The involvement of endoplasmic reticulum formation and protein synthesis efficiency in VCP- and ATL1-related neurological disorders

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Abstract

The endoplasmic reticulum (ER) is the biggest organelle in cells and is involved in versatile cellular processes. Formation and maintenance of ER morphology are regulated by a series of proteins controlling membrane fusion and curvature. At least six different ER morphology regulators have been demonstrated to be involved in neurological disorders—including Valosin-containing protein (VCP), Atlastin-1 (ATL1), Spastin (SPAST), Reticulon 2 (RTN2), Receptor expression enhancing protein 1 (REEP1) and RAB10—suggesting a critical role of ER formation in neuronal activity and function. Among these genes, mutations in VCP gene involve in inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD), familial amyotrophic lateral sclerosis (ALS), autism spectrum disorders (ASD), and hereditary spastic paraplegia (HSP). ATL1 is also one of causative genes of HSP. RAB10 is associated with Parkinson’s disease (PD). A recent study showed that VCP and ATL1 work together to regulate dendritic spine formation by controlling ER formation and consequent protein synthesis efficiency. RAB10 shares the same function with VCP and ATL1 to control ER formation and protein synthesis efficiency but acts independently. Increased protein synthesis by adding extra leucine to cultured neurons ameliorated dendritic spine deficits caused by VCP and ATL1 deficiencies, strengthening the significance of protein synthesis in VCP- and ATL1-regulated dendritic spine formation. These findings provide new insight into the roles of ER and protein synthesis in controlling dendritic spine formation and suggest a potential etiology of neurodegenerative disorders caused by mutations in VCP, ATL1 and other genes encoding proteins regulating ER formation and morphogenesis.

Keywords: Amyotrophic lateral sclerosis, Atlastin 1, Dendritic spine formation, Endoplasmic reticulum, Frontotemporal dementia, Hereditary spastic paraplegia, Protein synthesis efficiency, Valosin-containing protein

Background

The endoplasmic reticulum (ER) is a contiguous membrane network extending from the nuclear envelope to the entire cytoplasm and making contact with plasma membrane [1–4]. It is responsible for protein synthesis, modification and quality control. The ER also plays crucial roles in carbohydrate metabolism, control of lipid synthesis and delivery, formation of other membrane-bound organelles and lipid droplet and calcium homeostasis [1–3, 5]. The ER undergoes constant extension, retraction and membrane fusion [1, 6–8]. Biogenesis and maintenance of ER are complex and tightly controlled processes [8, 9], and many factors regulating ER formation and morphology have already been identified [1, 7, 8, 10]. Interestingly, mutations in genes involved in the regulation of ER biogenesis and maintenance, such as Valosin-containing protein (VCP), Atlastin-1 (ATL1), Spastin (SPAST), Reticulon 2 (RTN2), and Receptor expression enhancing protein 1 (REEP1) have been linked to neurological diseases. ATL1, RTN2, SPAST and REEP1 are the causative genes of hereditary spastic paraplegia (HSP) [8, 11, 12]. Mutations of the VCP (also known as p97) gene have been identified in patients with frontotemporal dementia [13, 14], amyotrophic lateral sclerosis (ALS) [15–17], autism spectrum disorders

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ATPases Associated with Membrane Degradation (UFD1L)-nuclear protein localization two most studied VCP cofactors are the ubiquitin fusion degradation processes, amongst others [24, 33, 34]. These diverse cellular processes, including ER-associated protein degradation [27, 28, 37] and chromatin-associated protein degradation [28, 38, 39]. Since VCP uses its N-terminal overlapping binding sites to interact with P47 and the UFD1L-NPL4 dimer [40], expression levels of VCP cofactors may alter complex formation and thereby influence the function of VCP in cells [23].

In 2004, Kimonis and colleagues provided the first evidence that mutations in the VCP gene result in inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD), which is a multiple tissue disorder associated with myopathy, bony defects and dementia [13]. Later, whole exome sequencing further revealed that VCP is associated with other neurological disorders, including familial ALS [15], ASD [18], and HSP [19]. It is unclear why mutations in a single gene, VCP, result in various neurological disorders. Perhaps it is due to the diverse activities of VCP in cells. Since the functions of VCP are determined by its interacting cofactors [26], the genetic diversity and/or expression levels of VCP cofactors likely influence the outcome of VCP deficiency, although direct evidence supporting this hypothesis is lacking.

VCP is involved in versatile cellular activities and multiple neurological diseases

VCP, a member of the AAA+ (ATPases Associated with diverse cellular Activities) protein family, acts as a molecular chaperon regulating multiple cellular processes [24–26], including ER-associated protein degradation [27, 28], the ubiquitin–proteasome system [24, 29], ER and Golgi morphogenesis [30–32], chromatin-associated processes, amongst others [24, 33, 34]. These diverse activities are determined by the cofactors of VCP [26]. The two most studied VCP cofactors are the ubiquitin fusion degradation 1-like (UFD1L)-nuclear protein localization homolog 4 (NPL4) heterodimer [35] and P47 [36]. The VCP-UFD1L-NPL4 complex is mainly involved in protein degradation [27, 28, 37] and chromatin-associated processes [24]. When VCP binds to P47, it regulates homotypic membrane fusion of ER and Golgi apparatus [30, 31, 36, 38, 39]. Since VCP uses its N-terminal overlapping binding sites to interact with P47 and the UFD1L-NPL4 dimer [40], expression levels of VCP cofactors may alter complex formation and thereby influence the function of VCP in cells [23].

ATL1, a causative gene of SPG3A, acts as a membrane fusogen controlling ER formation

Approximately 60% of HSP patients carry autosomal dominant mutations in one of four genes: ATL1, SPAST, RTN2 and REEP1 [10–12, 41]. These four genes work together to drive homotypic ER membrane fusion and coordinate microtubule interactions with the tubular ER network (Table 1) [42–45]. ATL1 acts as a membrane-anchored dynamin-like GTPase and directly interacts with SPAST [46, 47]. The ATL1-SPAST complex also interacts with RTN2 and REEP1 [45, 48, 49]. In addition, Drosophila Atlastin functionally associates with TER94 (Transitional endoplasmic reticulum ATPase 94), the VCP ortholog in Drosophila [50]. Mammalian VCP also co-immunoprecipitates with ATL1 [23]—the member of the Atlastin protein family predominantly expressed in the brain [42]—suggesting a physical association of VCP with ATL1 in mammalian brains. Since VCP mutation has been identified in patients with HSP [19], it seems plausible that VCP and ATL1 work together to control the function and activity of neurons. We discuss evidence supporting this possibility below.

Abnormal neuronal morphology as a feature of neurological disorders

Neurons are highly differentiated cells with specialized subcellular structures, including axon, dendrite and synapses.

**Table 1** Molecular functions and disease associations of ER morphology regulators

| Disease          | Molecular functions                                                                 |
|------------------|-------------------------------------------------------------------------------------|
| VCP              | AAA+ ATPase; molecular chaperon; cofactors guiding different functions [24–26]       |
| IBMPFD [13, 14]; ALS [15–17]; ASD [18]; HSP [19] | AAA+ ATPase; molecular chaperon; cofactors guiding different functions [24–26]       |
| ATL1             | Dynamin-like GTPase; driving homotypic membrane fusion by dimerization [79].         |
| SPG3A [78]       | ER shaping protein; interaction with spastin [80].                                    |
| RTN2             | ER-shaping protein; acts together with spastin and atlastin-1 [45].                  |
| REEP1            | ER-shaping protein; acts together with spastin and atlastin-1 [45].                  |
| SPAST            | AAA+ ATPase; microtubule-severing protein [83].                                      |
| SPG4 [82]        | Small GTPase; controls ER tubule extension and fusion [75].                          |
| RAB10            | PD-associated [84]                                                                   |
All these subcellular structures are essential for neurons to transmit signals among neurons and required for neuronal function and activity. In mammalian brains, excitatory synapses are mainly localized at the tips of dendritic spines, the tiny protrusions emerging from dendrites [51]. Thus, the morphological features of neurons, such as the size and density of dendritic spines, dendritic arbors and branching level and axonal length, are highly relevant to the function of neurons. The impairments of formation and/or maintenance of these structures result in neuronal defects and neurological disorders. Especially, synaptopathy, such as dendritic spine pathology, is most relevant to many psychiatric, neurodevelopmental and neurodegenerative disorders [52–54]. Morphological change (enlargement, shrinkage or elongation) of dendritic spines and/or alteration (increase or decrease) of dendritic spine density have been demonstrated in various neurological disorders, including Alzheimer’s disease, frontotemporal dementia, schizophrenia, ASD, etc. [52, 55, 56]. The morphological changes of dendritic spines are directly related to synaptic strength and the spine loss reflects a deficit of neuronal connectivity [57–59]. Though electrophysiological studies are still recommended to confirm the conclusion of synaptic deficits, morphological and density analyses of dendritic spines provides the easy and reliable ways to assess synaptic deficits and the potential impairment of neuronal activity. Dendritic spine deficits serve as useful indicator to evaluate pathological condition in various neurological disorders, including neurodevelopmental disorders as well as neurodegenerative diseases.

**Vcp deficiency impairs neuronal morphology**

Initial evidence supporting a role for VCP in regulating neuronal morphology came from a study about neurofibromin, a protein product encoded by the neurofibromatosis type 1 (Nf1) gene [60–62]. Using a series of biochemical analyses, VCP and P47 were shown to interact with neurofibromin in rat brain extracts and HEK293 cells [60]. Expression of individual VCP- and neurofibromin-interacting domains to disrupt complex formation of neurofibromin and VCP reduced the density of dendritic spines [60]. Furthermore, reduction of Nf1 and Vcp expression decreased dendritic spine density [60]. Thus, the neurofibromin-VCP complex in neurons regulates the formation of excitatory synapses. Since VCP overexpression rescues Nf1 haploinsufficiency [60] and because the subcellular distribution of VCP is altered in Nf1<sup>−/−</sup> mouse brains [60], it would appear that VCP acts downstream of neurofibromin in regulating dendritic spine density.

In addition to dendritic spine formation in mammalian brains, Drosophila Ter94 is required for dendritic pruning during metamorphosis [63]. Ter94 deficiency results in mislocalization and gain-of-function of the Drosophila homolog of the human RNA-binding protein TAR–DNA-binding protein of 43 k-Daltons. A protein degradation-independent pathway is suggested to be involved in the role of Ter94 in dendritic pruning [63].

Taken together, the studies in both rodents and Drosophila support the role of VCP in regulation of neuronal morphology. The morphological defects caused by VCP deficiency likely impair neuronal function and activity and result in pathological condition. However, the above studies were still limited to in vitro cultured neurons. More in vivo studies using mouse models or patients’ samples are required to verify the results of cultured neurons. It is also intriguing to explore where specific brain region(s) is more susceptible to Nf1 and VCP deficiency.

**Involvement of ER morphology and protein synthesis in regulating dendritic spine density**

Given the fact that VCP is involved in multiple cellular processes, it has been challenging to investigate the molecular etiology of VCP-related disorders. Since ubiquitin- and VCP-positive protein aggregations in muscle are a hallmark of patients with IBMPFD [13, 64], protein degradation defects caused by VCP deficiency have been recognized as an important pathogenic mechanism for VCP-related disorders. However, accumulated evidence suggests that the consequences of VCP deficiency in different types of cells vary. For instance, expression of VCP IBMPFD mutants induces polyubiquitinated protein aggregation in mouse myoblast C2C12 cells [65] but not in cultured hippocampal neurons [60], while still reducing dendritic spine density [23, 60]. These results suggest that another mechanism, in addition to the protein aggregation induced by VCP IBMPFD mutants, is critical for dendritic spine impairment.

Since the functions of VCP are determined by its cofactors, evaluating the roles of VCP’s cofactors in dendritic spine formation may reveal how VCP controls dendritic spine formation. Based on this rationale, two major cofactors of VCP—namely the UFD1L-NPL4 heterodimer and P47—have been knocked down individually in cultured hippocampal neurons. Although the UFD1L-NPL4 heterodimer is well-known to guide VCP’s regulation of protein degradation and chromatin-associated processes [24], knockdown of UFD1L to disrupt the function of the UFD1L-NPL4 heterodimer did not influence the dendritic spine density of cultured hippocampal neurons [23], suggesting that UFD1L-NPL4 heterodimer-dependent processes are not critical to dendritic spine formation. In contrast, knockdown of P47 reduced dendritic spine density [23]. Moreover, P47 overexpression rescued the spine phenotype caused by partially reduced VCP expression using a knockdown approach in cultured neurons, suggesting that P47 acts downstream in VCP-mediated dendritic spine formation [23].
Previous studies indicate a role for the VCP-P47 complex in homotypic membrane fusion of intracellular membrane-bound organelles, particularly ER [32, 36, 66]. Experiments using DsRed-ER (a red fluorescent protein fused with ER-targeting and -retention sequences) to label ER revealed that knockdown of VCP or P47, or overexpression of VCP IBMPFD mutants, indeed reduced the distribution of ER along dendrites in cultured neurons as well as in brains [23]. Further experiments using transmission electron microscopy to analyze knock-in mice carrying the R95G IBMPFD mutation in the Vcp gene demonstrated that the length and amounts of rough ER in soma are reduced by VCP IBMPFD mutation [23], supporting that neuronal ER is impaired by Vcp deficiency.

In addition to the reduced amounts of rough ER, attachment of ribosomes to rough ER also decreased under expression of VCP IBMPFD mutant [23]. Since ER is critical for the synthesis of membrane, secreted and cytosolic proteins [67–69], a reduction of ribosomal attachment on ER likely has a global effect on the protein synthesis of neurons. The effect of VCP deficiency on protein synthesis was directly investigated by bioorthogonal non-canonical amino acid tagging [70] and surface sensing of translation [71]; the former uses L-azidohomoalanine to label newly synthesized proteins, whereas puromycin is integrated into newly synthesized proteins in the latter. Both of these methods revealed that the amount of newly synthesized proteins within 1 h of labeling was reduced under VCP deficiency [23]. However, labeled protein amounts after 4 or 6 h were not obviously different between wild type and VCP-deficient neurons [23]. This finding indicates that VCP deficits impair the efficiency of protein synthesis but not total protein levels, implying that unstable proteins may be more sensitive to VCP deficiency.

By increasing protein synthesis to rescue the dendritic spine deficits caused by VCP deficiency can further strengthen the notion that inefficient protein synthesis is indeed the key downstream outcome of VCP deficiency. The branched-chain amino acid, especially the leucine, is well-known to activate the mTOR pathway that upregulates protein synthesis [72–74]. Adding extra leucine in cultured media increased the protein synthesis of VCP-deficient neurons [23]. Importantly, the dendritic spine defects caused by VCP deficiency were also effectively rescued to levels comparable to those of wild type neurons by leucine supplements [23]. The results of these leucine rescue experiments concluded that VCP mutation or deficiency result in impairment of ER formation and a reduction of protein synthesis efficiency and, consequently, impair dendritic spine formation.

Convergence of multiple ER formation pathways to control dendritic spine formation

If ER malformation is sufficient to impair protein synthesis efficiency and to result in reduced dendritic spine density, it is reasonable to speculate that other regulators of ER morphology also control protein synthesis efficiency and dendritic spine density. In addition to VCP, many other regulators of ER morphology have been identified. Two other ER morphology regulators, ATL1 and RAB10, have been assessed. RAB10, a small GTPase, regulates ER tubule growth, which is independent of the membrane fusion controlled by ATL1 [75]. Expression of the ATL1 SPG3A mutant or the GDP-locked T23 N mutant of RAB10 impairs ER formation in cultured neurons and reduces protein synthesis efficiency [23]. Importantly, dendritic spine density of cultured hippocampal neurons is reduced by At1l and Rab10 deficiencies [23]. These studies support the hypothesis that normal ER formation is critical for protein synthesis and for controlling dendritic spine formation.

A previous study suggested that the VCP-P47 complex acts with an unknown membrane fusogen to control homotypic membrane fusion [76]. Since ATL1 functions as an ER fusogen and because ATL1 interacts with VCP [23], ATL1 is therefore an excellent candidate as an interacting partner with VCP to control ER formation and dendritic spine formation. Indeed, in VCP-knockdown neurons, overexpression of wild-type ATL1 increases the density of dendritic spines of cultured hippocampal neurons. Expression of disease-associated mutants of both VCP and ATL1 does not further reduce dendritic spine density compared with single transfected neurons [23]. In contrast, expression of the GDP-locked T23 N mutant of RAB10 further reduces dendritic spine density of neurons expressing the VCP IBMPFD mutant [23]. Taken together, these experiments suggest that ER formation and associated protein synthesis efficiency is a common downstream pathway of multiple upstream regulators (such as VCP-P47-ATL1 and RAB10) controlling dendritic spine formation (Fig. 1).

Conclusion

Although VCP possesses multiple different functions in cells, its regulation of ER formation is critical for controlling dendritic spine density. Among ER-dependent cellular processes, protein synthesis is particularly important for VCP-, ATL1-, P47- and RAB10-regulated dendritic spine formation. Previous study indicated that tubular rough ER is concentrated at the bases of dendritic spines to meet their demands in response to synaptic stimulation [77]. The studies summarized above provide a mechanism underlying the role of ER and protein synthesis in controlling dendritic spine formation. Nevertheless, several questions remain unanswered. First, whether apart from VCP,
ATL1, P47 and RAB10, other regulators of ER morphology have a similar function in protein synthesis and dendritic spine formation. Second, are any specific proteins particularly sensitive to ER malformation? For instance, are short half-life proteins and/or membrane and secreted proteins more susceptible to VCP-, ATL1-, P47- and RAB10-related ER defects? Third, in vivo evidence to support the effect of ER malformation on dendritic spine formation is still lacking. Fourth, since VCP acts downstream of neurofibrin to regulate dendritic spine formation, it would be intriguing to explore whether ER formation and protein synthesis also contribute to neurofibrin-mediated dendritic spine formation. Finally, leucine supplementation seems to be potentially useful for increasing dendritic spine density in vivo. Investigation of the beneficial effects of leucine supplementation on mouse models of VCP- and HSP-related disorders is warranted, potentially providing research avenues for future therapeutics. If protein synthesis efficiency is indeed involved in the etiology of VCP- and HSP-related disorders, it suggests that nutrient and genetic factors may have synergistic effects on induction of these neurodegenerative disorders. Thus, environmental factors, such as nutrients, should also be taken into consideration when investigating VCP- and HSP-related disorders.

Abbreviations
AAAA + : ATPases associated with diverse cellular activities; ALS: amyotrophic lateral sclerosis; ASD: autism spectrum disorders; ATL1: atlastin-1; ER: endoplasmic reticulum; HSP: hereditary spastic paraplegia; IBMFD: inclusion body myopathy with Paget disease of bone and frontotemporal dementia; NPL4: nuclear protein localization homolog 4; PD: Parkinson’s disease; REEP1: receptor expression enhancing protein 1; RTN2: reticulon 2; SPAST: spastin; SPG: spastic paraplegia; Ter94: transitional endoplasmic reticulum ATPase 94; VCP: valosin-containing protein; UFD1L: ubiquitin fusion degradation 1-like

Acknowledgements
We thank Dr. John O’Brien for English language editing.

Funding
Y.-P.H. is supported by grants from Academia Sinica and the Ministry of Science and Technology, Taiwan (MOST 106–2321-B-001-019 and 105–2311-B-001-061-MY3). Y.-T.S. is supported by a postdoctoral fellowship from Academia Sinica.

Availability of data and materials
Not applicable.

Authors’ contributions
Y-TS and Y-PH wrote, read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 27 September 2017 Accepted: 26 December 2017
Published online: 08 January 2018

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