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·Review·

Chaperone-mediated autophagy and neurodegeneration: connections, mechanisms, and therapeutic implications

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Lysosomes degrade dysfunctional intracellular components *via* three pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Unlike the other two, CMA degrades cytosolic proteins with a recognized KFERQ-like motif in lysosomes and is important for cellular homeostasis. CMA activity declines with age and is altered in neurodegenerative diseases. Its impairment leads to the accumulation of aggregated proteins, some of which may be directly tied to the pathogenic processes of neurodegenerative diseases. Its induction may accelerate the clearance of pathogenic proteins and promote cell survival, representing a potential therapeutic approach for the treatment of neurodegenerative diseases. In this review, we summarize the current findings on how CMA is involved in neurodegenerative diseases, especially in Parkinson's disease.

Keywords: autophagy; chaperone-mediated autophagy; neurodegenerative disorder; Parkinson's disease; risk factors; mutant proteins; therapeutic target

Introduction

There are two major proteolytic systems in cells: the proteasome-based system and the lysosome-based system. The autophagy-lysosome pathway (ALP) is a dynamic process by which lysosomes dispose of subcellular components and has the capacity to degrade complete organelles in addition to proteins[1, 2]. This process has recently sparked great interest. Instead of being viewed as a passive process of degradation, the ALP is now recognized to be actively involved in the regulations of various cellular processes and the maintenance of cellular homeostasis. In mammals, depending on how the pathway substrates are delivered into the lysosomes, autophagy can be categorized into three main forms, macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (Fig. 1). In macroautophagy, a newly-formed isolation membrane sequesters cytosolic components and organelles that need to be degraded. This membrane then matures and seals to become a double-membrane vesicle named the autophagic vacuole[3]. This allows degradation of the contents and the inner membrane of the autophagic vacuole by the lysosome enzymes for recycling. On the other hand, in microautophagy, intracellular components are directly captured by the lysosomal membrane *via* invagination[4, 5]. The engulfed cargoes are delivered into the lumen by vesicle scission for subsequent degradation[6, 7]. Microautophagy is considered to be the form that participates in the constant removal of organelles and intracellular proteins[5]. In contrast, CMA does not require

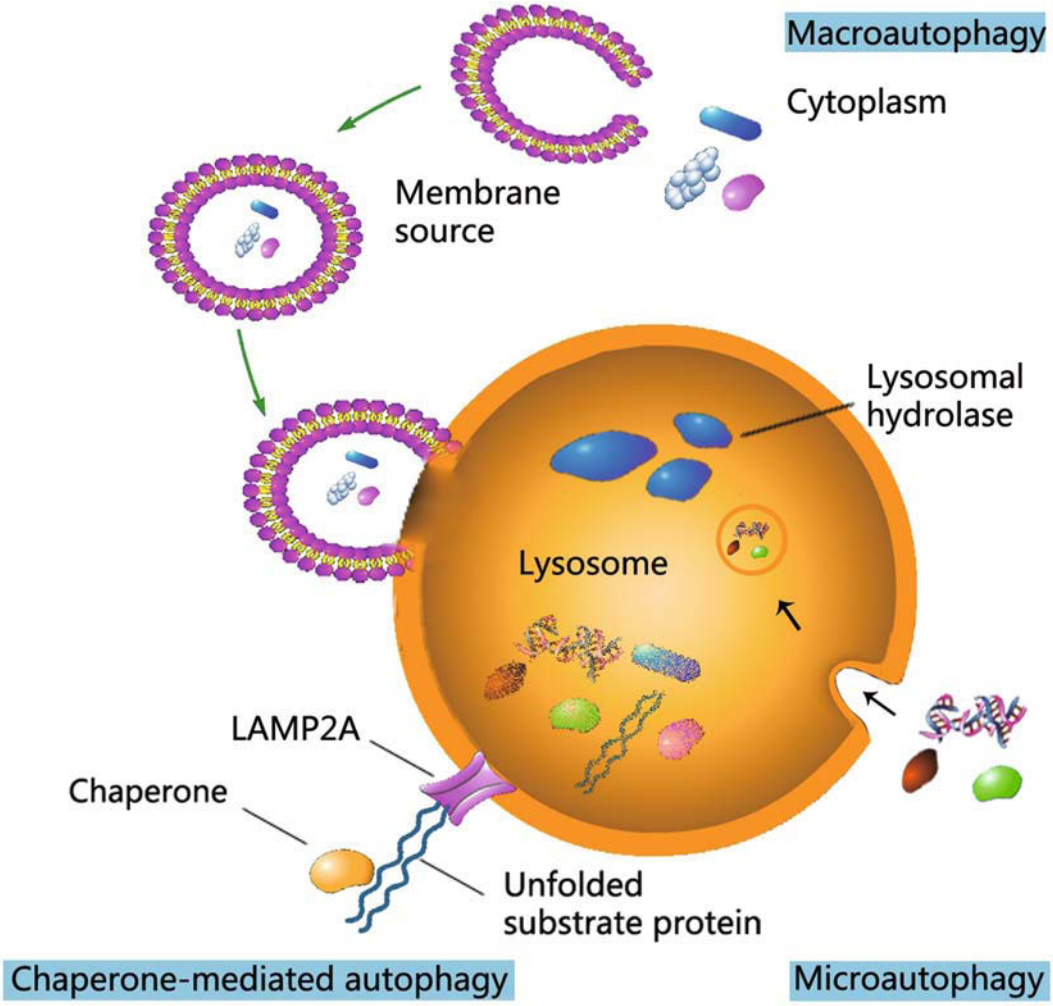

Fig. 1. A model of the three pathways for the degradation of intracellular proteins in lysosomes.

vesicle formation, but involves the capture and delivery of selected substrates to lysosomes for removal *via* chaperone proteins.

Dysfunction of the ALP plays critical roles in various pathological conditions including neurodegeneration. Many neurodegenerative diseases involve the aggregation of proteins, such as α-synuclein in Parkinson's (PD), amyloid-β and tau in Alzheimer's (AD), and huntingtin (Htt) in Huntington's disease (HD), which, in part, result from the failure of protein degradation systems. These proteins contain in their amino acid-sequences a KFERQ motif, the CMA substrate sequence, and have all been reported to be modulated by CMA[8, 9]. Changes of CMA activity, including both compensatory up-regulation and down-regulation, have been reported in models of neurodegeneration[10-12].

In this review, we summarize the current findings on CMA and neurodegeneration with a focus on PD, and highlight the potential of CMA modulation as a therapeutic approach for treating neurodegenerative diseases.

Chaperone-Mediated Autophagy

CMA described in mammalian cells is a highly selective form of autophagy. Its basic molecular components are known, but the detailed mechanisms by which it is regulated remain unclear[13]. One of the unique features of CMA is its selectivity and the fact that, in contrast to the other two autophagic pathways, it does not involve vesicle formation.

Instead, proteins with the recognition motif KFERQ in their amino-acid sequences are targeted individually by this process[14]. Through this unique mechanism, CMA functions as an efficient system to selectively remove damaged or abnormal proteins. Since ~30% of cytosolic proteins are reported to contain the KFERQ motif, it is reasonable to believe that CMA has the potential to regulate many cellular processes[15, 16].

Basic CMA Components

Chaperones Heat shock cognate 70 kDa protein (Hsc70) is a chaperone protein belonging to the heat shock protein 70 family[17, 18]. Cytosolic Hsc70 identifies a substrate protein *via* the KFERQ motif and transports it in complex to lysosomes for degradation[19]. Hsc70 in its ADP-bound form has a high affinity for CMA substrates[20] and also helps the docking of unfolded substrate proteins on the lysosomal membrane[21]. Besides Hsc70, other co-chaperones including Hsc40 and Hsc90 can interact with Hsc70 and facilitate this process[18, 22].

Receptor Lysosomal membrane glycoprotein (LAMP2A) functions as the receptor for CMA substrates[23]. Through its short cytosolic tail (GLKRHHTGYEQF), LAMP2A interacts directly with CMA substrate proteins and leads to their translocation into lysosomes[18]. The positively-charged residues located at the LAMP2A cytosolic tail are key to its interaction with substrates[24]. Overexpression of LAMP2A efficiently increases CMA activity, suggesting that its availability is rate-limiting for the entire process[18, 19, 25].

Substrates The KFERQ motif is the classic amino-acid sequence that mediates the interaction of a substrate protein with Hsc70[19, 26]. It includes a glutamine (Q) preceded or followed by a group of four amino-acids that contain a combination of acidic (D, E), basic (R, K), and bulky or hydrophobic (F, I, L, V) residues[27]. Such KFERQ motifs are found in ~30% of cytosolic proteins[28]. Some proteins such as the M2 isoform of pyruvate kinase have buried KFERQ motifs because of multimer formation. Other modifications such as ubiquitination can help dissociate the multimeric structure to expose the KFERQ motif, thus potentially regulating the spectrum of substrates[29].

Machinery

CMA uses a mechanism that is quite distinct from the other two autophagy pathways[19]. At the cellular level, the CMA process consists of four steps. The first includes substrate recognition and its targeting to the lysosomes. A typical CMA substrate protein with at least a KFERQ-like motif in its sequence is recognized and bound by Hsc70[20]. The second step involves substrate binding to LAMP2A and unfolding[24]. The CMA substrate protein in complex with Hsc70 binds to the C-terminal cytosolic tail of LAMP2A and this interaction quickly promotes the monomeric form of LAMP2A to assemble into a multimeric complex with a high molecular weight of ~700 KDa[24, 25]. The assembly of LAMP2A into this complex is dynamic and is in part initiated by the binding of the substrate to LAMP2A. The third step involves substrate translocation through the lysosomal membrane. It has been shown that both cytosolic and lysosome-associated Hsc70 participate in CMA translocation[13, 30-32]. Blockade of epitopes on lysosome-associated Hsc70 can interfere with the internalization. Thus, lysosome-associated Hsc70 plays an important role in substrate translocation after its delivery to the lysosomal surface by cytosolic Hsc70. The exact manner by which this translocation occurs has not yet been defined. The membrane complex may function actively to pull a substrate protein into the lumen or hold onto it to prevent its return to the cytosol[15]. The stability of lysosome-associated Hsc70 in lysosomes is highly influenced by the pH of this organelle, an increase in the lysosomal pH leading to its degradation. The fourth step is substrate degradation in the lysosomal lumen. After the substrate is pulled into the lumen of the lysosome, LAMP2A dissociates rapidly from the translocation complex into monomers, a state that allows it to bind the next substrate[25].

Regulation

CMA is influenced by multiple factors. For example, moderated levels of oxidative stress and neurotoxin treatment are known to increase the level of LAMP2A, the rate-limiting factor for CMA substrate flux, and enhance CMA activity[33]. Also, glial fibrillary acidic protein and elongation factor 1 alpha modulate CMA activity by regulating LAMP2A assembly/disassembly in a GTP-dependent manner[34]. Moreover, CMA can be regulated through the ubiquitin-proteasome system. When this system is compromised, part of the protective cellular response involves enhancing CMA to prevent the buildup of toxic species[19, 35]. On the contrary, blockade of CMA often leads to the accumulation of poly-ubiquitinated proteins[36].

In spite of these findings, the critical mechanisms by which CMA machinery responds to signals and is modulated remain to be determined.

Role of CMA in Neurodegeneration

Neurodegenerative diseases are associated with risk factors such as aging, exposure to neurotoxins, and oxidative stress[37, 38]. Some of these factors also interact with the CMA process. CMA dysfunction may be involved in the pathogenic process of several neurodegenerative diseases, and some of the more recent advances supporting this link are summarized below.

Effects of Neurodegeneration-Associated Risk Factors on CMA

Aging Aging is accompanied by a decline in the level of LAMP2A and CMA activity[39]. For example, in primary fibroblast cultures, CMA activity and lysosomal levels of LAMP2A are decreased in old-passage fibroblasts compared with early passages[40]. In the aged liver, although the transcription, synthesis, and lysosomal targeting of the LAMP2A protein appear to be the same in young and old rats, the stability of LAMP2A at the lysosomal membrane clearly decreases with aging[41]. The reason for this reduced stability is not clear, but it is possible that changes in the lipid microdomains of the lysosomal membrane contribute[41].

Neurotoxins Several neurotoxins modulate CMA activity. For example, 6-hyroxydopamine (6-OHDA) increases the levels of LAMP2A and Hsp90 in the nigral region in this rat model[42]. We showed that exposure to 6-OHDA increases the level of LAMP2A in a DA neuronal cell line and in the substantia nigra pars compacta (SNc) of mice and stimulates CMA activity[33]. Interestingly, toxins may alter the balance between CMA and substrate *via* mechanisms independent of a direct effect on lysosomes. Mitochondrial toxin also increases the levels of the CMA substrates α-synuclein and MEF2D[43]. But these changes seem to be due to changes in the regulation of their *de novo* synthesis but not their degradation by CMA. All of these findings provide examples of neurotoxin-induced changes in CMA activity and a shift of the balance between CMA and its substrates, suggesting a role for CMA as a cellular protective response to alleviate toxin-induced damage[44].

Oxidative stress Pro-oxidant compounds can cause changes in lysosomes isolated from cultured cells and rodent livers[45]. On the one hand, moderate oxidative stress seems to stimulate several key CMA components, including Hsp90, lysosomal Hsc70, and LAMP2A[33]. On the other hand, oxidative modifications of proteins may enhance their recognition by Hsc70. Together, these effects accelerate degradation *via* CMA, consistent with the evidence that antioxidants can partially prevent the CMA-mediated degradation of certain proteins following stress[46, 47]. In support of this, oxidative modifications of the neuronal survival factor MEF2D have been reported to promote its removal by CMA[33].

Role of CMA in PD

PD is one of the most common neurodegenerative diseases affecting the motor system. It results from the death of dopaminergic neurons in the SNc. Several gene mutations have been shown to cause familial PD[48]. Recent findings indicate that inhibition of CMA is a common mechanism by which the mutated proteins encoded by some of these genes exert their toxic effects.

α-synuclein and CMA PD is associated with an increase of α-synuclein protein[49]. α-synuclein contains a KFERQ-like motif and has been shown to be degraded by CMA[50]. Both monomers and dimers, but not oligomers, of α-synuclein are subjected to CMA regulation. Oxidation and nitration of α-synuclein may slightly hinder its degradation by CMA[51]. Furthermore, other post-translational modifications including phosphorylation and oligomerization may impair the turnover of α-synuclein, leading to its accumulation[52]. Interaction with oxidized dopamine (present in dopaminergic neurons) may induce a conformational change in the tertiary structure of α-synuclein, causing the protein to become misfolded and no longer internalizable by lysosomes. Pathogenic A30P and A53T α-synuclein mutants can bind to LAMP2A but are not internalized efficiently[8, 53]. Other mutant forms also hamper the CMA degradation pathway to varying degrees[54]. Therefore, all these conditions appear to impair CMA at the translocation and internalization step and may interfere with or inhibit the degradation of other CMA substrates.

Leucine-rich repeat kinase 2 (LRRK2) and CMA Mutation of the LRRK2 gene causes autosomal dominant PD. The LRRK2 protein is located in membrane microdomains, multivesicular bodies, and autophagic vesicles[55], and it

is also a substrate for CMA. However, the most common pathogenic mutant form of LRRK2, G2019S, is processed poorly through this pathway[56]. In the presence of other CMA substrates, the binding of both wild-type and several pathogenic mutant LRRK2 proteins to lysosomes is enhanced. This is thought to interfere with the organization of the CMA translocation complex, resulting in a CMA defect. In neuronal cultures, the brains of LRRK2 transgenic mice, induced pluripotent stem-cell-derived dopaminergic neurons, and the brains of PD patients carrying a LRRK2 mutation, cells respond to such LRRK2-mediated interference with CMA by increasing the level of LAMP2A. But this is not sufficient to overcome the blockade, resulting in the buildup of other CMA substrates including α-synuclein[56].

Mutant ubiquitin C-terminal hydrolase L1 (UCH-L1) and CMA UCH-L1 is a de-ubiquitinating enzyme expressed abundantly in neurons. Its oxidative/carbonyl damage has been reported in sporadic PD and its mutation is associated with familial PD[57]. UCH-L1 interacts with LAMP2A, Hsc70, and Hsp90. At the cellular level, the UCH-L1 I93M mutant shows an enhanced interaction with the CMA components independent of the mono-ubiquitin-binding activity but remains stuck in the membrane, blocking the CMA machinery[58]. Expression of the UCH-L1 I93M mutant in cells results in an increase of α-synuclein and possibly other substrates secondary to CMA inhibition. Therefore, the aberrant interactions between the UCH-L1 mutant and CMA machinery may underlie the pathogenesis of PD caused by this mutated protein[58].

Together, these findings indicate that several proteins whose mutation is associated with familial PD focus their toxic activity on CMA, implying that inhibition of CMA is a key and a common mechanism in the pathogenic process of familial forms of PD caused by the mutation of multiple genes.

Neuronal survival factor myocyte enhancer factor 2D (MEF2D) MEF2D, a transcription factor, plays an essential role in neuronal survival[59-61]. Our studies indicated that MEF2D is a CMA substrate[62]. Under normal conditions, non-functional MEF2D is transported to the cytoplasm and degraded by CMA and a decrease in CMA activity causes MEF2D accumulation in the cytoplasm. Both wild-type and A53T α-synuclein disrupt the CMA-mediated degradation of MEF2D and sensitize cells to stress[62, 63]. Consistent with this, cytoplasmic accumulation of MEF2D correlates positively with the level of α-synuclein in the brains of both α-synuclein transgenic mice and PD patients. Furthermore, our recent findings revealed that the neurotoxin 6-OHDA leads to oxidative modification of MEF2D and inhibits its activity[33]. Oxidatively-damaged MEF2D has a higher affinity for Hsc70 and is readily removed by CMA. Importantly, 6-OHDA induces MEF2D oxidation and increases LAMP2A in both cultured cells and the SNc region of the mouse brain. Similarly, the levels of oxidized MEF2D are much higher in postmortem PD brains than in controls. Functionally, reducing the levels of either MEF2D or LAMP2A exacerbates 6-OHDA-induced death in a dopaminergic neuronal cell line. An MEF2D mutant that is resistant to oxidative modification protects cells from 6-OHDA-induced death[33]. These findings indicate that removal of damaged MEF2D by CMA maintains cellular homeostasis and is protective against oxidative stress.

Alternations of CMA components The LAMP2 protein has three isoforms, LAMP2A, B, and C, which are affected differently in the early stages of PD[18]. The CMA receptor LAMP2A is selectively reduced related to the levels of α-synuclein. It has been reported that in PD brains, the levels of LAMP2A and Hsc70 are significantly reduced in the SNc and amygdala compared with those in age-matched AD and control brain samples[11, 64].

CMA in Other Neurodegenerative Diseases

Varying degrees of evidence indicate that dysfunctional CMA is also implicated in neurodegenerative diseases other than PD, including HD, AD, frontotemporal dementia, and amyotrophic lateral sclerosis.

In AD and tauopathies, CMA has been shown to influence the levels of neurofibrillary tangles resulting from the accumulation of mutant tau protein. The degradation of normal tau by CMA is mediated by its binding to Hsc70 *via* one of the two targeting motifs in its C-terminus[65]. Interestingly, it appears that it is the fragments of mutant tau, but not the full-length protein itself, that translocate to the lysosomal membrane through Hsc70 recognition to undergo CMA-dependent cleavage by cathepsin L[65].

Hsc70 and LAMP2A play roles in the degradation and clearance of Htt, whose gene mutation causes HD[52]. It has been reported that macroautophagic dysfunction triggers

an increase in CMA in the early stage of HD, which may be a protective measure[12]. Moreover, phosphorylation of Htt by inhibitor of kappa B kinase appears to promote its degradation in the lysosomes of unaffected neurons[66]. Therefore, a decline in CMA activity may function as one of the aggravating factors in the progression of HD[10, 19].

In frontotemporal dementia and amyotrophic lateral sclerosis, the pathogenic protein Tar-DNA binding protein 43 (TDP-43) forms cytosolic aggregates. TDP-43 is degraded by both ubiquitin proteasome and macroautophagy pathways, but there is also a report supporting the involvement of CMA in its degradation. This requires an interaction between Hsc70 and ubiquitinated TDP-43[67].

CMA as a Therapeutic Target in Treating Neurodegenerative Diseases

One of the most common characteristics among the different neurodegenerative diseases is the aggregation of deleterious proteins or inclusions in neurons[45, 68], including Htt protein in HD, amyloid-β in AD, and α-synuclein in PD. Many studies have established a strong link between a decline of CMA and the abnormal aggregation of such proteins in various models of neurodegenerative diseases[62, 69], suggesting that CMA is a new and promising target for treating multiple neurodegenerative disorders.

Increased or restored CMA activity could be protective in several pathological conditions. As summarized above, the involvement of CMA in neurodegenerative diseases is complex. It is responsible for the clearance of aggregated or harmful proteins, and it is often the target of the toxic effects of these deleterious proteins. Rigorously designed studies are needed to thoroughly evaluate and determine whether enhancing CMA activity can provide substantial therapeutic benefits.

For the pharmacological manipulation of CMA activity, several molecules and their analogues have been reported to enhance autophagy with verified efficacy in several animal models of disease[68, 69]. Genetically, LAMP2A overexpression in mouse liver improves lysosomal as well as liver functions[45]. This suggests that reversing the aging-related impairment of CMA could have functional consequences. Similarly, studies on models of neurodegeneration also confirm the efficacy of targeting LAMP2A. Overexpression of LAMP2A in both cell lines and primary neurons efficiently increases the CMA activity and decreases the amount of α-synuclein[70]. More remarkably, in the rat SNc, LAMP2A overexpression effectively reduces the levels of total α-synuclein and related aberrant inclusions, increases the survival of dopaminergic neurons, and alleviates the α-synuclein-induced neurodegeneration[71, 72]. In addition, experimentally increasing CMA activity seems to provide therapeutic benefits in HD models[10]. Overall, these findings strongly suggest that manipulation of CMA activity may be an effective therapeutic strategy for treating neurodegenerative diseases.

Summary and Direction

To date, over 30 CMA substrates have been identified, a significant portion of which is involved in the regulation of critical neuronal functions. This highlights the importance of CMA in the nervous system under both physiological and pathological conditions. As discussed above, there is a particularly strong link between CMA impairment and PD. Accumulating evidence indicates that loss of CMA activity is associated with multiple toxic and genetic conditions associated with PD, suggesting that a decrease in CMA activity may underlie in part the pathogenic process of PD. However, several issues remain to be resolved. First, a better understanding of the specific stage(s) when the CMA defect occurs in models and human tissues of PD is needed. Second, a better understanding of the critical mechanism(s) underlying how the CMA defect contributes to or even triggers the neurodegenerative process is needed. Third, although still preliminary, manipulating CMA has been shown to alleviate PD-related neurodegenerative changes in several experimental models. Future studies are clearly needed to validate these findings and optimize strategies aimed at selectively and potently modulating CMA function. The selective modulation of CMA will not only allow a more precise assessment of the possible side-effects of such therapies, but also aid investigations into the mechanisms of how the pathological factors cause degenerative alterations through inhibition of the CMA pathway.

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