Mini-review

The interconnection of endoplasmic reticulum and microtubule and its implication in Hereditary Spastic Paraplegia

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ABSTRACT

The endoplasmic reticulum (ER) and microtubule (MT) network form extensive contact with each other and their interconnection plays a pivotal role in ER maintenance and distribution as well as MT stability. The ER participates in a variety of biological processes including protein folding and processing, lipid biosynthesis, and Ca^{2+} storage. MTs specifically regulate cellular architecture, provide routes for transport of molecules or organelles, and mediate signaling events. The ER morphology and dynamics are regulated by a class of ER shaping proteins, which also provide the physical contact structure for linking of ER and MT. In addition to these ER-localized and MT-binding proteins, specific motor proteins and adaptor-linking proteins also mediate bidirectional communication between the two structures. In this review, we summarize the current understanding of the structure and function of ER-MT interconnection. We further highlight the morphologic factors which coordinate the ER-MT network and maintain the normal physiological function of neurons, with their defect causing neurodegenerative diseases such as Hereditary Spastic Paraplegia (HSP). These findings promote our understanding of the pathogenesis of HSP and provide important therapeutic targets for treatment of these diseases.

1. Introduction

Eukaryotic cells are compartmentalized into different organelles in order to perform complex cellular functions. The endoplasmic reticulum (ER) is one of the largest and most complicated organelles, and is composed of several subdomains including perinuclear membrane sheets as well as tubule-like structures that extend from the nuclear envelope all the way to the cell membrane [1]. This ER tubule network is linked to several other organelles and participates in numerous biological processes. Studies have consistently found that the ER forms contacts with other organelles, including mitochondria, Golgi apparatus, and endosomes [2]. Along with many other organelles, the ER is closely associated with the microtubule (MT) network [3,4]. ER tubules transport along MTs bidirectionally by binding to the positive end of an MT or sliding along the MT using molecular motor proteins [5]. Nocodazole-induced MT depolymerization results in the retraction of peripheral ER tubules and their interconversion to perinuclear ER cisternae [3]. MTs therefore play a

pivotal role in ER morphogenesis. Numerous studies have identified a number of ER-localized proteins directly interacting with MTs, including the ER membrane protein CLIMP-63 [4], and p180 [6], receptor expression-enhancing protein 1 (REEP1) [7], and Sec61β [8], all of which contain MT-binding domains. These findings highlight the physiological importance of the ER-MT association.

Hereditary spastic paraplegias (HSPs) are a diverse group of heritable neurological disorders, which are clinically characterized by spastic and weakness in lower-extremities, and pathologically by retrograde axonal degeneration. To date, no etiological treatments for prevention or reversal of motor neuron degeneration in HSP have been documented [9]. Symptomatic treatments via physical or occupational therapy and drugs have improved muscle spasticity only temporarily and improved balance, strength, and agility. Over 80 genetic forms of HSP have been defined by genetic linkage analyses [10]. Gene functional studies have led to several hypotheses regarding the pathogenesis of HSP including ER morphology defect, axonal transport dysfunction, and axonal myelin abnormality [11]. More than half of HSP cases result from autosomal dominant mutations in *ATL1*, *REEP1*, *SPAST*, and *RTNs*, all of which are either ER-localized or MT-binding proteins. These results indicate that dysfunctional ER-MT interaction may be a major pathogenesis factor of HSP [10,12].

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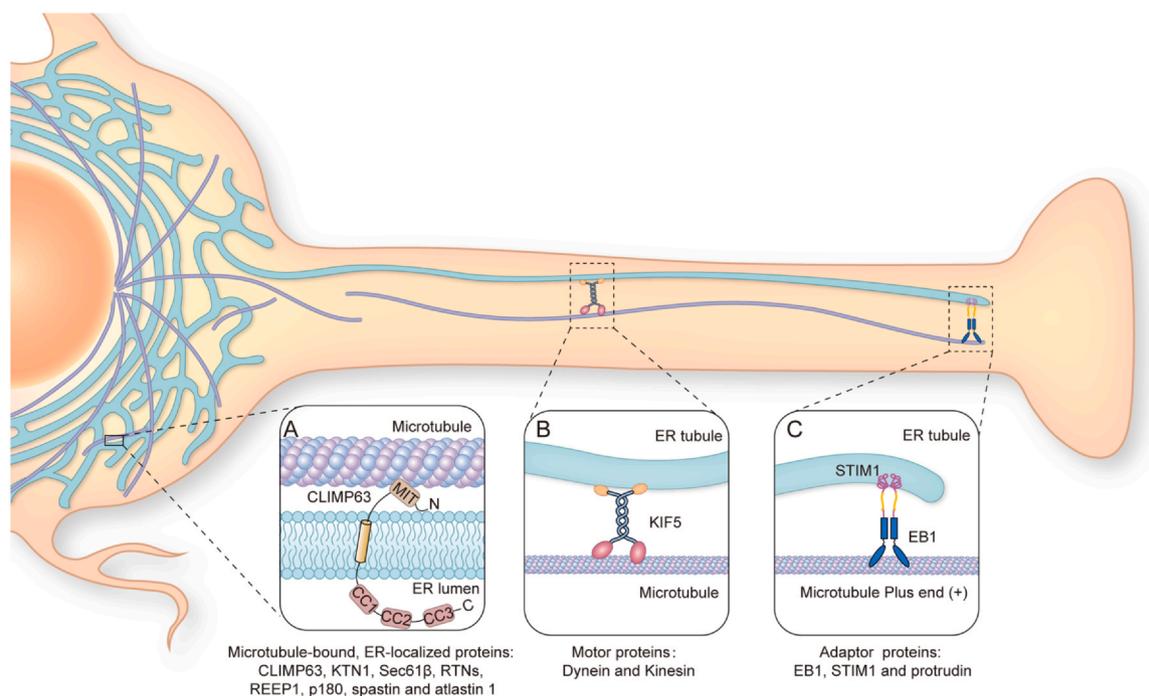
Fig. 1. Three kinds of molecules mediate ER-MT connections: ER-localized MT-binding proteins, motor proteins, and adaptor linking proteins. Three enlarged detail diagrams are presented separately: **A)** ER-localized MT-binding protein CLIMP-63 has a MT-interacting and trafficking (MIT) domain that binds MT; **B)** KIF5 (a Kinesin) mediates ER movement along a microtubule; **C)** 'TAC' (tip attachment complex) mediates ER moving with the elongating plus end of a microtubule, through interaction between STIM1 on the ER and EB1 on the microtubule.

In this review, we focus on the interconnection between the ER and MTs as well as the linking molecules responsible for ER distribution and integrity and their role in maintenance of ER position and MT stability. We provide an overview of three kinds of molecules which mediate ER-MT connections, which work together to sustain a normal ER distribution, particularly for axon development and maintenance. We further highlight how impaired maintenance of axonal ER-MT interaction, local ER stress, MT instability, and protein synthesis failure act as prominent contributors to the pathogenesis of HSP. In conclusion, we provide perspectives on potential therapeutic strategies targeting ER-MT interaction.

2. ER and MT contacts

2.1. Structure and function of the ER and MTs

The ER network, including the nuclear envelope, as well as a connected peripheral network of tubules and interspersed sheets is highly dynamic, with tubules continuously forming and retracting [13]. Network formation and maintenance involves curvature stabilizing proteins such as RTNs, and proteins participating in ER re-modeling, including atlastins and Lunapark (LNP) [14]. RTNs and REEPs generate curves of membrane [15,16]. Atlastins tether and fuse the ER tubules together to form three-way junctions, which are thereafter stabilized by LNP [17]. Atlastins and RTNs counterbalance one another dynamically to regulate ER fragmentation and fusion [18]. The integrity of ER is essential for synthesis of transmembrane and secreted protein, as well as folding and processing of proteins, and finally plays a role in lipid synthesis and Ca^{2+} homeostasis [19,20].

MTs cooperate with actin filaments and intermediate filaments to construct the cytoskeletal system, provide routes for transport of molecules or organelles, and maintain cell shape, division, and polarity [21–24]. MTs are composed of α- and β-tubulin, which are heterodimeric and noncovalently join to form a stable hollow tubular structure [25]. MTs are initially nucleated from MT organizing centers that contain γ-tubulin, which serves as a structural template for high-efficiency nucleation [26,27]. Tubulin dimers hydrolyze GTP to GDP, facilitating addition to the growing MT lattice, which weakens the affinity for adjacent dimers and favors disassembly. The GDP attached to β-tubulin which is incorporated into an MT can only be replaced by GTP when tubulin returns to its heterodimeric form during the continuous cycling of MT polymerization and depolymerization [28]. Both extrinsic [MT-associated proteins (MAPs) and physical barriers] and intrinsic [tubulin concentration, isoforms, and posttranslational modifications (PTMs)] factors control MT dynamics, enabling both global and local tuning of MT growth and shrinkage. MTs are modified with distinct PTMs, including acetylation, tyrosination, and glutamylation, and some of these PTMs influence the binding affinity and/or activity of several MAPs [29,30].

2.2. Molecules involved in ER-MT connections

The ER network forms abundant membrane contacts with other organelles, such as mitochondria, Golgi apparatus, and endosomes [2]. Electron microscopy in the 1970 s and 1980 s revealed close connections between the ER and MT. Further, live-cell fluorescence microscopy has been used to visualize the dynamic changes taking place across ER and MT interactions. In the lamellipodia of thinly spread cells, Mark Terasaki and his colleagues observed that MTs and ER tubules have similar distributions, however, this is not observed with respect to intermediate filaments [3]. Other evidence of ER-MT connections are evident in the tip of ER tubule being linked to the tip of a MT, and the fact that ER distribution and retraction occurs concurrently with MTs [31,32]. During this activity, the interactions mediating movement of ER along MT are linked through motor proteins or other linking proteins. In recent years, a growing body of evidence has suggested that the ER is closely associated with MTs [33–35]. It was found in 1998 that p63, an integral membrane protein within the reticular subdomain of the rough ER, directly binds MTs both in vivo and in vitro, providing evidence of ER-MT connections at the molecular level. Functional domain analysis of p63

revealed an ER rearrangement domain near the N-terminus and a central MT-binding domain. Due to its similar functioning to cytoskeleton-linking membrane proteins (CLIMPs), it was renamed CLIMP-63 [4]. Thereafter, more proteins with similar features were identified (this will be explored in more detail in the following section).

Current investigation has found that three kinds of molecules mediate ER-MT connections: microtubule-bound ER-localized proteins, motor proteins, and adaptor proteins (Fig. 1). The microtubule-bound, ER-localized proteins include CLIMP-63, p180, REEP1, and Sec61β. Mutation and functional-domain truncation experiments have demonstrated that CLIMP-63 has an ER-rearrangement domain near the N-terminus and a central MT-binding region [4] (Fig. 1A). A recent study also demonstrated that CLIMP63 is able to bind the centrosomal MT in the perinuclear region [36]. Another integral ER membrane protein, p180, contains a short luminal and single-transmembrane domain followed by a long cytosolic tail featuring a basic decapeptide repeat at the N-terminus and an acidic coiled-coil (CC) domain at the C terminus. The highly basic N-terminal region of p180 is responsible for binding to MTs [6]. REEP1 remodels the ER network by directly interacting with MTs through its C-terminal cytoplasmic domain [7].

The tubular ER network is dynamic and constantly rearranges its structure along the MT cytoskeleton [13]. In animal cells, these rearrangements, referred to as ER sliding, occur bidirectionally and are mediated by motor proteins [37]. The MT-associated protein families of kinesins and dyneins serve to distribute intracellular cargo along MTs, with kinesins working in the anterograde direction and dyneins working in the retrograde direction [38]. The typical protein used is Kinesin-1 (also known as KIF5) [39] (Fig. 1B). Immunofluorescence experiments using anti-Kinesin-1 antibodies illustrate punctate staining patterns which are often strongly associated with ER membranes. Suppression of kinesin heavy chain expression within cultured rat hippocampal neurons by short hairpin RNA (shRNA) results in the retraction of the ER network from the periphery into the center of the cell, without impacting the distribution of MTs [40]. Furthermore, a variety of tubulin isoforms, posttranslational modifications, and MT-associated proteins create a tubulin code and provide a precisely regulated mechanism of organelles and molecules transported in an orderly fashion along MTs [36,41]. For example, KIF5 binds and moves along MTs marked with acetylated tubulin. Acetylated microtubules are predominantly bundled, which allows for this movement. This bundling enhances kinesin run lengths and provides a greater number of available kinesin binding sites [42].

The tip attachment complex (TAC) is important for the linkage of the ER and MTs; the tip of the ER tubule is bound to the tip of a dynamic MT, and the ER tubule grows and shrinks in concert with the dynamics of the plus-end of the MT [43,44]. The integral ER membrane protein STIM1 mediates TAC formation, however it lacks a MT-binding domain. In contrast, STIM1 interacts with MT indirectly through the STIM1-MT end binding protein 1 (EB1) complex [45] (Fig. 1C). STIM-EB1 regulates ER tubule localization at the cell periphery, and the KIF5 complex transports ER tubules to the cell periphery. Additionally, a complex structure can be formed between three proteins: protrudin, a vesicle-associated membrane protein-associated protein (VAP), and the kinesin protein KIF5. VAP is present in ER where it can bind to protrudin and protrudin binds to KIF5 thereafter [46,47]. Protrudin then facilitates the interaction of KIF5 with VAP family proteins including Rab11, Surf4, and RTNs. This suggests that protrudin serves as an adaptor protein and that the protrudin-KIF5 complex contributes greatly to the transport of proteins in neurons [47].

2.3. ER and MTs are interdependent

In vitro studies have demonstrated that the *de novo* formation of the ER is possible using small membrane vesicles prepared from *Xenopus laevis* eggs independent of MTs [48]. However, some studies have demonstrated that the interaction between the ER and MTs regulates the eventual shape of the ER. For example, nocodazole-induced MT depolymerization leads to the retraction of peripheral ER tubules and their interconversion to perinuclear ER cisternae [3]. The MT cytoskeleton helps position and support ER membranes, as well as actively participating in remodeling the ER. During nutrient starvation, cells increase CLIMP63 protein levels to shuttle ER towards the perinuclear region, thereby clustering lysosomes for efficient autophagic degradation. Thereafter, cells harness enhanced p180–MT binding to redistribute ER for a proper reset [36]. Conversely, a recent study demonstrated that MT dynamics and numbers are affected by the ER. Researchers used the Streptavidin-SBP system to produce immediate and sustained retention of ER tubules within the soma. They found that retraction of ER tubules to the perinuclear area results in a drastic increase in the number of the characteristic comets (marking MT plus-end tips) in regions of the soma lacking ER tubules, highlighting a decrease in MT stability [40].

MT diversity can be achieved via numerous post-translational modifications including acetylation, tyrosination, and glutamylation, all of which together constitute key elements of the tubulin code [41]. Modifications are dynamic and rapidly reversible. ER distribution is mediated via specific membrane-bound proteins, which bind to distinct levels and types of glutamylated MTs. For example, Kinectin 1 (KTN1) preferentially binds to perinuclear, poly-glutamylated MTs with long glutamate chains, whereas p180 binds glutamylated MTs with either short or long chains. Therefore, MTs have key roles in ER distribution [36]. Cells dynamically tune ER distribution through differential MT modifications, which is certainly important functionally. Dysregulation of ER shaping and MT polyglutamylation may lead to different neurodegenerative diseases [49,50].

Within a polarized neuron ER tubules can access the axon, while ER cisternae are excluded from the axon through retrograde transport back to the soma [40]. Tubular ER is able to recognize the tubulin code and move along MTs to establish and maintain a proper distribution and function; altered ER distribution is mediated by ER tubule-shaping proteins such as p180 alongside the MT. p180 induces MT acetylation through MTB-1, which further stabilizes p180 MTs [6]. Therefore, tubular ER selectively moves along acetylated MT, further demonstrating that ER distribution is broadly sensitive to MT modifications. Under nutrient starvation conditions, cells modulate CLIMP63 protein levels and p180–MT binding to glutamylated MT to move ER and lysosomes bidirectionally for adequate autophagic responses [36]. Nonetheless, the mechanisms underlying the dynamic regulation of ER and MT across different physiological and pathological conditions are topics of future study.

3. Physical functions of ER-MT interactions in axon development and maintenance

A neuron consists of a single axon and multiple dendrites, forming a highly polarized structure which establishes a complex network. During neuronal development, axon formation is critical for neuronal polarity establishment [51]. The neurites are indistinguishable until one of them develops into the axon, while the remaining ends develop into dendrites. Local cytoskeleton rearrangements determine the neuronal symmetry, in which a stable and uniform pool of parallel MT bundles is reorganized with their plus ends towards the axon terminus [52,53]. The specialized axon and dendrites form separate compartments which depend on a unique set of motors and cargos to transport organelles and

molecules. The preference of KIF5 for modified MTs is also important for axon specification in developing neurons, which extend multiple MT-rich neurites outward from the cell body [54]. Outside of motor proteins, MT-associated proteins have been proposed to be the major players in regulating MT remodeling within axons [55]. Nevertheless, numerous studies have indicated that specific ER membrane proteins can directly bind MTs and regulate their reorganization and stability [56]. We further summarize the mechanism of ER-MT connections regulating axon specification and development below.

The ER is organized into two distinct structural and functional interconnected domains determined by their membrane shape: ER cisternae and ER tubules [1]. In mature neurons, the two different ER shapes are asymmetrically distributed within the axonal, dendritic, and soma compartments. The ER distributes along the entire axon mainly in its tubular form [57]. ER tubules localize to the axon at very early stages of neuronal development. Alongside axon growth, the localization of the axonal ER depends on the cooperation of the MT plus-end motor protein KIF5 and various ER tubule-shaping proteins (Fig. 1B). Accordingly, different ER-shaping proteins are distributed throughout dendrites and axons. For example, Sec61β, RTN4A, DP1, and atlastin 1 are all distributed in both the axon and dendrites, whereas the ER cisternae proteins CLIMP63 and KTN1 are distributed only in the soma and dendrites [40]. Specific ER-resident transmembrane proteins control the shape of the ER. For example, CLIMP63, p180, and KTN1 induce a flattened structural organization of ER cisternae, whereas proteins such as RTNs and REEP1 promote highly curved ER [15,58]. Therefore, ER-shaping proteins control ER cisternae and tubule-level balance and determine the ER distribution across the axon. A recent study reported a specialized ER structure called an ER ladder in axons. ER ladders are composed of rungs that wrap tightly around MT bundles and dynamic rails for sliding across MT [34].

Recent studies suggest that neuronal polarity establishment depends upon axonal ER-MT connections. Disrupting the MT cytoskeleton alters ER tubule organization, and these alterations in the ER disrupt proper MT organization. Throughout unpolarized neuron stages, recruitment of ER tubules to one neurite initiates primary axon formation, whereas ER retention in the perinuclear area or disruption of ER tubules inhibits neuronal polarization. When ER tubules reach the axon initiation site, those containing proteins, such as p180, further induce MT stabilization which fully supports axon formation [40]. Consistently, KIF5 is found more frequently in developing axons than dendrites [59]. This is likely induced by increased posttranslational modifications of axonal MTs and the preference of KIF5 to bind to these modified tracks [42]. These studies suggest that axon developmental initiation requires proper ER-MT connections.

ER-MT connections are essential for achieving neuron polarization, and importantly, for maintaining it. MTs are required for stabilization of ER tubules and their transport along the axon. Additionally, ER tubules are critically important for organizing axonal MTs [60]. A balance between ER cisterna and ER tubule-shaping proteins as well as fusion between ER membranes regulates ER organization within the mature neuron [14,58]. Investigating the precise role of ER-MT connections in axon formation and maintenance in response to extracellular signals will be of interest to future studies.

4. The implication of ER-MT contact defects in HSP

HSP is an inherited neurodegenerative disease defined by a length-dependent axonopathy of corticospinal motor neurons, resulting in prominent lower extremity spasticity and gait difficulties [11]. Because of the remarkable genetic heterogeneity across HSPs, a genetic classification scheme has emerged and HSPs are commonly identified by their affected genes and spastic gait (SPG) loci,

SPG1–80, assigned in order of locus identification [10,61]. Over half of HSP genes result from autosomal dominant mutations in *SPAST (SPG4), ATL1 (SPG3A),* or *REEP1 (SPG31)*. More importantly, mutations in *SPAST* cause 40% of the autosomal dominant cases of HSP. Overall, the common feature of these gene products is that they are ER-localized and MT-associated, leading us to therefore speculate that these gene mutations may disrupt ER-MT interaction [7,62].

### 4.1. Disassociation of ER-MT contact is implicated in HSP pathogenesis

As ER-MT interaction is essential to axon development and maintenance, observance of HSP-associated proteins indicates developmental defects or retrograde degeneration of the long corticospinal tract axons, thus implying pathogenesis of HSP [62]. A recent study demonstrated that the HSP proteins atlastin 1 and REEP1 interact within the tubular ER membrane in corticospinal neurons to coordinate ER shaping and MT dynamics [56]. Indeed, the same ER-resident protein mutations have been reported to cause neurological disorders through significant MT disorganization mediated by spastin [63], ER stress mediated by REEP1 [64], and severe axonal degeneration mediated by RTNs, spastin, and atlastin 1 [7,65], similar to spastic paraplegia syndrome in humans. Thus, defects in tubular ER shaping and network interactions with the MT cytoskeleton appear to be the predominant pathogenic mechanism of HSP.

Spastin is an ATPase protein which severs MTs and plays a critical role in cytoskeleton regulation [66]. Spastin contains four functional domains: N-terminal sequence (only in M1 isoform), an ATPase domain, a MT-binding domain, and a MT-interacting and trafficking (MIT) domain [67,68] (Fig. 2). M1 Spastin inserts itself into the ER membrane and interacts with RTNs, atlastin 1, and REEP1 to regulate ER morphogenesis [7,69]. Functionally, spastin breaks longer MTs into shorter ones, which is critical for efficient MT transport in a rapid and concerted fashion within the axon [70]. Functional studies have demonstrated that spastin is an MT-severing enzyme. Most of its missense gene mutations are clustered within the AAA domain. *In vivo* these mutations lead to at least a partial loss of microtubule-severing activity [67,71]. Outside of its function with respect to MTs, numerous studies have shown that the long M1 spastin isoform physically interacts with REEP1 and atlastin 1 to coordinate ER morphogenesis and localization along MTs, which plays a critical role in axon branching, elongation, and regeneration [7,72]. M1 spastin is especially enriched within the spinal cord [73] and the three most prevalent SPG mutations consistently lead to HSP disease, highlighting the critical role of the integrity of ER-MT in disease pathogenesis [7].

ATL1 is identified as the second-most abundant mutant gene that causes an HSP [12]. *ATL1* encodes the dynamin-family GTPase. Atlastin 1 interacts with spastin in their N-termini [66] (Fig. 2). A mutation of *ATL1* outside of the GTPase domain causing HSP has been demonstrated functionally to prevent interaction with spastin [74], suggesting that HSP could result from a lack of normal interaction between these two proteins. The ER tubule shaping protein REEP1 also binds to MTs (Fig. 2) and interacts robustly with atlastin 1 [7]. Therefore, atlastin 1 may also disrupt the balance of ER tubules by influencing their distribution, shape, and function. Importantly, treatment with MT-targeting drugs has a demonstrated beneficial effect on axon development of human *SPG3A* neurons [75]. Through knockdown of *SPAST* or overexpression of disease mutant *SPAST*, animal models mimicking human HSP phenotypes in *Drosophila*, zebrafish, *C. elegans*, and mouse, our understanding of disease pathogenesis and the development of effective therapeutic drugs has been deepened. Recently, vinblastine, a MT-destabilizing drug, consistently alleviates synapse and muscle defects in the *Drosophila* model of *ATL1* mutants [60]. It has also been shown that either *SPAST* knockdown or mutation significantly relieves the loss of synapses
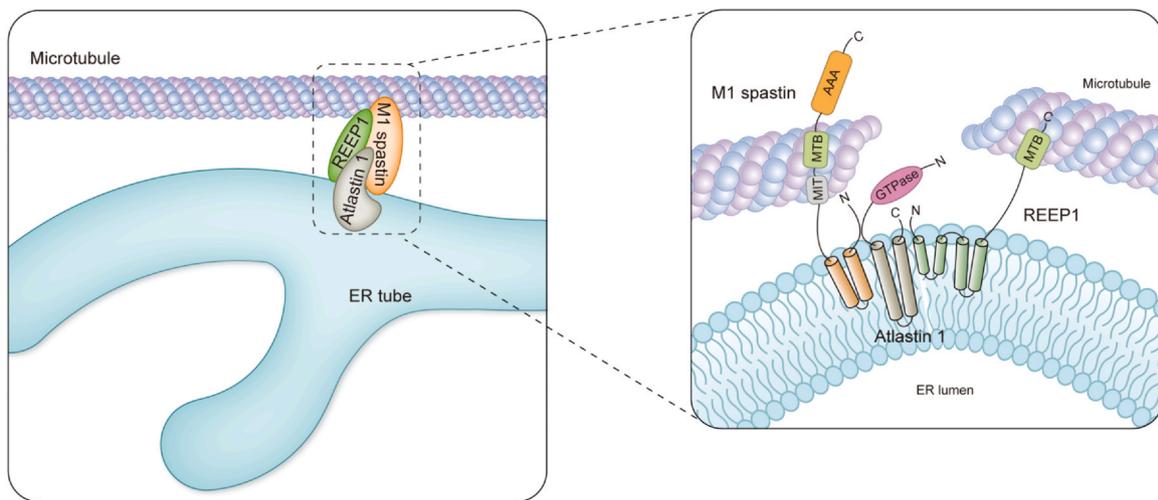
Fig. 2. HSP proteins spastin, atlastin 1, and REEP1 interact within the tubular ER membrane to coordinate ER shaping and MT dynamics (modified from Park et al. [7]). The three proteins (M1 spastin, atlastin 1, and REEP1) form protein complexes within the tubular ER, interacting with each other through hydrophobic hairpin domains in each of these proteins. The enlarge image shows the membrane topology of spastin, atlastin 1, and REEP1. The M1 isoform of the spastin ATPase binds to microtubules through its MIT domain and MTB domain, and is involved in microtubule severing, coupling changes in ER morphology with microtubule dynamics. Atlastin 1 interacts with spastin in their N-termini. REEP1 makes direct contact with the microtubular cytoskeleton through its C-terminal cytoplasmic domain.
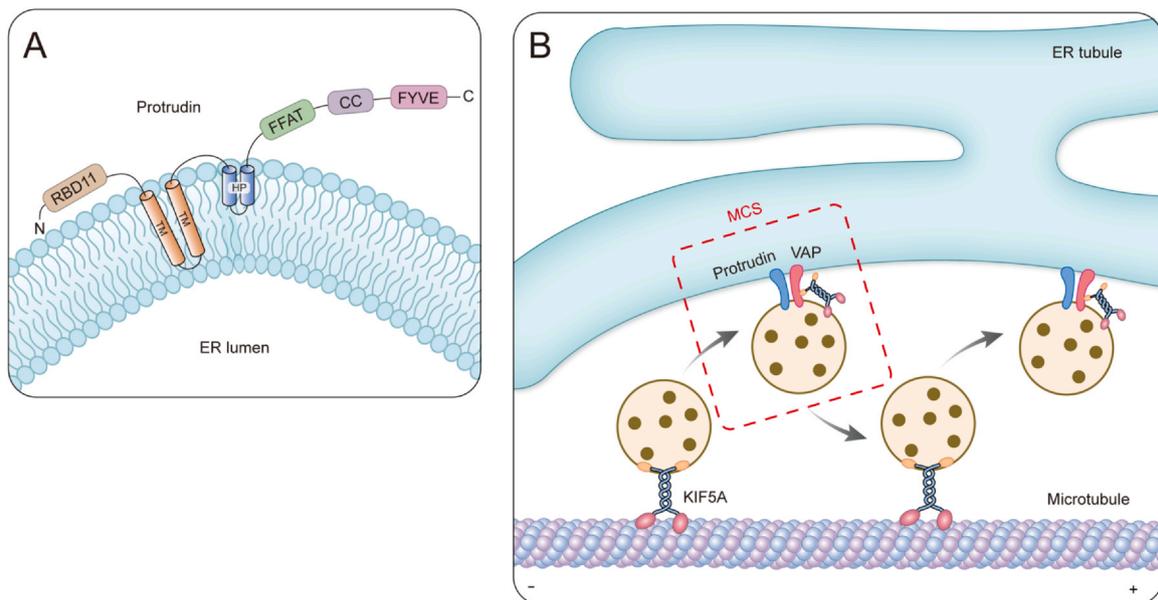
Fig. 3. Protrudin regulates KIF5-dependent endosome trafficking along microtubules at MCSs. **A)** Membrane topology of protrudin. Mutations in the HP domain of protrudin give rise to ER stress in neurons and eventual axonopathy. **B)** At MCSs formed between the ER and endosomes, protrudin promotes the transfer of endosomes from the ER to MTs for their polarized transport. Protrudin and VAP tether the endosome to the ER, and then charge with KIF5. The KIF5 binding endosome is released to the microtubule for KIF5-mediated transport toward the plus-end of the MT. Protrudin plays a vital role in directional endosomal trafficking through its function as a tethering factor at MCSs.

and ameliorates eclosion ratios in the *Drosophila* model [76]. Moreover, similar results are obtained using a mouse model. Vinblastine treatment rescues axonal swelling pathology of iPSCs from *SPAST* mutant patients and *SPAST*-knockdown hESCs derived neurons [77–79]. Studies from other authors have suggested that only mild stabilization may be beneficial [80]. However, this result is disputed [81]. Vinblastine dosage and multi-target response may contribute to the different outcomes seen in different prior publications. Taken together, these results suggest that integrity of the ER-MT interaction may be one of the predominant pathogenic mechanisms of HSP, and targeting ER-MT is a potential therapy for HSP.

4.2. ER-MT contact deficiency contributes to ER morphology and distribution

Shaping and positioning of organelles, signaling complexes, and other molecules properly within highly polarized neurons all depend on motor proteins [82]. An autosomal dominant forms, SPG10, is caused by mutations in *KIF5A* [83], one of the three KIF5 heavy chain proteins in mammals which act as a plus end–directed MT motor protein involved in anterograde transport of membranous organelles in nerve axons including ER [38] (Fig. 1B). Most mutations are missense changes in the motor domain with sites in the vicinity of the MT and nucleotide binding regions. For example, the N256S mutation results in substitution of a highly conserved asparagine residue in the switch II loop/helix motif of the MT binding site with a serine residue, preventing stimulation of motor ATPase activity by MT-

binding [83]. *In vitro* studies have shown that N256S-KIF5A is unable to generate single-motor processive motion in MT gliding and bead motility assays, resulting in decreased gliding and transport velocities [84,85]. Mutations in the *KIF5A* gene with SPG10 have given direct evidence for motor-based transport impairments in HSP.

Protrudin is an ER resident membrane protein, which regulates polarized vesicular trafficking in neurons. Mutations within the protrudin gene *(ZFYVE27*, also known as *SPG33)* are found in a subset of individuals [86]. However, the sequence variant has also been identified in several normal control populations across different ethnic backgrounds. The p.G191V mutation was a benign polymorphism or variant in different ethnic backgrounds and still requires further investigation and validation [87]. Protrudin has a FFAT motif responsible for binding the ER protein VAP, a coiled-coil domain, and a FYVE domain with Zn^{2+}-binding motifs at its C-terminal end [88] (Fig. 3A). Protrudin has a hydrophobic HP domain that shapes high-curvature ER tubules. Mutations in this domain give rise to abnormal ER morphology and increased susceptibility to ER stress [89]. This may be a major contributor to HSP pathogenesis. Animal and cell culture studies have demonstrated that protrudin plays a central role in directional endosomal trafficking through its function as a tethering factor at membrane contact sites (MCSs). At MCSs formed between the ER and endosomes, protrudin promotes the transfer of endosomes from the ER to MTs for their polarized transport [46] (Fig. 3B). VAP residing in the ER binds to the consistent FFAT (two phenylalanines in an acidic tract) motif of protrudin. Protrudin is then able to bind to KIF5 through its FFAT motif and coiled-coil domain, serving as an adaptor protein, which links the motor protein KIF5 and its cargo molecules including Rab11, VAP family members, and Surf4. The protrudin-KIF5 complex contributes to the transport of these proteins in neurons and is essential for neurite elongation [47]. Research has demonstrated that protrudin contributes to the regulation of ER morphology and function through interactions with other HSP-related proteins including spastin, PLP1, atlastin 1, REEP1, REEP5, KIF5A, KIF5B, KIF5C, and RTNs [89,90]. VAP is an important determinant of the subcellular localization of protrudin within the ER. Given that mutations in genes for *ZFYVE27, KIF5A*, and *RTNs* give rise to HSP, protrudin-containing complexes appear to be fundamental to neuronal function associated with HSP pathogenesis [47,89].

4.3. ER-MT contact deficiency contributes to ER dysfunction

Aside from the role of MT-ER interacting proteins in regulating ER structure and distribution, these proteins are also involved ER functioning, including promoting ER stress resistance, Ca^{2+} homeostasis, and autophagic degradation of ER, which may also implicate in the pathogenesis of HSP. Knockdown of REEP1 aggravates Tau-mediated neurodegeneration. Conversely, the overexpression of REEP1 protein rescues these phenotypes through resistance to ER stress [64]. These results suggest that improving ER stress is a promising treatment strategy for HSP disease. Liang and his colleagues demonstrate that atlastin deficiency inhibits ER-autophagy and that atlastins participate downstream of the FAM134B ER-autophagy receptor. The similarities between FAM134B and *ATL1* mutations in the context of clinical manifestation, suggest that mutations of MT-ER interacting proteins impair ER-autophagy, thus contributing to HSP [91]. In addition, the expression of spastin K467R, lack of spastin [92], and mutant atlastin 1 or depletion of atlastin 1 [93] reduce calcium entry capacity and thereby result in enlarged ER. Importantly, both enlarged ER and improper Ca^{2+} homeostasis can be rescued by the MT-destabilizing drug vinblastine [92]. It remains to be seen whether HSP is caused by deficits in MT-ER interacting proteins inducing ER dysfunction. Several mechanistic studies will hopefully shed light on the molecular basis of the disease and provide an effective therapeutic strategy.

5. Summary and outlook

Recent improvements in the ability to visualize the dynamic interaction of ER-MT within live cells have revealed ER-MT interaction domains and functions that were not fully understood even a few years ago. While we have come a long way in our understanding of factors that influence the ER-MT shape and function, it is clear that the responsibilities of ER-MT extend well beyond traditionally studied functions. The ER-MT interaction platform is extraordinarily dynamic, but information about the physical cues and molecular pathways that regulate these connections is lacking. Outside of ER morphology imbalance and intracellular trafficking defects, other molecular pathogenesis, such as mitochondrial dysfunction, abnormalities in axonal pathfinding or myelination and lipid metabolism, also play critical roles in HSP. As we know, all membrane-bound organelles in the cytoplasm (cell membranes, Golgi apparatus, vacuoles, endosomes, peroxisomes, and mitochondria) are a continuous structure. Previous studies have also demonstrated that ER-microtubule interactions specify the cellular distribution of many other organelles [36], however, it is still unclear whether the formation of ER/MT connections also requires the participation of other membrane-bound organelles. MT code diversity may provide the regulatory basis for these complex biological processes. Future work addressing these questions will greatly improve our understanding of ER-MT functions in the cell.

CRediT authorship contribution statement

Conceptualization, X.W., Y.L. and Y.Z.; Writing - original draft, X.W. and C.F.; Writing - review & editing, X.W., Y.L. and Y.Z.; Funding acquisition, Y.Z.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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