autophagy controls proteomic and transcriptomic pathways to maintain glioma stem cell activity. Cancer Res. 2022;82(7):1283–1297. doi: 10.1158/0008-5472.CAN-21-2161
[64] Desideri E, Castelli S, Dorard C, et al. Impaired degradation of YAP1 and IL6ST by chaperone-mediated autophagy promotes proliferation and migration of normal and hepatocellular carcinoma cells. Autophagy. 2022;19(1):1–11. doi: 10.1080/15548627.2022.2063004

[65] Lv L, Li D, Zhao D, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell. 2011;42 (6):719–730. doi: 10.1016/j.molcel.2011.04.025
[66] Ali AB, Nin DS, Tam J, et al. Role of chaperone mediated autophagy (CMA) in the degradation of misfolded N-CoR protein in non-small cell lung cancer (NSCLC) cells. PLoS One. 2011;6(9): e25268. doi: 10.1371/journal.pone.0025268
[67] Li L, Fang R, Liu B, et al. Deacetylation of tumor-suppressor MST1 in Hippo pathway induces its degradation through HBXIP-elevated HDAC6 in promotion of breast cancer growth. Oncogene. 2016;35(31):4048–4057. doi: 10.1038/onc.2015.476
[68] Quintavalle C, Di Costanzo S, Zanca C, et al. Phosphorylation-regulated degradation of the tumor-suppressor form of PED by chaperone-mediated autophagy in lung cancer cells. J Cell Physiol. 2014;229(10):1359–1368. doi: 10.1002/jcp.24569
[69] Xie W, Zhang L, Jiao H, et al. Chaperone-mediated autophagy prevents apoptosis by degrading BBC3/PUMA. Autophagy. 2015;11(9):1623–1635. doi: 10.1080/15548627.2015.1075688
[70] Wang R, Liu Y, Liu L, et al. Tumor cells induce LAMP2a expression in tumor-associated macrophage for cancer progression. EBioMedicine. 2019;40:118–134. doi: 10.1016/j.ebiom.2019.01.045
[71] Filali-Mouncef Y, Hunter C, Roccio F, et al. The ménage à trois of autophagy, lipid droplets and liver disease. Autophagy. 2022;18 (1):50–72. doi: 10.1080/15548627.2021.1895658
[72] Sooparb S, Price SR, Shaoguang J, et al. Suppression of chaperone-mediated autophagy in the renal cortex during acute diabetes mellitus. Kidney Int. 2004;65(6):2135–2144. doi: 10.1111/j.1523-1755.2004.00639.x
[73] Liu C, Sun W, Zhu T, et al. Glia maturation factor-β induces ferroptosis by impairing chaperone-mediated autophagic degradation of ACSL4 in early diabetic retinopathy. Redox Biol. 2022;52:102292. doi: 10.1016/j.redox.2022.102292
[74] Miller WP, Sha CM, Sunilkumar S, et al. Activation of disulfide redox switch in REDD1 promotes oxidative stress under hyperglycemic conditions. Diabetes. 2022;71(12):2764–2776. doi: 10.2337/db22-0355
[75] Zhou D, Li P, Lin Y, et al. Lamp-2a facilitates MHC class II presentation of cytoplasmic antigens. Immunity. 2005;22 (5):571–581. doi: 10.1016/j.immuni.2005.03.009
[76] Wang F, Muller S. Manipulating autophagic processes in autoimmune diseases: a special focus on modulating chaperone-mediated autophagy, an emerging therapeutic target. Front Immunol. 2015;6:252. doi: 10.3389/fimmu.2015.00252
[77] Wilhelm M, Bonam SR, Schall N, et al. Implication of a lysosomal antigen in the pathogenesis of lupus erythematosus. J Autoimmun. 2021;120:102633. doi: 10.1016/j.jaut.2021.102633
[78] Xu Y, Zhang Y, García-Cañaveras JC, et al. Chaperone-mediated autophagy regulates the pluripotency of embryonic stem cells. Science. 2020;369(6502):397–403. doi: 10.1126/science.abb4467
[79] Juste YR, Kaushik S, Bourdenx M, et al. Reciprocal regulation of chaperone-mediated autophagy and the circadian clock. Nat Cell Biol. 2021;23(12):1255–1270. doi: 10.1038/s41556-021-00800-z
[80] Konecki DS, Foetisch K, Zimmer KP, et al. An alternatively spliced form of the human lysosome-associated membrane protein-2 gene is expressed in a tissue-specific manner. Biochem Biophys Res Commun. 1995;215(2):757–767. doi: 10.1006/bbrc.1995.2528
[81] Pérez L, McLetchie S, Gardiner GJ, et al. LAMP-2C inhibits MHC class II presentation of cytoplasmic antigens by disrupting chaperone-mediated autophagy. J Immunol. 2016;196 (6):2457–2465. doi: 10.4049/jimmunol.1501476
[82] Maron BJ, Roberts WC, Arad M, et al. Clinical outcome and phenotypic expression in LAMP2 cardiomyopathy. JAMA. 2009;301(12):1253–1259. doi: 10.1001/jama.2009.371
[83] Nishino I, Fu J, Tanji K, et al. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature. 2000;406(6798):906–910. doi: 10.1038/35022604
[84] Furuta K, Ikeda M, Nakayama Y, et al. Expression of lysosome-associated membrane proteins in human colorectal neoplasms and inflammatory diseases. Am J Pathol. 2001;159 (2):449–455. doi: 10.1016/S0002-9440(10)61716-6
[85] Trincheri NF, Follo C, Nicotra G, et al. Resveratrol-induced apoptosis depends on the lipid kinase activity of Vps34 and on the formation of autophagolysosomes. Carcinogenesis. 2008;29 (2):381–389. doi: 10.1093/carcin/bgm271
[86] Nishikawa R, Osaki M, Sasaki R, et al. Splice variants of lysosome-associated membrane proteins 2A and 2B are involved in sunitinib resistance in human renal cell carcinoma cells. Oncol Rep. 2020;44(5):1810–1820. doi: 10.3892/or.2020.7752
[87] van Dommelen SM, Vader P, Lakhal S, et al. Microvesicles and exosomes: opportunities for cell-derived membrane vesicles in drug delivery. J Control Release. 2012;161(2):635–644. doi: 10.1016/j.jconrel.2011.11.021
[88] Wang X, Chen Y, Zhao Z, et al. Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. J Am Heart Assoc. 2018;7(15):e008737. doi: 10.1161/JAHA.118.008737
[89] Laakkonen P, Vuorinen K. Homing peptides as targeted delivery vehicles. Integr Biol (Camb). 2010;2(7–8):326–337. doi: 10.1039/c0ib00013b
[90] Simhadri VR, Reiners KS, Hansen HP, et al. Dendritic cells release HLA-B-associated transcript-3 positive exosomes to regulate natural killer function. PLoS One. 2008;3(10):e3377. doi: 10.1371/journal.pone.0003377
[91] Alvarez-Erviti L, Seow Y, Yin H, et al. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011;29(4):341–345. doi: 10.1038/nbt.1807
[92] Tian Y, Li S, Song J, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35(7):2383–2390. doi: 10.1016/j.biomaterials.2013.11.083
[93] Bellavia D, Raimondo S, Calabrese G, et al. Interleukin 3- receptor targeted exosomes inhibit in vitro and in vivo chronic myelogenous leukemia cell growth. Theranostics. 2017;7(5):1333–1345. doi: 10.7150/thno.17092
[94] Kim G, Kim M, Lee Y, et al. Systemic delivery of microRNA-21 antisense oligonucleotides to the brain using T7-peptide decorated exosomes. J Control Release. 2020;317:273–281. doi: 10.1016/j.jconrel.2019.11.009
[95] Wang C, Li N, Li Y, et al. Engineering a HEK-293T exosome-based delivery platform for efficient tumor-targeting chemotherapy/internal irradiation combination therapy. J Nanobiotechnology. 2022;20(1):247. doi: 10.1186/s12951-022-01462-1
[96] Yang J, Zhang X, Chen X, et al. Exosome mediated delivery of miR-124 promotes neurogenesis after ischemia. Mol Ther Nucleic Acids. 2017;7:278–287. doi: 10.1016/j.omtn.2017.04.010
[97] Kim M, Kim G, Hwang DW, et al. Delivery of high mobility group box-1 siRNA using brain-targeting exosomes for ischemic stroke therapy. J Biomed Nanotechnol. 2019;15(12):2401–2412. doi: 10.1166/jbn.2019.2866
[98] Zhang R, Fu Y, Cheng M, et al. sEvsrvg selectively delivers antiviral siRNA to fetus brain, inhibits ZIKV infection and mitigates ZIKV-induced microcephaly in mouse model. Mol Ther. 2022;30 (5):2078–2091. doi: 10.1016/j.ymthe.2021.10.009
[99] Mentkowski KI, Lang JK. Exosomes engineered to express a cardiomyocyte binding peptide demonstrate improved cardiac retention in vivo. Sci Rep. 2019;9(1):10041. doi: 10.1038/s41598-019-46407-1
[100] Kim H, Mun D, Kang J-Y, et al. Improved cardiac-specific delivery of RAGE siRNA within small extracellular vesicles engineered to express intense cardiac targeting peptide attenuates myocarditis. Mol Ther Nucleic Acids. 2021;24:1024–1032. doi: 10.1016/j.omtn.2021.04.018
[101] Mao L, Li Y-D, Chen R-L, et al. Heart-targeting exosomes from human cardiosphere-derived cells improve the therapeutic effect on cardiac hypertrophy. J Nanobiotechnology. 2022;20(1):435. doi: 10.1186/s12951-022-01630-3

[102] Wang B, Zhang A, Wang H, et al. MiR-26a limits muscle wasting and cardiac fibrosis through exosome-mediated microRNA transfer in chronic kidney disease. Theranostics. 2019;9(7):1864–1877. doi: 10.7150/thno.29579
[103] Wang H, Wang B, Zhang A, et al. Exosome-mediated miR-29 transfer reduces muscle atrophy and kidney fibrosis in mice. Mol Ther. 2019;27(3):571–583. doi: 10.1016/j.ymthe.2019.01.008
[104] Liu S, Lv K, Wang Y, et al. Improving the circulation time and renal therapeutic potency of extracellular vesicles using an endogenous ligand binding strategy. J Control Release. 2022;352:1009–1023. doi: 10.1016/j.jconrel.2022.11.017
[105] Kim G, Lee Y, Ha J, et al. Engineering exosomes for pulmonary delivery of peptides and drugs to inflammatory lung cells by inhalation. J Control Release. 2021;330:684–695. doi: 10.1016/j.jconrel.2020.12.053
[106] Yu Y, Liu X, Zhao Z, et al. The extracellular matrix enriched with exosomes for the treatment on pulmonary fibrosis in mice. Front Pharmacol. 2021;12:747223. doi: 10.3389/fphar.2021.747223
[107] Liang Y, Xu X, Xu L, et al. Chondrocyte-specific genomic editing enabled by hybrid exosomes for osteoarthritis treatment. Theranostics. 2022;12(11):4866–4878. doi: 10.7150/thno.69368
[108] Liu X, Zhang L, Xu Z, et al. A functionalized collagen-I scaffold delivers microRNA 21-loaded exosomes for spinal cord injury repair. Acta Biomater. 2022;154:385–400. doi: 10.1016/j.actbio.2022.10.027
[109] Houseley J, Tollervey D. The many pathways of RNA degradation. Cell. 2009;136(4):763–776. doi: 10.1016/j.cell.2009.01.019
[110] Dengjel J, Schoor O, Fischer R, et al. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc Natl Acad Sci U S A. 2005;102(22):7922–7927. doi: 10.1073/pnas.0501190102
[111] Pérez L, Sinn AL, Sandusky GE, et al. Melanoma LAMP-2C modulates tumor growth and autophagy. Front Cell Dev Biol. 2018;6:101. doi: 10.3389/fcell.2018.00101
[112] Jalali Z, Parvaz N. Molecular evolution of autophagy rate-limiting factor LAMP2 in placental mammals. Gene. 2020;727:144231. doi: 10.1016/j.gene.2019.144231
[113] Guarnieri FG, Arterburn LM, Penno MB, et al. The motif Tyr-X-X-hydrophobic residue mediates lysosomal membrane targeting of lysosome-associated membrane protein 1. J Biol Chem. 1993;268(3):1941–1946. doi: 10.1016/S0021-9258(18)53945-4
[114] Williams MA, Fukuda M. Accumulation of membrane glycoproteins in lysosomes requires a tyrosine residue at a particular position in the cytoplasmic tail. J Cell Bio. 1990;111(3):955–966. doi: 10.1083/jcb.111.3.955
[115] Rout AK, Strub M-P, Piszczek G, et al. Structure of transmembrane domain of lysosome-associated membrane protein type 2a (LAMP-2A) reveals key features for substrate specificity in chaperone-mediated autophagy. J Biol Chem. 2014;289(51):35111–35123. doi: 10.1074/jbc.M114.609446
[116] Bandyopadhyay U, Kaushik S, Varticovski L, et al. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. Mol Cell Biol. 2008;28(18):5747–5763. doi: 10.1128/MCB.02070-07
[117] Russ WP, Engelman DM. The GxxxG motif: a framework for transmembrane helix-helix association. J Mol Biol. 2000;296(3):911–919. doi: 10.1006/jmbi.1999.3489
[118] Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. Cell Death Differ. 2005;12(Suppl 2):1542–1552. doi: 10.1038/sj.cdd.4401765
[119] Tekirdag K, Cuervo AM. Chaperone-mediated autophagy and endosomal microautophagy: Joint by a chaperone. J Biol Chem. 2018;293(15):5414–5424. doi: 10.1074/jbc.R117.818237
[120] Lescat L, Herpin A, Mourot B, et al. CMA restricted to mammals and birds: myth or reality? Autophagy. 2018;14(7):1267–1270. doi: 10.1080/15548627.2018.1460021
[121] Herpin A, Lescat L, Bobe J, et al. Lighting chaperone-mediated autophagy (CMA) evolution with an ancient LAMP: the existence of a functional CMA activity in fish. Autophagy. 2020;16(10):1918–1920. doi: 10.1080/15548627.2020.1797344
[122] Yu TT, Zhou X, Vakifahmetoglu-Norberg H. CRISPR-Cas9 gene editing to generate isoform-specific LAMP-2A knockout in human cancer cells. Methods Mol Biol. 2022;2445:39–50.
[123] Eskelinen EL, Schmidt CK, Neu S, et al. Disturbed cholesterol traffic but normal proteolytic function in LAMP-1/LAMP-2 double-deficient fibroblasts. Mol Biol Cell. 2004;15(7):3132–3145. doi: 10.1091/mbc.e04-02-0103
[124] Rothaug M, Stroobants S, Schweizer M, et al. LAMP-2 deficiency leads to hippocampal dysfunction but normal clearance of neuronal substrates of chaperone-mediated autophagy in a mouse model for Danon disease. Acta Neuropathol Commun. 2015;3(1):6. doi: 10.1186/s40478-014-0182-y
[125] Anguiano J, Garner TP, Mahalingam M, et al. Chemical modulation of chaperone-mediated autophagy by retinoic acid derivatives. Nat Chem Biol. 2013;9(6):374–382. doi: 10.1038/nchembio.1230
[126] Wu H, Chen S, Ammar AB, et al. Crosstalk between macroautophagy and chaperone-mediated autophagy: implications for the treatment of neurological diseases. Mol Neurobiol. 2015;52(3):1284–1296. doi: 10.1007/s12035-014-8933-0
[127] Kaushik S, Massey AC, Mizushima N, et al. Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. Mol Biol Cell. 2008;19(5):2179–2192. doi: 10.1091/mbc.e07-11-1155