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Chaperone-mediated autophagy as a therapeutic target for Parkinson disease

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Abstract

Introduction: Parkinson disease (PD) is the most common neurodegenerative movement disorder. Currently only symptomatic treatments exist for PD, and so the search for potential neuroprotective drug targets is of great importance. Chaperone mediated autophagy (CMA) is one of the key cellular mechanisms in protein homeostasis. Many of the pathogenic pathways thought to be important in PD converge on CMA, thus rendering it an attractive therapeutic target.

Areas covered: In this review we will discuss current up-to-date knowledge of the molecular mechanisms involved in CMA as well its regulation. Furthermore, we will go on to discuss the links between CMA and PD including CMA's role in α-synuclein processing, oxidative stress and mitochondrial function. We will finish by exploring the potential benefits of how upregulation of CMA may be beneficial in PD as well as strategies to achieve this. A comprehensive literature search has been carried to include relevant *in vitro*, *in vivo* and patient based studies.

Expert opinion: Upregulation of CMA is an attractive therapeutic target in PD due to its links with several pathogenic pathways implicated in PD. Currently more knowledge of the precise mechanisms that regulate CMA is required to allow for the development of specific CMA modulators. However, recent studies demonstrating the role of retinoic acid derivatives and miRNAs in regulating CMA are promising, and indirect upregulation of CMA by modulating other lysosomal pathways may be helpful. Furthermore, the discovery of reliable biomarkers of CMA dysfunction will be of great help in assessing future therapeutic strategies.

Article highlights

- Chaperone-mediated autophagy (CMA) is implicated in many of the pathogenic pathways associated with Parkinson Disease (PD), including proteostasis and mitochondrial dysfunction.
- Key components of the CMA pathway are found to be dysregulated in the brains of PD patients.
- Up-regulation CMA in *in vitro* and *in vivo* models appears to confer protection against α-synuclein accumulation and associated cell death.
- The use of miRNAs, retinoic acid derivatives and lipid modification are current potential therapeutic strategies to modify CMA activity.
- Further work is required to fully elucidate the regulatory mechanisms of CMA, which in turn will generate further potential therapeutic targets.

1. Introduction

Parkinson disease (PD) is the most common neurodegenerative movement disorder with an estimated incidence ranging from 5 to >35 new cases per 100,000 individuals per year [1]. PD is rare before the age of 50 years but incidence increases 5-10 fold from the sixth to ninth decade of life [2], with >3% of those over 80 years of age affected by PD globally [3]. PD is characterised by progressive neuronal loss in specific areas of the brain, such as brainstem monoaminergic nuclei as well as, most characteristically, the dopaminergic neurons of the substantia nigra pars compacta (SNpc). Besides the motor symptoms of tremor, rigidity, bradykinesia and postural instability, PD patients often experience a range of non-motor symptoms such as fatigue, depression, sleep disturbance and dementia which are associated with reduced quality of life [4]. The only causes of PD identified to date are genetic and it remains unclear whether the environment plays any role in aetiology. Research has highlighted several dysregulated pathways implicated in PD pathogenesis, including oxidative stress, mitochondrial function and impaired proteostasis [5]. It is likely that better insights into the aetiopathogenesis of PD offers the best hope for the development of successful neuroprotective treatments that will slow or prevent the progression of both motor and non-motor features. Disease heterogeneity, the lack of reliable animal models and suitable clinical trial design are just some of the proposed reasons for the lack of success in producing disease modifying therapies [6–8].

A characteristic morphological feature of PD is the presence of intraneuronal protein aggregates (Lewy bodies, LB), mainly composed of the protein α-synuclein. The long lifespan of post-mitotic neurons in human brain means that they are particularly dependant on efficient intra-cellular handling of abnormal proteins. Chaperone mediated autophagy (CMA) is one of the main mechanisms cells employ to maintain protein turnover [9]. There is an ever growing body of evidence from genetic, *in vitro* and *in vivo* studies, that link CMA to PD. In this review we will outline the basic mechanisms that underlie CMA before going on to discuss the current evidence that links

CMA to PD pathogenesis. We will then discuss the proposed benefits of manipulating CMA activity in PD, as well as review current knowledge on CMA regulation, highlighting the current and potential future strategies to target CMA therapeutically, as well as the current obstacles to using such a strategy.

2. CMA - key proteins and molecular mechanisms

Protein homeostasis, or proteostasis, plays a crucial role in maintaining a vast number of cellular processes [10]. Protein degradation is a key component of proteostasis but it is also essential for the removal of damaged or altered proteins that may lead to cellular toxicity. The two major pathways employed by cells to achieve this are the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP) [11, 12]. The ALP can be further subdivided into three main types; macroautophagy (MA), microautophagy and chaperone-mediated autophagy (CMA), which differ in their selectivity and the mechanism by which they deliver substrates to the lysosome [13]. Whilst macro and microautophagy sequester and deliver entire regions of the cytosol to the lysosome, which can include complete organelles such as mitochondria, CMA only targets and delivers selected proteins [13, 14]. This selectivity allows for CMA to remove damaged or misfolded proteins without removing normally functioning neighbouring proteins, as well as play a regulatory role in many cellular processes by selective removal of transcription factors and cell maintenance proteins [15].

The CMA process is divided into four steps (fig.1): (I) recognition of substrate proteins and targeting them to the lysosome; (II) binding of substrate to lysosomal receptor and substrate unfolding; (III) translocation of the substrate into the lysosome; and (IV) degradation of substrate in the lysosomal lumen [9]. Recognition of substrates takes place in the cytosol where the constitutive chaperone, heat shock cognate protein of 70KDa (hsc70), binds to a pentapeptide amino acid motif (a KFERQ-like sequence) present in all CMA substrates [16]. Such motifs may only become accessible after protein unfolding or after proteins disassemble from multiprotein complexes that expose the

previously hidden motif. The CMA-targeting motif is based on the charge of the amino acids and so an incomplete motif can be made into a complete motif by post translational modification (e.g. acetylation or phosphorylation) [17, 18]. The binding of hsc70 targets the substrate to the lysosomal membrane where it interacts with the cytosolic tail of the lysosome-associated membrane protein type 2A (LAMP2A) [19]. LAMP2A exists as a monomer at the lysosomal membrane but on binding to a CMA substrate it undergoes multimerization to form a multiprotein translocation complex that facilitates the translocation of substrates into the lysosomal lumen [20]. The mechanism by which substrates are internalised is not fully understood but it is clear that substrates must undergo unfolding before translocation across the lysosomal membrane [21]. The unfolding process is likely facilitated by hsc70 as well as a number of co-chaperones [22]. Furthermore a lysosomal form of hsc70 (lys-hsc70) is required for substrate translocation into the lysosomal lumen, but the mechanism by which this occurs has not been fully elucidated [22]. Once the substrate has been translocated into the lysosomal lumen, LAMP2A is disassembled from the translocation complex to form LAMP2A monomers, allowing for further substrate binding [20]. The rate of CMA can be modified by the rate of assembly/disassembly of the translocation complex as well as the abundance of LAMP2A at the lysosomal membrane. The relative amount of LAMP2A at the lysosomal membrane it a finely controlled process balancing de novo synthesis and degradation. It has been shown that LAMP2A is dynamically sub-compartmentalised in the lysosomal membrane, with a proportion being associated with cholesterol and glycosphingolipid-rich lipid microdomains [23]. This association is thought to facilitate LAMP2A's cleavage by cathepsin A and an as yet unknown metalloproteinase, after which is it released into the lysosomal lumen to undergo degradation [23, 24]. LAMP2A not associated with such lipid microdomains is protected from such degradation and so can form LAMP2A multimers required for CMA activity. As such altering the proportion of LAMP2A associated with lipid microdomains in the lysosomal membrane can alter the rate of CMA activity [23].

3. CMA activity is altered in both genetic and sporadic forms of PD

There is now a large body of evidence, comprising both animal and human studies, that links CMA dysfunction to PD pathogenesis [25, 26].

3.1 α-synuclein and CMA:

It is known that single nucleotide variants, duplications and triplications of SNCA (the gene that encodes α-synuclein) cause familial forms of PD [27, 28]. Also, age of onset of PD and disease severity appears to be linked to the overexpression rate with patients carrying triplications of SNCA developing a more severe form of the disease earlier [28]. Finally, genome wide association studies have found that single-nucleotide variants at the SNCA locus increase the risk for sporadic PD [29]. Therefore, neuropathological and genetic evidence strongly support the notion that α-synuclein is important in PD pathogenesis.

It has been shown that α-synuclein contains a KFERQ-like motif and is a bona-fide substrate for CMA in *in vitro* isolated liver lysosomes [30] as well as in neuronal cell lines and primary neuronal cultures [31]. Furthermore, it has been shown that the downregulation of CMA, via interference with LAMP2A expression in both neuronal cell models and rat midbrain, leads to the accumulation of high molecular weight and detergent-insoluble species of α-synuclein [31, 32]. Interestingly it was found that reducing LAMP2A expression in rat midbrain led to a reduction in striatal dopamine content followed by progressive loss of nigral neurons with concurrent accumulation of α-synuclein, as well as lysosomal dysfunction and behavioural deficits [32]. In addition it has been shown that silencing of hsc70 gene expression leads to a significant rise in α-synuclein protein levels in neuronal cell models [33]. Taken together these results show that reducing key components of the CMA pathway, namely LAMP2A and hsc70, leads to accumulation of α-synuclein highlighting the importance of CMA in α-synuclein homeostasis.

The relationship between CMA and α-synuclein is however not unidirectional, as it has been shown that CMA can be a direct target of the toxic effects of α-synuclein. Thus a detrimental loop would ensue where CMA inhibition would lead to further α-synuclein accumulation. PD linked mutations in the SNCA gene (A30P and A53T) produce α-synuclein protein that binds LAMP2A with a higher affinity than wild type protein thus preventing its translocation across the lysosomal membrane [30]. This toxic interaction with aberrant α-synuclein not only precludes its own degradation but also other CMA substrates. Similar impairment of CMA function has been observed with α-synuclein that has undergone post-translational modification [34]. Such post-translational modification of α-synuclein by environmental or cellular stressors (such as pesticides or oxidative stress) as well as interactions with dopamine are thought to be important in the pathogenesis of sporadic PD [35, 36]. Whilst oxidised and nitrated forms of α-synuclein adversely alter their own degradation by CMA, dopamine-modified α-synuclein reduces CMA activity in a similar fashion to mutated forms of the protein. The tight binding and subsequent poor translocation of dopamine-altered α-synuclein by the CMA translocation complex inhibits its own degradation but also that of other CMA substrates. As for the PD linked mutations in α-synuclein, the persistence of post-translationally modified α-synuclein bound to the lysosomal membrane seeds the formation of potentially toxic α-synuclein oligomers [34]. Interestingly, there are also indications that overexpression of unmodified wild-type (WT) α-synuclein may have a detrimental effect on CMA activity [37, 38] (Fig 1.).

3.2 Other genetic mutations linking PD and CMA

Leucine-rich repeat kinase 2 (LRRK2) mutations are the most common familial cause of PD, with the most common G2019S mutation accounting for up to 30-40% of North African and Jewish PD cases as well as occurring in 1% of sporadic European PD patients [39]. It has recently been shown that LRRK2 is a CMA substrate and that both increased levels of the wild type protein as well as mutant forms (including the G2019S mutation) lead to impaired CMA processing [40]. A direct consequence of this toxic effect on CMA was an accumulation of α-synuclein at the lysosomal membrane which

promoted the formation of α-synuclein oligomers [40], which are commonly thought to be the toxic form of the protein [41]. Similar abnormal interactions with key CMA components have been reported for PD associated mutations in the ubiquitin C-terminal hydrolase L1 (UCH-L1) protein. The PD linked mutation of UCH-L1 (I93M) leads to a protein that abnormally interacts with LAMP2A, hsc70 and hsp90, leading to reduced CMA function and α-synuclein accumulation [42]. Finally, recent reports have demonstrated that both deficiency and autosomal dominant PD-linked mutation (D620N) of vacuolar protein sorting-35 (VPS35) lead to α-synuclein accumulation in mouse dopaminergic neurons. This effect was mediated by impaired endosome-to-Golgi retrieval of LAMP2A, leading to reduced LAMP2A levels and so reduced CMA activity [43] (Fig 1.).

All these data together strongly support the concept that impaired CMA function is important in the pathogenesis of PD, with the inhibition of CMA appearing as a common mechanism through which multiple proteins linked to familial PD could exert their toxic effect. Furthermore, it highlights potential toxic synergies between proteins known to be important in PD, for example LRRK2 and α-synuclein. In this scenario mutated, post-translationally modified or even potentially WT α-synuclein may lead to a reduction in CMA activity with a subsequent accumulation of LRRK2 protein which has been shown to inhibit CMA and in turn lead to further detrimental α-synuclein accumulation. Therefore restoring CMA activity to remove these toxic proteins is a valid target for future therapeutic research in PD.

3.3 Evidence of reduced CMA in idiopathic PD

In further support for a direct role of CMA in PD pathogenesis, key components of the CMA pathway have been found to be reduced in post mortem brain tissue from idiopathic PD patients. Both LAMP2A and hsc70 were found to be reduced in both the substantia nigra and amygdala of PD patients compared to aged matched controls and patients with Alzheimer's disease (AD) [44]. This is surprising given that the cellular environment in PD, such as high levels of oxidative stress, would

likely increase CMA activity. Indeed, increased LAMP2A and hsc70 have been found in the brains of PD mouse models [45]. Furthermore, analysis of post-mortem brain samples from patients suffering from dementia with Lewy bodies (DLB), a related synucleinopathy, revealed increased LAMP2A levels [46]. This would suggest differential CMA activity and regulation within the synucleinopathies. It is known that CMA activity decreases with age, with reduced LAMP2A stability at the lysosomal membrane being the most likely mechanism for this [9, 47]. It is therefore tempting to think that the reduced CMA proteins seen in PD brains may be a consequence of a gradual reduction due to ageing, however given that Alvarez-Erviti et al compared PD brains to aged matched AD and healthy controls it suggests other factors are likely to contribute to the observed reduction in CMA markers. A potential candidate are microRNAs (miRNAs) which are single stranded RNA molecules that have the ability to downregulate protein translation or promote degradation of specific mRNA molecules [48, 49]. Eight miRNAs, that were known to be upregulated in PD from previous studies [50], and that were predicted to interact with either LAMP2A or hsc70 have been identified [51]. Transfection of seven of these miRNAs into SHSY-5Y cells was able to produce dose-dependent reductions in endogenous LAMP2A and hsc70 proteins, with associated α-synuclein accumulation [51]. This reduction in protein level was not due to reduced mRNA levels, suggesting the miRNAs are acting at the translational level. Furthermore, the six of the seven miRNAs capable of producing this effect were found to be increased in the substantia nigra and amygdala of post-mortem brain tissue from PD patients [51]. In support of this a recent analysis of early PD brain tissue has shown a specific reduction in LAMP2A protein that precedes neuronal loss and Lewy body formation. This correlated with decreased hsc70 levels and increased levels of α-synuclein and other CMA substrates (including MEF2D) [52]. Of note, mRNA expression of all LAMP2 isoforms were not different from controls, supporting the idea that reductions in LAMP2A protein level must be mediated by processes other than reduced transcription, such as miRNA interference with translation [52]. It is tempting to speculate that the differences in LAMP2A levels found in PD and DLB brains may be in part due to differing miRNA expression profiles between these synucleinopathies.

4. CMA, MEF2D, mitochondrial dysfunction and oxidative stress

The transcription factor myocyte enhancer factor 2 (MEF2), which has 4 isoforms (MEF2A-D) was first described as a transcription factor involved in muscle cell differentiation [53]**.** It has been subsequently shown that MEF2s are highly expressed in brain and play a role in neuronal differentiation and survival. [54, 55]**.** It is now known that one isoform of MEF (MEF2D) is regulated, at least in part, by CMA with inhibition of CMA leading to an accumulation of cytoplasmic MEF2D [37]. Although this would intuitively suggest that a reduction in CMA would beneficial, it has been shown that the accumulated cytosolic MEF2D has reduced DNA binding and is therefore less functional. This suggests CMA preferentially removes non-functional MEF2D and that accumulation of the non-functional form compromises the action of active MEF2D [37]. The same study went on to show that both mutant (A53T) and WT α-synuclein disrupted MEF2D binding to hsc70, leading to MEF2D accumulation and cell death. Such toxicity could be attenuated by maintaining nuclear MEF2D levels by modification of MEF2D to impede its export from the nucleus [37]. These results therefore provide a link to how accumulation of WT α-synuclein may exert its toxic effects as well as the potential role of MEF2D in PD pathogenesis. In support of this Yang et al. went on to show that MEF2D levels were increased in both the brains of α-synuclein transgenic mice and PD patients, suggesting MEF2D-α-synuclein interactions may be important *in vivo [37]*.

Aside from being a nuclear transcription factor, MEF2D has been shown to regulate mitochondrial DNA (mtDNA) expression. Specifically MEF2D binds to mtDNA in the coding region of the ND6 gene to control its transcription [56]. ND6 encodes the protein NADH dehydrogenase 6, which is an essential component of Complex I of the mitochondrial transport chain [57]. Reducing mitochondrial MEF2D activity, via genetic manipulation of MEF2D, leads to a reduction in ND6 mRNA with subsequent reduction in complex 1 activity, reduced ATP levels and increased hydrogen peroxide formation [56]. This demonstrates that MEF2D directly regulates mitochondrial function.

Mitochondrial dysfunction has long been implicated in the pathogenesis of PD [58, 59]. Furthermore, reduced complex 1 activity has been described in substantia nigra of sporadic PD patients [60] and is felt to be a key pathogenic 'milestone' in PD. Reduction in mitochondrial MEF2D is therefore able to recreate features of PD pathogenesis. Interestingly mitochondrial MEF2D and ND6 proteins were greatly reduced in the brains of both MPTP-treated mice and human PD patients [56]. As mentioned previously total cellular MEF2D levels were found to be increased in the brains of PD patients, therefore this indicates a build-up of non-functional cytosolic MEF2D with a reduction in both DNA and mtDNA binding in PD. Since CMA inhibition leads to such a cytosolic accumulation of MEF2D it suggests a key role for CMA in regulating functional mitochondrial MEF2D levels and so provides another possible link to PD.

Oxidative stress, where excessive oxidant production and/or defective antioxidant systems lead to a redox imbalance, is another factor implicated in the pathogenesis of PD [61]. Oxidative stress has also been shown to upregulate CMA function via transcriptional upregulation of LAMP2A [62], suggesting a protective role for CMA in the selective removal of abnormal or damaged proteins to prevent toxic accumulation. In support of this argument selective reduction in CMA activity in cellular models increases vulnerability to stressors, in particular to those that induce oxidative stress [63]. The mechanism by which CMA reduction could lead to such vulnerability was not suggested. However, evidence demonstrating CMAs ability to degrade oxidised, damaged proteins linked to cell survival (MEF2D) and the oxidative stress response (DJ-1) may in part explain the observed reduction in cell viability in CMA deficient cells under stress. Furthermore, the fact that these proteins are linked to PD pathogenesis provide more evidence of CMAs role in PD.

Oxidative insults such as 6-hydroxydopamine (6-OHDA) have been shown to oxidise MEF2D in neuronal cell lines. Such oxidised MEF2D was found to have reduced DNA binding and be preferentially degraded by CMA [64]. The same authors also demonstrated that accumulation of

oxidised MEF2D led to cell death, which was exacerbated by reducing CMA activity and diminished using mutated MEF2D resistant to oxidative damage [64]. This demonstrates a direct relationship between reduced clearance of oxidised MEF2D and cell death. The finding that total and oxidised MEF2D levels are increased in the brains of PD patients [64] suggests that an accumulation of damaged MEF2D leading to reduced nuclear and mitochondrial binding may be important in PD pathogenesis, and that CMA appears to be key to maintaining MEF2D homeostasis by selectively removing damaged forms of the protein.

A very similar role for CMA in maintaining protein function has recently been described for DJ-1, another protein linked closely to PD [65]. Daisuke-Junko-1 (DJ-1/PARK7) is a multifunctional protein, with its principal roles being antioxidant defence and maintenance of mitochondrial function [66, 67]. Mutations in DJ-1 are associated autosomal recessive early onset PD [68], and lead to the destruction of DJ-1's functional homodimeric structure. Furthermore, accumulation of non-functional, extensively oxidised DJ-1 is found in the brains of sporadic PD patients [69] as well as induced pluripotent stem cell (IPSC) derived dopaminergic neurons from sporadic PD patients [70]. It has been demonstrated that DJ-1 is a CMA substrate but also that extensively oxidised DJ-1 is preferentially degraded by CMA under conditions of increased oxidative stress [65]. In addition the same study found that reduction of CMA activity led to both an accumulation of oxidised DJ-1 monomers that alters the balance of DJ-1 dimerisation and increased mitochondrial damage and cell death under stress conditions [65]. Thus a reduction in CMA activity could explain the increased levels of oxidized DJ-1 found in sporadic PD patients. A recent study has linked reduced DJ-1 function to a pathological chain of events beginning with increased oxidative stress leading to oxidised dopamine accumulation, ultimately leading to lysosomal dysfunction and α-synuclein accumulation. This cascade was found not only in DJ-1 mutant IPSC derived neurons but also in those derived from sporadic PD patients [70]. This clearly implicates reduced DJ-1 activity in PD pathogenesis, and given

CMAs apparent role in removing non-functional DJ-1 protein, provides another link between CMA and PD.

5. Can upregulation of CMA be beneficial in PD?

The evidence presented so far demonstrates a clear link between PD and CMA, as well the detrimental effects reduced CMA activity has in the context of PD pathogenesis. It is therefore plausible that upregulation of CMA would be beneficial in PD. In support of this it has been shown that genetic manipulation to preserve CMA function in the liver of aged mice by expressing an exogenous copy of LAMP2A led to enhanced resistance to stressors and general improvements in liver function [71]. Importantly the study demonstrated that increasing LAMP2A levels once the age related decline in CMA had already begun was able to improve CMA activity and organ function. Subsequently it has been shown that employing a similar strategy in the brain can have equally beneficial outcomes. Using an *in vivo* adeno-associated virus (AAV) model of PD in rats Xilouri et al. were able to demonstrate that genetic upregulation of LAMP2A was not toxic and more importantly that it greatly reduced α-synuclein levels (including toxic species) and consequently ameliorated α-synuclein-induced dopaminergic neurodegeneration [72]. The fact that upregulation of CMA is capable of alleviating toxicity associated with α-synuclein supports the notion that changes in CMA observed in PD are not just a consequence of the disease but contribute to its pathogenesis. It also demonstrates that *in vivo* modulation of CMA is a very promising therapeutic for PD as well as other related synucleinopathies.

6. Regulation of CMA - obstacles and potential therapeutic targets:

Understanding of the regulatory and signalling pathways that control CMA are likely to provide targets for therapeutic intervention. Despite the molecular components of CMA targeting and translocation being well characterised the signalling pathways that mediate stress induced CMA upregulation are largely unknown. As previously stated is known that the rate of CMA is proportional

to the amount of LAMP2A at the lysosomal membrane and can be modulated by the rate of assembly and disassembly of the LAMP2A translocation complex [20]. As such the molecular regulators of LAMP2A levels at the lysosomal membrane, either through increased transcription or reduced degradation, as well as regulators of LAMP2A translocation complex assembly are important therapeutic targets (Fig. 1).

6.1 Targeting LAMP2A translocation complex assembly:

It has been shown that a pair of proteins, GFAP and EF1α, acting in a GTP dependent manner can modulate the assembly/disassembly rate of the LAMP2A translocation complex [73]. Association of GFAP to the translocation complex contributes to its stabilization. Once the substrate has passed through the complex GFAP dissociates and binds to phosphorylated forms of GFAP which are found bound to EF1α, this promotes disassembly of the translocation complex and so a reduction in CMA flux [73]. A further layer of complexity has been added recently with the finding that CMA activity is regulated by the lysosomal mTORC2/PHLPP1/Akt axis [74]. Here mTORC2 acts as an inhibitor with PHLPP1 acting as a stimulator of CMA under stress conditions, likely mediated through the phosphorylation of GFAP [74]. The number of cellular processes involving PHLPP1 is enlarging [75] which may well limit it as a target for therapeutic intervention due to off target effects. Similarly, unlike the closely related mTORC1 (which interestingly is involved in the regulation of macroautophagy), mTORC2 signalling is less well understood but appears to be involved in a diverse range of processes that suggest inhibition may have detrimental consequences [76,77]. Currently there are no small molecules that specifically target mTORC2, but encouragingly competitive inhibitors of mTOR that inhibit both mTORC1 and mTORC2 are being investigated in oncology clinical trials and so appear to be tolerated by human subjects [78].

6.2 Targeting LAMP2A degradation

As highlighted, degradation of LAMP2A requires its association with cholesterol rich lipid microdomains within the lysosomal membrane. Therefore the lipid composition of the lysosomal membrane is able to modulate CMA activity [23]. It has been shown that exposure to a high cholesterol diet accelerates LAMP2A degradation, thus reducing CMA activity, due to increased trapping of LAMP2A in lipid microdomains [79]. Conversely, cholesterol depleting agents were able to increase CMA activity by reducing LAMP2A degradation [23]. It is postulated that changes in the lipid composition of the lysosomal membrane may account for the reduction in CMA seen with ageing [79, 80]. These findings hint that lipid lowering medications could potentially improve CMA activity. Controversy still exists regarding the relationship between statins and PD risk, with conflicting results showing, increased, decreased or no change in risk of PD in those taking statins [81–83]. However this still represents an interesting area for further exploration and the effect of simvastatin in disease modification of PD is currently being explored in a therapeutic trial.

6.3 Targeting LAMP2A transcription

When targeting CMA it also needs to be kept in mind that the three ALP systems (CMA, macroautophagy and microautophagy) and the UPS do not act independently from one another, with multiple points of interaction existing between them. As such these interactions need to be taken into consideration when modulating any if the individual pathways. However, the signalling pathways that control these relationships have yet to be fully elucidated [84]. Accordingly some of the small molecules that were initially described to modulate CMA [85] have proven rather non-specific, having effects on multiple proteostatic mechanisms such as macroautophagy. With reference to this there is conflicting evidence regarding whether upregulation of MA, which is seen with CMA inhibition [63], is beneficial [86] or detrimental [38]. As such the recent finding that retinoic acid derivatives can specifically regulate CMA without affecting other autophagic pathways is of interest [87]. Anguiano et al. identified that disruption of signalling at the retinoic acid receptor alpha (RARα) had a stimulatory effect on CMA by increasing transcription of LAMP2A and other CMA

components, which was able to protect cells from oxidative stress and proteotoxicity [87]. Retinoic acid derivatives are attractive compounds as they are already used in human disease such as in the treatment of certain leukaemias [88]. Although further pre-clinical models utilising these compounds to boost CMA are still awaited, this is a promising therapeutic avenue.

6.4. Targeting miRNAs

The finding that miRNAs that target and reduce the expression of key CMA components are increased in PD brains [51], raises them as a potential therapeutic target. In support of this, upregulation of a miRNA resulting in the reduction of hsc70 was shown to increase α-synuclein accumulation in SHSY-5Y cells [89]. Furthermore a recent study has shown that the selective reduction of miRNA-21, was able to counter the reduction LAMP2A expression caused by miRNA-21 and reduce α-synuclein accumulation in both a cellular model and in the brains of MPTP treated mice [90]. Thus miRNA-21 appears a promising new therapeutic target. Therapeutic strategies to target miRNAs have already reached clinical trials in both oncology and hepatology [91], which suggests such a strategy would be plausible for neurodegenerative disorders such as PD.

7. Conclusion

PD is a progressive neurological disorder that currently has no neuroprotective treatment options. As one of the main autophagy pathways, CMA is a crucial component to maintain proteostasis. There is an ever enlarging body of evidence that suggests that several processes implicated in PD pathogenesis converge on impaired CMA function, including α-synuclein accumulation, mitochondrial dysfunction and oxidative stress. Furthermore, CMA has been shown to have close interactions with several PD related genes (SNCA, LRRK2, VPS35, UCH-L1 and DJ-1) as well as being reduced in the brains of those with sporadic PD. All of this evidence suggests improving CMA activity may have multiple potential benefits for PD patients and so is an attractive therapeutic target. Indeed the genetic manipulation of CMA has been shown to be beneficial in both *in vitro* and *in vivo*

models of PD. The intricate pathways that regulate CMA activity as well as those that mediate the 'cross-talk' between CMA and the other proteostatic pathways are yet be fully elucidated and so currently the use of small molecules to selectively to upregulate CMA is in its infancy. However, RARα signalling and miRNA interactions are showing promise that selective CMA upregulation may be possible. As more is learnt about CMA's signalling pathways, new therapeutic targets are likely to be emerge that could potentially have a great impact on PD treatment.

8. Expert opinion

CMA is clearly implicated in PD pathogenesis and so is a potential target for therapeutic intervention. However, the pathogenic sequence of events that leads to a person developing PD has yet to be fully delineated [92]. There have been a number of putative neuroprotective compounds that have shown promise in pre-clinical models of PD but have failed to show benefit when tested in human clinical trials. Suggested reasons for such disappointing human translation include unreliable pre-clinical models of PD as well as the lack of appreciation of disease heterogeneity when planning clinical trials [6–8]. The latter point is especially interesting as it suggests that the 'road' to neurodegeneration in PD may be different between subgroups of patients. The appreciation of such disease heterogeneity has led to great therapeutic advances in the field of oncology, so perhaps the same could occur in the field of neurodegeneration. Given this, it is possible to speculate that CMA dysfunction may be more important in some PD patients that others. Therefore the development of reliable biomarkers of CMA dysfunction will likely prove important in the future development of therapeutics aimed at improving CMA function. Interestingly, studies have shown evidence of impaired CMA function (via reduced levels of LAMP2A and hsc70) in lymphocytes from PD patients. Two studies have found reduced hsc70 levels idiopathic PD lymphocytes, with no changes in LAMP2A levels [93,94], whereas another found reduced LAMP2A levels but did not examine hsc70 [95]. Interestingly Papagiannakis et al also found decreased hsc70 protein levels in lymphocytes isolated from PD patients with SNCA and GBA mutations [94]. These studies suggest a systemic

dysfunction in CMA is apparent in PD. Furthermore the disagreement between the studies could hint to the heterogeneity discussed above, indeed even within the two studies that show consistent results there is a large overlap of hsc 70 levels in PD patients with controls implying CMA impairment may not universal in PD [93,94]. The search for reliable biomarkers will be key to directing which PD patients should enrol in trials of CMA altering therapies as well as acting as makers of target engagement thus representing a key future area of research.

As compounds to selectively regulate CMA are still in their infancy, strategies to indirectly improve CMA function, for example substrate reduction, may warrant further exploration. Given the evidence presented regarding α-synuclein's relationship with CMA, specifically that even wild type α-synuclein can inhibit CMA at high levels, reducing α-synuclein is likely to improve CMA function. Such a reduction in α-synuclein may be achieved by targeting glucocerebrosidase. Mutations in the glucocerebrosidase gene (GBA) are numerically the most important risk factor developing PD [94]. GBA encodes glucocerebrosidase (GCase), an important lysosomal enzyme, which has been shown to be reduced in the brains of sporadic PD patients [95]. The relationship between α-synuclein and GCase is complicated but defective GCase activity has consistently been linked with α-synuclein accumulation [94]. Interestingly it has been found GCase activity is selectively reduced in the early stages of PD in regions with increased α-synuclein levels that have yet to develop significant Lewy pathology. Furthermore, the loss of GCase activity and increase in α-synuclein were shown to be directly related to reduced LAMP2A levels in PD brains, suggesting impaired CMA [96]. Thus, finding agents that are able to increase levels of functional properly folded GCase would enhance α-synuclein degradation and therefore potentially preserve CMA function.

Overall the modulation of CMA activity either directly or indirectly is likely to be of great benefit to PD patients. Fully elucidating the mechanisms of CMA regulation, as well the discovery of reliable accessible biomarkers of CMA dysfunction are important future areas of research as this will not only

foster the discovery of therapeutic targets but also highlight the group of PD patients at whom those experimental compounds should be targeted.

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Abbreviations:

6-OHDA	6-hydroxydopamine
ALP	Autophagy-lysosome pathway
CMA	Chaperone-mediated autophagy
EF1α	Elongation factor 1-α
GFAP	Glial fibrillary acidic protein
hsc70	Heat shock cognate protein of 70KDa
IPSC	induced pluripotent stem cell
LAMP2A	Lysosome-associated membrane protein type 2A
LRRK2	Leucine-rich repeat kinase 2
LB	Lewy bodies
mTORC2	mammalian target of rapamycin complex 2
miRNA	mircoRNA
mtDNA	mitochondrial DNA
MEF2	myocyte enhancer factor 2
PD	Parkinson disease
PHLPP1	PH domain leucine-rich repeat-containing protein phosphatase 1

shRNA	Short hairpin RNA
SNpc	Substantia nigra pars compacta
UCH-L1	Ubiquitin C-terminal hydrolase L1
UPS	Ubiquitin-proteasome system
VPS35	Vacuolar protein sorting-35
WT	Wild type

Figure 1 – Steps of chaperone mediated autophagy (CMA). Proteins to be degraded by CMA are recognised by a chaperone complex containing hsc70 (Heat shock cognate protein of 70KDa) which bind to a KFERQ-like motif in the substrate; this starts a cascade of events leading to protein degradation within the lysosome via the lysosomal receptor protein, LAMP2A (Lysosome-associated membrane protein type 2A). Many of the genetic mutations associated with Parkinson disease, including VPS35 (Vacuolar protein sorting-35), UCHL-1 (Ubiquitin C-terminal hydrolase L1), LRRK2 (Leucine-rich repeat kinase 2), GBA (glucocerebrosidase) and SNCA (alpha synuclein), all have detrimental effects to proper CMA functioning (red arrows). Therapeutic targets include up-regulation of LAMP2A levels at the lysosomal membrane via increased transcription and/or reduced degradation, increased rate of assembly/disassembly of the translocation complex and substrate reduction (green arrows).