Role of chaperone-mediated autophagy in degrading Huntington’s disease-associated huntingtin protein

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Mutant N-terminal huntingtin (Htt) protein resulting from Huntington’s disease (HD) with expanded polyglutamine accumulates and forms aggregates in vulnerable neurons. Both ubiquitin proteasomal and autophagic pathways contribute to the degradation of mutant Htt. Here, we focus on the involvement of chaperone-mediated autophagy (CMA), a selective form of autophagy in the clearance of Htt. Selective catabolism in CMA is conferred by the presence of a KFERQ-like targeting motif in the substrates, by which molecular chaperones recognize the hydrophobic surfaces of the misfolded substrates, and transfer them to the lysosomal membrane protein type-2A, LAMP-2A. The substrates are taken into the lysosomes through LAMP-2A and are rapidly degraded by the lysosomal enzymes. Taken together, we summarize the recent evidence to elucidate that Htt is also a potential substrate of CMA. We propose that the manipulation of CMA could be a therapeutic strategy for HD.

Keywords chaperone-mediated autophagy (CMA); Huntington’s disease (HD); chaperones; lysosome-associated membrane protein 2A (LAMP-2A)

Received: August 21, 2013 Accepted: November 6, 2013

Introduction

Neurodegenerative diseases are harmful for human beings more and more because of the increasing population and prolongation of age. Several neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyloid lateral sclerosis, have many unique features but have one in common, accumulation and aggregation of the misfolded proteins. Under normal circumstances, proteins should be folded correctly to become functional inside the cell, and the misfolded proteins are targeted for degradation by the proteolytic systems [1]. However, it does not go well sometimes. The misfolded and aggregated proteins, which are a pathogenic hallmark in neurodegenerative diseases, can cause dysfunction and death of specific populations of neurons. With the development of the pathology itself or aging, which causes the decrease of the ability of the proteolytic systems to get rid of the mutated proteins, these proteins start to accumulate inside the cells and exert their toxic effects. Therefore, neurodegenerative diseases such as HD with mutant huntingtin (Htt) protein are considered to be age-related disorders [2].

Autophagy Is the Most Important Metabolic Pathway of the Misfolded and the Aggregated Proteins of Neurodegenerative Diseases

Huntingtin is mainly distributed in the cytosol, and a small portion is localized in the nucleus. Htt is involved in a variety of cellular functions including vesicle transport, transcription, and energy metabolism [3,4]. Wild-type Htt has ~20 polyglutamine (polyQ) repeats encoded by CAG trinucleotide in its N-terminus. HD is caused by a more than 36 expanded CAG trinucleotide repeats abnormal tract and is characterized by the accumulation and aggregation of mutant Htt protein in diseased neurons, resulting in cellular toxicity and organ damage because of the ubiquitous expression of Htt [5,6]. The major pathways of protein clearance are the proteasome and the lysosome systems in the cell, both are affected more or less by aging [7–9]. The wild-type Htt is degraded through the ubiquitin–proteasome system (UPS), which is mainly involved in the degradation of short-lived regulatory proteins [10]. In the UPS, useless and misfolded proteins are tagged for degradation by ubiquitin. Then, the unwanted protein was moved into the proteosome and then broken down into amino acids which can be recycled. It has been reported that the UPS is impaired in...
HD [10,11]. The long polyQ (exceeds a length of 22 amino acids) is the main reason for ineffective degradation through the clogging of the proteasomes in mHtt [10,12]. Indeed, the UPS will be impaired by mHtt which is diffuse and oligomeric, not huge aggregative mHtt. The huge aggregation could frustrate the UPS directly in the late step of HD [13]. According to the theory, disease can be developed because of the damage of the UPS. However, there were also studies showing that proteasome impairment was controversial in HD. For example, the experiments did not show any impairment when the proteasomes were incubated with isolated mHtt aggregates in vitro [14,15]. Whether or not the UPS itself is impaired in HD, there was evidence to suggest that aggregative HD protein clearance was not mediated by the UPS, but by an alternate method called autophagy [10,16,17]. The reasons preferring to transport the misfolded and aggregated proteins like Htt to the lysosomes for degradation are as follows: first, the proteolytic system of the lysosome is mostly affected by age [18]. The direct evidence is that the degradation of long-lived proteins, which may mainly be degraded by lysosome, is retarded in old cells and animals [19,20], and there is an age-related increase in the number and size of the lysosomes in most cells [21]. However, not all these lysosomal pathways of proteolysis are equally affected by age. The lysosomal pathways of macroautophagy and chaperone-mediated autophagy (CMA) are conspicuously related to old organisms [22]. The level of ubiquitin mRNA and the ability of the old cells to degrade ubiquitinated proteins do not change with age [23] and no adequate clear evidence for such age-related changes in proteasome activity has been found. Secondly, in contrast to the ubiquitin–proteasomes, the major inducible pathway autophagy is likely to be the primary mechanism involved in the degradation of bigger proteins such as mutated proteins and is the only mechanism by which entire organelles such as mitochondria are degraded. Large membrane proteins, oligomers, and aggregates fail to pass through the narrow proteasome barrel [24]. For example, mutant Htt was found to be degraded by lysosomal proteases [25–28].

Autophagy can be divided into three distinct pathways based on the ways reaching the lysosomal lumen of the substrates: macroautophagy, microautophagy, and CMA [29,30]. Macroautophagy is a multi-step process, involving the formation of double membrane structures known as autophagosomes. Then, the autophagosome fuses with the lysosomes to form autophagolysosomes, which are considered to be the characteristic components of autophagy [31]. The contents are then degraded by the hydrolytic enzymes in the autophagolysosomes. The substrates of microautophagy are directly swallowed from the lysosome membrane even under resting conditions. CMA is a selective autophagy to degrade one-by-one the pathogenic substrates that have special amino acid sequence (KFERQ), and a specific cytosolic chaperone protein (heat-shock cognate protein of 70 kDa, Hsc70) binds to the lysosomal membrane protein, LAMP-2A, and is then transported into the lysosomes for degradation by the hydrolases [32]. CMA constitutively exists in many types of cells, and CMA is maximally activated under stress conditions such as nutritional stress or starvation to protein damage, like macroautophagy [33]. Often, macroautophagy is first activated, and then CMA would be activated for degradation with starvation persisting. At this time, the amino acids from CMA selective degradation could be used for the synthesis of essential proteins [34,35]. The advantage of CMA is its intrinsic selectivity, which persists to degrade the useful and normal proteins [36]. Blockage of CMA in cultured cells can affect specific transcriptional programs, metabolic pathways such as glycolysis, and modify cell growth and cell survival [35,37–39]. Up to now, the degradation of Htt by macroautophagy has been investigated by several groups [25,40], whereas the involvement of CMA has received less attention.

Since HD is a neurodegenerative disease associated with aging, researchers proposed that there is a relationship between CMA and HD. Currently, the supporting evidence on a role of CMA in HD is scarce. Here, we review the preliminary evidence that Htt can be degraded by CMA in cellular and animal models of HD.

**CMA Is Involved in Neurodegenerative Diseases**

The selective recognition of the substrates by the chaperone proteins is the feature that distinguishes CMA from macroautophagy and microautophagy [41]. Base CMA activity can be detected in most types of mammalian cells, but maximal activation of CMA is triggered by stressors, such as long-term starvation, oxidative stress, or exposure to toxic compounds that induce abnormal conformational changes of cytosolic proteins [42–44]. Under those conditions, CMA accounts for the degradation of 30% of cytosolic proteins. The identified substrates for this pathway include some glycolytic enzymes, transcription factors and their regulatory proteins, cytosolic proteases, cytosolic forms of secretory proteins, and lipid- and calcium-binding proteins, specially, the aggregated proteins [45]. All of the substrate proteins of CMA contain a motif biochemically related to the pentapeptide KFERQ that targets the proteins to the lysosomes [46]. This motif is recognized by Hsc70 as described before, which interacts with the substrate proteins in the cytosol [41,43]. All of the CMA folded substrates once recognized by the cytosolic Hsc70 in the cytosol [41] were targeted to the lysosomes, where they bound to LAMP-2A which could organize into a multimeric complex. Cytosolic Hsc70 always binds to the substrate until that substrate attaches multimeric-LAMP-2A [47] and then cytosolic Hsc70 releases the substrates. The complex of LAMP-2A can mediate the substrate protein into the unfolded ones and then
across the membrane with the help of Hsc70 in the lysosomal lumen (lys-Hsc70) [47,48]. No transport of the substrates will take place if Hsc70 is absent or experimentally blocked. The chaperone–substrate protein complex binds to LAMP-2A [49]. The level of LAMP-2A on the lysosomal membrane is the rate-limiting step for protein binding and uptake by the lysosomes [45,50]. The level of LAMP-2A is regulated by LAMP-2A dynamic changes between the lysosomal membranes and the matrix [51]. Macroautophagy is often activated in the early step of the stimulus. Once the time of the stimulus is prolonged, CMA works for long-term as compensation [37]. Neurodegenerative disease as a long-time stimulus should be associated with CMA, which is associated with age.

The relationship of CMA and neurodegenerative diseases such as AD and PD has been studied for a long time, and it was demonstrated that CMA can significantly contribute to the removal of proteins in neurodegenerative diseases [37]. PD is the most common neurodegenerative movement disorder. The increase in the amount of α-synuclein proteins causes PD and the mutation in α-synuclein is associated with familial PD. α-synuclein is degraded, at least partially, by CMA. PD-associated mutations in the later stage of morbidity could block the degradation pathway of CMA [29]. Furthermore, a mutant form of Tau of AD is also targeted to the lysosomes via CMA. Once the Tau at the membrane is mutated, it results in CMA blockage with the decrease of the clearance of the mutated proteins [52]. In these cases, the toxicity resulting from the CMA blockage is caused by the lysosomal enzymes that produce shorter amyloidogenic fragments which destruct the stability of their membranes. The leakage of the lysosomal enzymes probably accelerates cell death [52], and the expression of mutant Htt increases the levels of the lysosomal proteases [26–28]. Koga et al. [2,53] found that fluorescent puncta labeled in the Htt-cell with KFERQ, the photoswitchable cyan fluorescent protein (KFERQ-PS-CFP2), which was a monomeric photoconvertible fluorescent protein as a photoswitchable reporter increased after the removal of serum, implying that CMA activity was more in the HD cells. So far, how mHtt was degraded through CMA is still not very clear. We will focus on CMA and its unique role in HD.

The Relationship between CMA and HD

The vacuole targeting (Cvt) pathway in yeast, which is similar to the selective autophagy, may decline with age and be related to the degradation of polyQ repeat Htt [54]. Furthermore, Bauer et al. [55] and Thompson et al. [56] have reported the association of CMA and HD, and a recent publication from Koga et al. [2] showed that CMA activity is increased in response to macroautophagic dysfunction in the early stages of HD as a physiological self-treatment. Then, our group also proved that Hsc70 and LAMP-2A through CMA play a role in the clearance of Htt and suggested a novel strategy to target the degradation of mutant Htt [57]. However, the mechanism of CMA involved in HD is still not fully understood.

The substrates by CMA should first bind to Hsc70 by recognizing the pentapeptide KFERQ that targets the proteins to LAMP-2A, and is then transported into the lysosomes for degradation by the lysosome hydrolases. If Htt could be degraded by CMA, Htt should have a KFERQ-like pentapeptide which interacts with Hsc70 and LAMP-2A. All of these components are required for confirming the role of CMA in HD, thus there is a possibility for using CMA to eliminate the pathogenic proteins in HD (Fig. 1).

Htt is related to the chaperone

The aggregation of proteins could disrupt the normal functions of the proteins. Several chaperones play an important role in handling the misfolded and the aggregated proteins.

Figure 1. CMA for mHtt degradation mHtt in cytosolic can expose the ‘KFERQ’-like motifs for CMA targeting under some modifications by binding cytosolic Hsc70. The complex between mHtt, Hsc70, and its co-chaperones is delivered to the surface of the lysosomal membrane where it interacts with LAMP-2A, the receptor for CMA. Once bound to LAMP-2A, mHtt protein is separated with cytosolic Hsc70 and co-chaperones. Then, mHtt completely unfolds and crosses the lysosomal membrane with the help of LAMP-2A and luminal form of Hsc70 (Lys-Hsc70). At last, mHtt could be rapidly degraded in the lumen.
through refolding and assisting the degradation of the aggregated proteins [49,50].

The increase of the chaperones would preferentially reduce aggregation and mutant Htt-mediated toxicity to suppress the process of neurodegeneration in mouse and HD models [58–61]. The mechanism to decrease the toxicity of mutant Htt proteins is that the chaperones trap them by hydrophobic interactions and transfer Htt to the lysosome for degradation [62]. For example, the cytosolic chaperonin-containing t-complex polypeptide 1 (CCT) is a chaperon that localizes in the eukaryotic cytosol, similar to the mitochondrial chaperonin Hsp60 [63,64]. CCT alters the aggregation state of the mutated Htt and prevents the cytotoxicity of this Htt in the neuronal cells [65]. Huntingtin yeast two-hybrid proteins (HYPs) possess a novel chaperone-like activity and play an effective role in protecting the neuronal cells against death induced by mutant N-terminal Htt. HYPK alters the numbers and the distribution of the aggregates formed by N-terminal mutant Htt with 40Q [66].

Chaperones, such as Hsp70s and Hsp90, could combine together with the substrate-binding domain region of the misfolded proteins. The binding of Htt with the chaperones is the first step toward degradation through CMA.

The chaperones contain hsp70, hsp90, hsp40, hsp60, Hsc70-hsp90 organizing protein (Hop), and Hsc70-interacting protein (Hip), many of which are relevant to CMA. Interestingly, the CMA substrates are first recognized in the cytoplasm by the Hsp70s family of chaperones [67]. Hsp70s, which are the most important chaperones of CMA, immediately combine with the substrate, and Hsp70s also recognizes the hydrophobic surfaces of the proteins controlled by their ATP-binding and hydrolysis activities [50]. The expression of Hsp70s has been reported to progressively suppress the development in mouse models of HD by antagonizing the conformational effect on Htt, as Muchowski’s group has revealed that hsp70s prevents Huntingtin to form oligomers [68–70]. A number of studies supported the notion that hsp70s protects the neurons in the polyQ pathology [71,72]. Therefore, the up-regulation of hsp70s in cells is considered to reduce the toxicity of mutant Htt oligomers and to recover the physiological functions of various proteins.

Although Hsp70 and Hsc70 of the Hsp70s are highly homologous at 85% and almost indistinguishable in their chaperone activity, studies have found that the Hsc70 is the only chaperone that interacts directly with the substrate in a manner regulated by the ATP/ADP binding cycles [67]. The percentage of Hsc70-containing lysosomes, which is no more than 40% under resting conditions, escalates to 80% in the liver under certain conditions such as during prolonged starvation or mild oxidative stress, in which CMA is up-regulated, [73]. A form of Hsc70 resident in the lysosomal lumen (lys-Hsc70) is needed to complete translocation of the substrate proteins into the lysosomes [48,74].

Blocking Hsc70 that reaches the lysosomal lumen exerts a strong inhibitory effect on the CMA in the cells [47]. Furthermore, the levels of lys-Hsc70 can represent different ability of the subgroups of the lysosomes to perform CMA [73], for example, Hsc70 can bind to Htt-46Q as a type of mHtt [75]. At the same time, Nihar also verified that the transient overexpression of Hsc70 suppresses the aggregation of Htt [62]. The binding of Hsc70 and Htt appears to be highly dynamic and transient, which is consistent with the Hsp70 family proteins binding the substrates by dynamic on–off cycling [76]. The efficacy of Hsc70 in the traffic and binding to Htt-46Q may be limited by other components of the quality control network, such as the Hsp40 co-chaperones [62,70]. The role of Hsc70 for degradation of CMA is that it acts actively by facilitating substrate internalization in an energy-dependent manner, and passively by binding a portion of the substrate to prevent its retrograde movement to the cytoplasm. The regulation by which lys-Hsc70 mediates substrate translocation may be dependent on the pH of the lysosomes. Lys-Hsc70 is stable in the lysosomal lumen at a pH of around 5.2 but not stable at a slight increase of lysosomal pH. The optimal pH helps the proteins to be degraded by the lysosomal proteases [73]. Furthermore, increase of binding for both polyQ and Hsc70 has revealed the targeting of mutant Htt to CMA, and reduced cell toxicity in mouse models [55]. In our study, the immunoprecipitation and the co-immunoprecipitation results confirmed that Htt could interact with Hsc70. Specifically, it was noticed that mutant Htt had stronger interactions with the critical components of CMA than wild-type Htt did, and the manipulation of the Hsp70 levels had no significant effect on Htt accumulation. These data suggested that Htt is degraded by an Hsc70-dependent mechanism through CMA [57].

In addition to Hsc70, Hsp90, hsp40, Hop, and Hip are relevant to the CMA at the lysosomal membrane [77]. Sometimes those proteins do not participate in targeting to the substrates directly, but they help the substrates unfold to pass the lysosomal membrane [78]. For example, Hip can promote the degradation of cytosolic proteins [79]. Therefore, it is speculated that those proteins should indirectly help CMA in degrading Htt. Further studies are required to understand whether other chaperones could also participate in targeting of Htt.

**Htt is a substrate of LAMP-2A**

CMA activity can be represented by LAMP-2A, which determines the CMA substrates [80]. Plenty of evidence supports the fact that the CMA substrates directly pass the lysosomal membrane and it is a saturable process to bind to LAMP-2A. The substrate proteins need to be completely unfolded before reaching the lysosomal lumen [44,81,82]. CMA activity declines with age due to a gradual decrease of LAMP-2A levels in the lysosomes [83], whereas artificial overexpression
of LAMP-2A in old rat liver similar to those in young animals can restore CMA activity and improve organ functions [35,84]. LAMP-2A is one of the three splice variants of the LAMP2 gene including LAMP-2b/c. LAMP-2A has a special transmembrane region different from others [48,85] and contains heavily glycosylated luminal region and 12-amino acid C-terminus tail exposed on the surface of the lysosomes, and these two are the places where the substrate proteins bind. There is a report revealing that the CMA substrates only bind to the region of LAMP-2A through four positively charged residues [84]. It has been confirmed that only LAMP-2A is related to CMA [35]. LAMP-2A could respond the activity of CMA and the relation to LAMP-2A should be an important evidence of its involvement in CMA. If Htt as a substrate could be bound to LAMP-2A, then it has been explained that Htt is not only degraded by macroautophagy since LAMP-2A is not involved in macroautophagy at least.

A recent study has revealed a close relationship of phosphorylated Htt and LAMP-2A [56]. Thompson et al. [56] have found that the clearance of phosphorylated Htt dependent on LAMP-2A and the reduction of the LAMP-2A levels with age may be related to HD pathogenesis. IkB kinase (IKK) can phosphorylate Htt and this post-translational modification accelerates the degradation of Htt in the lysosomes in the unaffected neurons [85]. It was proposed that in a presymptomatic HD neuron, the mutant Htt could induce IKK [56]. As long as the level of LAMP-2A is maintained high, mutant Htt could be degraded before it caused toxicity. As aging and mutant Htt together progressively impair proteasome and overall lysosomal activity, and as LAMP-2A levels decline with age, mutant Htt may accumulate, exacerbating HD pathogenesis. Thompson et al. have found that mimicking Htt phosphorylation increases its toxicity which has been shown to regulate additional post-translational modifications in Htt (ubiquitination, SUMOylation, and acetylation) [86] and aggregation in Drosophila photoreceptor neurons where the components of the mammalian machinery to degrade phosphorylated Htt, specifically LAMP-2A, are not present [56]. They suggested that it could be harmful to cells by increasing IKK activation in late stage HD, whereas in the presymptomatic stages of HD, the activation of IKK to modify Htt may be protective. There is also an example that Yamamoto et al. [87] knocked out the LAMP-2A and LAMP1 mRNAs and discovered that the reduction in LAMPs increases the accumulation of Htt. Bauer et al. [55] silenced LAMP-2A with shRNA in mutant Htt with 150Q. All of the three combinations of LAMP-2A-specific shRNAs increased the aggregation of mutant Htt. Koga et al. [2] have reported that a marked decrease in the lysosomal levels of LAMP-2A is the most pronounced difference in the lysosomes from 12-month-old HD mice compared with control mice. In our study, immunoprecipitation results confirmed that Htt can interact with LAMP-2A. Changing the levels of CMA-related LAMP-2A affects the accumulation of Htt-552 [57].

Binding of the CMA substrate proteins to LAMP-2A is a limiting step for CMA [84]. To study the process of degradation of Htt via CMA, we should know how LAMP-2A is regulated. Up-regulation of LAMP-2A has been described in response to stimulus such as mild oxidative stress [88]. In most instances, LAMP-2A levels are controlled through the distribution of LAMP-2A between the lysosomal membrane and the matrix [83] and do not require de novo synthesis of the protein [67]. The half-life of LAMP-2A can regulated by membrane proteases such as cathepsin A. Cathepsin A is located at the lysosomal membrane where it cleans LAMP-2A and negatively regulates CMA [89]. The up-regulation of CMA observed in mice knocked out for cathepsin A results from decreased rates of the degradation of LAMP-2A [89]. There is a special mechanism for the translocation of substrate proteins across the lysosomal membrane via CMA, which involves the multimerization of LAMP-2A [48,88]. Formation of this 700 kD complex containing LAMP-2A is essential for substrate translocation, because mutations in the transmembrane region of LAMP-2A, which prevent multimerization, do not affect substrate binding but block substrate translocation. The chaperones only associate with the lower-order complexes of LAMP-2A, not the 700 kD complex [48,88].

**Htt is degraded by the lysosomal enzymes**

The lysosome is the digestive place of the CMA substrate by the enzymes. It is a membrane-bound intracellular organelle containing a number of hydrolytic enzymes synthesized in the rough endoplasmic reticulum and then modified in the Golgi apparatus. The lysosomes combine with membrane-bound vacuoles which contain material to be digested. The main class of lysosomal proteases is the family of cathepsins, which is divided into three subgroups: cysteine (cathepsins B, C, F, H, K, L, N, O, S, T, U, W, and X), aspartyl (cathepsins D and E), and serine cathepsins (cathepsins A and G). Cathepsins are first synthesized in the membrane-bound ribosomes as N-glycosylated precursors, and then transferred into the endoplasmic reticulum and later into the Golgi complex. In the process of transporting to the Golgi complex, the carbohydrate moieties of the procathespins are modified. After binding to the mannose 6-phosphate-specific receptors, the procathespins are transported to the late endosomes [65]. The cathepsin activity is regulated by several mechanisms including regulation of synthesis, zymogen processing, endogenous inhibitors such as steins and cystatins, and pH stability [90,91]. The cathepsins play important roles in many physiological and pathological processes such as protein degradation, tumor, antigen presentation, and hormone processing [90].

Cathepsin D plays the most important roles in HD compared with the other cathepsins. Therefore, cathepsin D is
more intensively studied for its relationship with HD. When Htts, especially mutant N-Htt fragments, are less efficiently degraded by cathepsin D, HD pathogenesis will be worsened. It is explored in cells that the autophagic pathways process N-terminal Htt fragments through the comparison of the cells with and without cathepsin D [27]. The expression of Htt increased the levels of the lysosomal enzyme cathepsin D by an autophagy-dependent pathway. Cells without cathepsin D accumulated more N-terminal Htt fragments and cells with cathepsin D are more efficient in degrading Htt in vitro. The levels of cathepsin D were lower after treating the Htt-expressing cells with 3-methyladenine, which is a macroautophagy inhibitor [27]. Stable mutant Htt fragments formed in the presence of cathepsin D at a weakly acidic pH. These results suggested that cathepsin D plays a critical role in the pathway of degradation of N-terminal Htt. Altered processing of mutant Htt by cathepsin D may contribute to HD pathogenesis. Cathepsin D has broad substrate specificity and may recognize sites in Htt that are in the same or nearby sites cleaved by a more selective protease. Cathepsin D cleaves amyloid precursor protein near the beta secretase sites [92].

In addition to cathepsin D, cathepsin E (another protease) is also responsible for Htt aggregation formation. In aging rat brain, levels of cathepsin E are increased preferentially in the cortex and the striatum [93], which are the regions most affected in HD [93]. It has been demonstrated that cathepsins B and L are essential for maturation and protection of the central nervous system. Cathepsins B and L can compensate for each other in vivo [94]. In contrast to the inefficient clearance of mutant Htt by cathepsin D at neutral and weakly acidic pH, mutant Htt was effectively degraded by cathepsin L at the acidic pH that is optimal for its lysosomal enzymatic activity. In old PC12 cells, the up-regulation of cathepsin L has also been observed [27]. Access of mutant Htt to cathepsin L within the endosomes and the lysosomes may be important for Htt degradation. Koga et al. found a consistent marked increase of Hsc70 in lysosomes from Htt with 111Q mouse liver. Higher levels of Hsc70 were observed in lysosomal membranes from young Htt with 111Q mice, but no longer consistent in the older ones [2]. In our study, we successfully isolated intact lysosomes from mice liver for studying the lysosomal uptake of Htt with 552 aa (Htt-552). Addition of isolated lysosomes to cell lysates containing exogenously expressed Htt-552 can robustly reduce the Htt-552 levels. Otherwise, GAPDH which is a CMA substrate reduces the lysosome-mediated Htt-552 degradation by competing for LAMP-2A, suggesting that Htt-552 is also a substrate of CMA as GAPDH [28,68]. Furthermore, more mutant Htt-552 is accumulated in the lysosomal membranes but little change occurs in the matrix after inhibiting lysosomal degradation. In contrast, more WT Htt-552 was accumulated in the matrix but little change happened in the lysosomal membranes. Perhaps, the reason is that mutant Htt-552 is less able than WT Htt-552 to cross the lysosomal membranes and is degraded by the lysosomal enzymes. These data suggested that the expansion of a polyQ tract impairs Htt clearance through CMA [57].

In summary, the findings show that the lysosomal proteases cathepsins B, D, E, and L may play an important role in processing mutant Htt in HD brain. The identification of the compounds which inhibit the formation of stable mutant Htt fragments by cathepsin D could be an important therapeutic target in the treatment of HD.

The recognition of the pentapeptide motif of CMA in Htt

The selectivity of CMA is conferred by the recognition and binding ‘KFERQ’ pentapeptide motif of the substrate proteins by the chaperone proteins. This binding results in the targeting of the substrates to the lysosomes [46]. KFERQ pentapeptide was first found in the degradation of RNase A, which is the earliest and valid CMA substrate [95]. The motif may occur in either direction, meaning that the peptide may be related to KFERQ or QREFK [96]. The amino acid composition of the pentapeptide motif is also not exact. Studies have revealed the basic requirements of the motif [46,96]. The CMA targeting motif always begin with a glutamine (Q), and contains one acidic residue (D or E), a basic residue (K or R), a hydrophobic residue (F, I, L, or V), and an unimportant fifth residue that can be either basic or hydrophobic but cannot be omitted [96]. Glutamine is very important for CMA recognition to degrade CMA-mediated protein in response to serum withdrawal [95]. In particular contexts, asparagines (N) can replace glutamine in the interaction with the chaperones. The motif does not depend on where it is located in the protein (N-terminus, C-terminus, or internally) as far as it is accessible to the chaperone. When correctly folded, the motif is often hidden inside the protein core, and it only becomes exposed when the protein is unfolded. The motif does not depend on the quantity either. It has been revealed that the presence of multiple motifs does not increase the efficiency of the substrate protein for the chaperone or its rate of degradation through CMA [42,78].

The neurodegenerative disease-associated proteins degraded by CMA often contain the KFERQ-like pentapeptide motif. Clarification of more substrates of the CMA pathway will help to reveal the involvement of CMA in neurodegeneration. Regulator of calcineurin 1 (RCAN1) is a gene identified from the critical region of Down syndrome and has been implied in the pathogenesis of AD. Interestingly, RCAN1 is associated with CMA through 31–46 aa of RCAN1 which contains the putative KFERQ-like motif [97]. It has also been found that the KFERQ-like motif in synuclein associates with PD [29]. The motif is VKKDQ (176–180 aa) and is identified as the putative CMA target motif [74].
Post-translational modification including the phosphorylation, ubiquitination, SUMOylation, and acetylation of proteins is an important mechanism that helps metabolism by some kinds of physiological reactions including autophagy. Although Htt does not directly contain a ‘KFERQ’ Hsc70-binding motif in its sequence, phosphorylation of Htt serine 16 where the phosphorylated serine resembles a glutamic acid [E] can provide a similar motif. The ability of phosphomimetic Htt to interact with Hsc70 in vitro was tested and it has been found that mimicking the phosphorylation of Htt serines 13 and 16 on the unexpanded polyQ Htt increases the in vitro binding of Htt to Hsc70 by a specific ADP-dependent mechanism [56]. This is the only directly enunciable evidence of how the molecular mechanism associates with CMA at the present by post-translational modification. To look for more useful sites in mHtt with ‘KFERQ’-like motif for the design of therapies, other post-translations become urgently needed. Besides the phosphorylation sites existing in Htt, acetylation at K444 of Htt can directly influence mHtt in HD by facilitating the autophagic clearance, but without affecting the normal Htt [98]. K9 is an additional acetylation site of mHtt, which needs co-expression of kinase IKK in the same cells [56]. Our group also found two ‘KFERQ’-like motifs [99–103 (99KDRVN103) and 248–252 (248 NEIKV252)] in the 552 amino acids of N-Htt. This is the key step to further study the ability of CMA in Htt degradation. The mutation of the CMA recognition motif was achieved by changing the hydrophilic and the hydrophobic amino acids in the KFERQ-like motif located between aa99–103 and 248–252. These results showed that the mutation of amino acids in 99–103 but not in 248–252 reduced the Htt-552 interaction with Hsc70 and also reduced the clearance of Htt-552. These data suggested that a KFERQ-like motif located between aa99–103 is a functional site for recognition by Hsc70 [57].

The Regulation of CMA for Therapy of HD

Knowledge of HD has advanced rapidly in the past few years. It is believed that activating autophagy, especially CMA, may have beneficial effects on the clearance of misfolded and aggregated proteins and the prevention of neurodegeneration, and may become a new therapeutic target in HD. The regulation of CMA is an important way as a therapeutic application. There is more support evidence for cross-talk among autophagic pathways, which means that one autophagic pathway is activated when another one is blocked [67]. Therefore, it is possible that the activation of macroautophagy contributes to the endogenous inhibitors of CMA. Of course, blocking Hsc70 and LAMP-2A exerts a strong inhibitory effect on CMA [38]. In addition, the activation of CMA is triggered in response to the stressors, such as long-term starvation, oxidative stress, or exposure to toxic compounds that induce abnormal conformational changes in cytosolic proteins [42]. These interactions help the investigators to seek a useful way to treat HD by affecting CMA. Inappropriate or prolonged activation of CMA may lead to the complete demise of the cells maintained, and will be another major challenge in the future development of therapeutic strategy. The confirmation of the involvement of the CMA pathway in Htt clearance is significant due to the selectivity of CMA for the substrates. Thus, developing therapies that can induce an enhancement of CMA-mediated clearance of mutant Htt may have great value.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 81201861 and 81272476), the Postdoctoral Science Foundation of China (2011M500949), and the Postdoctoral Science Foundation of Jiangsu Province (1102139C).

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