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REVIEW

Cytoskeleton dysfunction of motor neuron in spinal muscular atrophy

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Abstract

Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by deletions or mutations of survival of motor neuron 1 (*SMN1*) gene. To date, the mechanism of selective cell death of motor neurons as a hallmark of SMA is still unclear. The severity of SMA is dependent on the amount of survival motor neuron (SMN) protein, which is an essential and ubiquitously expressed protein involved in various cellular processes including regulation of cytoskeletal dynamics. In this review, we discuss the effect of SMN ablation on cytoskeleton organization including actin dynamics, growth cone formation, axonal stability, neurite outgrowth, microtubule stability, synaptic vesicle dynamics and neurofilament protein release in SMA. We also summarized a list of critical proteins such as profilin-2 (PFN2), plastin-3 (PLS3), stathmin-1 (STMN1), microtubule-associated protein 1B (MAP1B) and neurofilament which play an important role in modulating cytoskeleton in SMA. Our aim is to highlight how cytoskeletal defects contribute to motor neuron degeneration in SMA disease progression and concentrating on cytoskeleton dynamics may be a promising approach to develop new therapy or biomarker.

Keywords Spinal muscular atrophy · Cytoskeleton defect · Actin · Microtubule · Profilin-2 · Plastin-3 · Microtubule-associated protein

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive inheritance disease, characterized by progressive muscle weakness due to the degeneration of spinal α motor neurons. Symmetrical motor function impairment initially happens in proximal skeletal muscles and subsequently spreads to distal muscles [1, 2]. In addition, other tissues in SMA are also affected with the increasing severity of the disease [3]. According to onset time, survival and motor function, SMA is clinically divided into five subtypes from severe type 0 to mild type IV [4, 5]. It is caused by mutations or deletions of survival of motor neuron 1 (*SMN1*) gene, while the severity of SMA is negatively regulated by the copy number of survival of motor neuron 2 (*SMN2)* as a paralogous backup gene [6–8]. Despite encoding SMN protein and being highly similar, *SMN2* and *SMN1* exhibit differences at five bases [9]. One of these differences is C-to-T transition in exon 7 of *SMN2* contributing to predominant skipping of exon 7 during pre-mRNA splicing [9–11]. Consequently, 90% *SMN2* products are truncated protein called SMNΔ7, whereas 10% transcripts are translated into full-length SMN protein which is insufficient to compensate for the loss of *SMN1* [6, 7, 9, 12, 13](Fig. 1A).

Therefore, increasing SMN level is a mainstream treatment for SMA, especially concentrating on modulating *SMN2* alternative splicing. In 2016, U.S. Food and Drug Administration approved Nusinersen (Spinraza®), an antisense oligonucleotide (ASO) to correct splicing by blocking an intronic silencer in intron 7 of *SMN2*. In addition, Risdiplam (Evrysdi®) as an *SMN2* pre-mRNA splicing modifier was approved to treat SMA by promoting the recruitment of U1 small nuclear ribonucleoprotein particles (U1-snRNPs). Moreover, onasemnogene abeparvovec (Zolgensma®) as a gene therapy for SMA patient delivers a functional copy of

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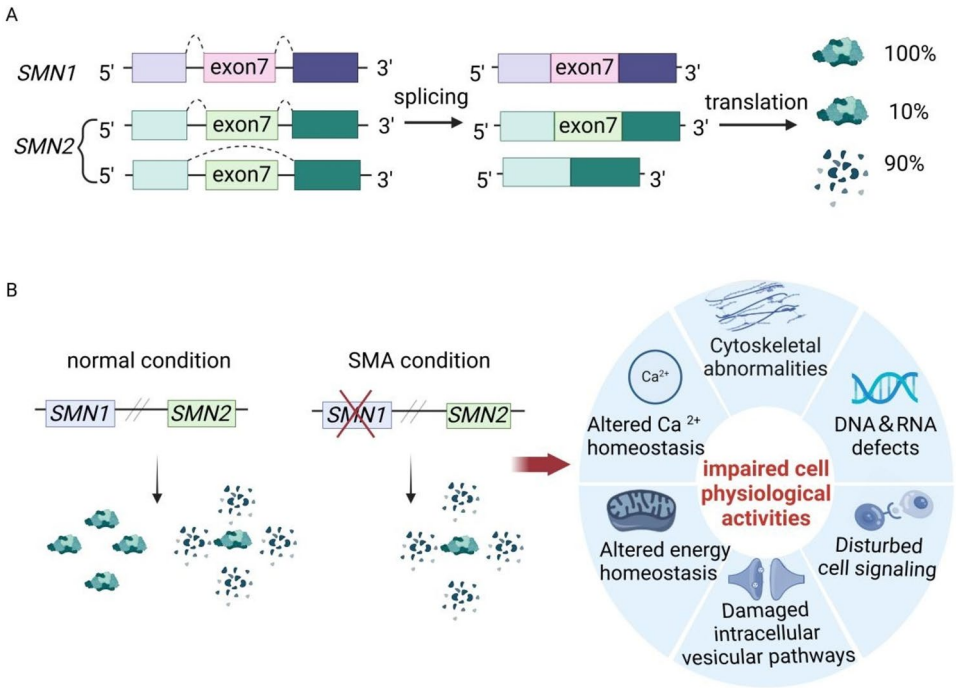

Fig. 1 **A** Both *SMN1* and *SMN2* genes are located close together on chromosome 5q13, encoding for SMN protein. Normally, *SMN1* transcripts are correctly spliced and translated into a full-length SMN protein, while 90% *SMN2* are translated into a truncated and non-functional SMN protein due to the exon 7 skipping caused by alternative splicing. **B** Under normal condition, the physiological behaviors of healthy individual are supported by the abundant SMN proteins which mainly derive from *SMN1* gene. In SMA, the limited SMN protein encoded by *SMN2* gene is insufficient to counterbalance the loss of *SMN1*, impairing various cell physiological activities such as cytoskeleton regulation, Ca^{2+} and energy homeostasis, intracellular vesicular pathways, cell signaling, RNA metabolism and DNA recombination

human SMN with adeno-associated virus vector [14–19]. Although this therapeutics made impressive strides in extending patient lifespan and improving neurodevelopmental outcome and motor function, current treatments do not allow to thrive and live a normal life for most SMA patients. Defects of motor axon development in SMA begin prenatally, associated with postnatal degeneration of motor neurons [20]. Thus, as suggested in clinic trials, the earlier treatment can be initiated, the better to attain stabilization of motor function [21]. However, delays in treatment are widespread and some therapies may be currently limited to a certain age population [22]. Even for the patients treated early, the disease burden remains due to irreversible neuronal cell death [23].

Therefore, to understand the mechanisms of selective cell death of motor neurons as a hallmark of SMA is important for SMA patient treatment and diagnosis. SMN is an essential and ubiquitously protein in all cell and tissue types, not just in motor neurons. SMN protein plays a housekeeping role in the regulation of snRNP biogenesis, as well as intracellular homeostasis including cytoskeletal dynamics, RNA metabolism, DNA recombination, cell signaling, Ca^{2+} homeostasis, intracellular vesicular pathways, ubiquitin–proteasome pathway and mitochondrial activity (Fig. 1B) [24–27]. Hence, SMN plays a major role in SMA pathology and specific vulnerability to motor neurons in this disease. Due to extraordinary extended axon length and their dependency on the cytoskeleton for its stability, signaling, and axonal transport, spinal motor neurons are particularly susceptible to selective and early degeneration compared with other neurons in SMA [28–30]. Moreover, Sumner et al. identified impaired radial growth and Schwann cell ensheathment of motor axons initiated during embryogenesis and caused reduced acquisition of myelinated axons that impeded motor axon function neonatally in SMA [20]. Cytoskeletal proteins have been demonstrated to fulfill crucial functions in various signaling pathways [31–35]. Accumulated evidence suggest that integrity of cytoskeleton is closely linked to cell death [35–43]. Hence, therapeutic strategies targeting cytoskeletal disorders have the potential to be valuable supplements to current treatment of SMA.

In this review, the altered dynamics of cytoskeleton and its mechanisms in SMA were comprehensively described, which explained how potential modifiers might improve phenotypes of SMA. The aim was to provide new insights into

SMA pathogenesis and emerging therapies by summarizing the latest advances in the cytoskeletal role of SMA.

Role of cytoskeletal dynamics in neurons

The neuronal cytoskeleton is an adaptive and dynamic structure consisting of three major components: actin-based microfilaments, microtubules and intermediate filaments (neurofilaments) (Fig. 2) [44–47]. Filamentous actin (F-actin) is a heterodimer structure and is composed of α- and β-actin. β-Actin is known as globular actin (G-actin). Microtubule is a cylinder comprising 13 protofilaments which are heterodimers formed by the alternating arrangement of α- and β-tubulin. Actin and microtubule proteins are indispensable for regulating neuron structure and function by influencing cellular motility processes such as growth cone formation, axonal stability, neurite outgrowth and synaptic vesicle dynamics [44, 48]. Actin cytoskeleton plays a vital role in pathfinding of neuronal axon and control of microtubule dynamics [49–51]. The assembly of microtubule in growth cone promotes the axon extension, influencing neuronal polarization and regeneration [52–54]. Moreover, both actin-based microfilaments and microtubules support the dynamic function and high energy demands of pre- and postsynaptic structures by binding with mitochondria and driving plastic changes [47, 55–57].

Therefore, it is perhaps not surprising that cytoskeleton defects may be a common characteristic among neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and SMA. In some neurodegenerative diseases, the alteration of cytoskeleton is considered as a potential etiological factor in part [58–60]. However, some cytoskeletal proteins abnormalities may not be causative factors of neurodegenerative disorders because that interventions targeting these proteins fail to rescue the survival of diseased mice [61]. Although the causal link between neurodegenerative diseases and cytoskeletal dysfunctions is unclear, the overwhelming majority of cytoskeleton defects related to neurodegenerative diseases contain the impairments of microtubule stability and actin dynamics [57, 62–69]. In addition, the aggregation of neurofilaments, a class of intermediate filaments found primarily in axons of nerve cells, can be observed in several neurodegenerative diseases [46, 70] and may be related to the hyperphosphorylation of intermediate filaments [46, 70, 71]. In recent years, neurofilament concentrations in biofluids have emerged as a promising clinical biomarker for neurodegeneration [71]. However, compared to actin-based microfilaments and microtubules, the mechanism of neurofilament changes in neurodegenerative diseases remains largely unknown.

SMN protein is not only localized primarily in actin-abundant neurites and growth cones but also regulates the distribution of β-actin within growth cones, which suggests the possible regulatory effects of SMN on cytoskeleton [72–77]. In SMA mouse model, morphological defects of nerve cell, which contributes to neurologic disorder, are often accompanied by cytoskeletal impairments such as destabilization of microtubules and imbalance of F/G-actin levels [45, 78]. In most instances, the depletion of SMN indirectly affects the cytoskeleton through a number of proteins that play a regulatory role in the cytoskeleton. Here, we discuss cytoskeleton-related proteins including PFN2,

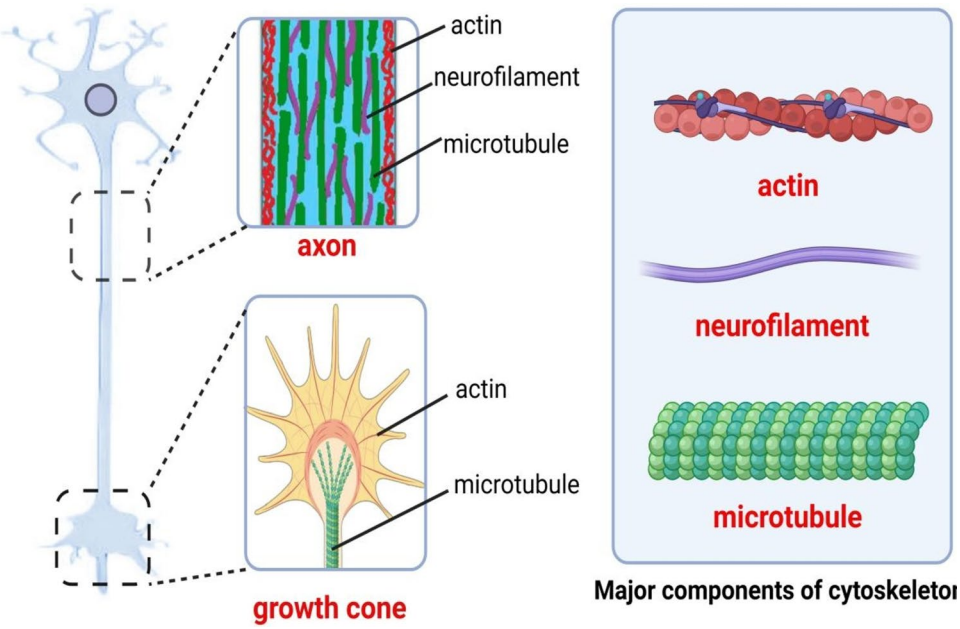

Fig. 2 The neuronal cytoskeleton is an adaptive and dynamic structure consisting of three major components: actin-based microfilaments, microtubules and intermediate filaments (neurofilaments). Actin and microtubule proteins are indispensable for regulating neuron structure and function by influencing cellular motility processes such as growth cone formation, axonal stability, neurite outgrowth and synaptic vesicle dynamics. Neurofilaments are a class of intermediate filaments found primarily in nerve cells, especially in axons. They are essential for the structure of neuronal axons and play a crucial role in maintaining the shape and function of neurons

PLS3 and microtubule-associated proteins (MAPs) that were affected by SMA (Table 1) and highlight the more extensively studied proteins that regulate cytoskeleton in the following sections.

PFN2, an important regulator of actin dynamics in SMA

Encoded by four genes *PFN1–PFN4*, profilins have a variety of expression profiles in mammals. Profilins possess a binding site for actin, two binding sites for phosphatidylinositol 4,5-bisphosphate (PIP2) [79] as well as poly-L-proline (PLP) binding domains, allowing for localization to membranes, interaction with PLP-containing proteins and regulation of actin dynamics [80, 81]. Therefore, profilins are of importance to regulate cellular processes related to actin cytoskeleton, such as cytokinesis, neuronal differentiation, synaptic plasticity, membrane trafficking and nuclear transport [82–86].

PFN2, as a major splice variant of *PFN2* gene, is primarily restricted in neuronal cells [82] and is a critical regulator of neuronal growth, development, and dendritic spine formation. PFN2 primarily inhibits actin polymerization by binding with actin monomers alone, whereas SMN protects F-actin from destabilization in the presence of urea, showing positive effect on actin polymerization [87]. In motoneurons, PFN2 is highly concentrated and colocalizes with SMN in the cytoplasm and nuclear gems through binding with PLP domain encoded by exon 5 of *SMN1* [88–90], producing more F-actin [87]. Sharma et al. revealed that increased PFN2 caused neurite outgrowth inhibition and axon pathfinding defects by disturbing the normal regulation of dynamic actin cytoskeleton in SMA PC12 cell model [87]. However, decrease of PFN2 via the heterozygous or homozygous knockout (KO) of PFN2 alleles in the intermediate SMA mouse model was not enough to rescue SMA phenotypes [91], indicating that other factors related to actin dynamics may be involved in the development of SMA. Moreover, PFN2 is abundant in postsynaptic structures [72, 76, 92] and actin predominately localizes at presynaptic terminal and postsynaptic density in neurons [47]. These distribution patterns indicate that actin cytoskeleton regulated by PFN2 may play a part in synaptic dysfunction of SMA. The ability of vesicle release was damaged along with a reduction in the size of the readily releasable pool in SMA mouse model [93, 94]. It also has a direct interaction with the PLP domain of Piccolo, a protein regulating neurotransmitter release by promoting F-actin assembly [95, 96]. PFN2 KO mouse exhibited deficiencies in the polymerization of synaptic actin and release of more neurotransmitters, indicating that PFN2 may play a functional role in the exocytosis of vesicles [91, 97]. In SMA Caenorhabditis elegans model, the changes in synaptic endocytic proteins and the deficiencies of endosomal compartments were observed, indicating that SMN depletion impairs synaptic endocytosis [98, 99].

The underlying mechanism of free PFN2 modulating neuronal actin cytoskeleton is through the interaction with ras homolog family member A (RhoA) kinase (ROCK) [100]. PFN2 is phosphorylated at serine 137 (Ser137) close to the PLP-binding domain by ROCK, decreasing combination capacity of PFN2 for some polyproline-rich proteins and

Table 1 The list of cytoskeleton-related proteins that were affected by SMA

Protein	Roles in cytoskeleton	Changes in SMA
PFN2	Regulates F-actin polymerization by binding with G-actin [82–86]	With the reduction of binding to SMN, available PFN2 is increased and hyperphosphorylated by ROCK [87–90]
PLS3	Binds and bundle actin filaments, and offset the actin depolymerization activity of cofilin and PFN [128–130]	Compared to SMA-affected siblings, some asymptomatic female siblings show high PLS3 level, while PLS3 is decreased in some SMA models [113–125]
MAP1B	Serves as a constituent of crossbridge between microtubules in neuron [157–160]	In SMN-depleted cells, increased MAP1B induces down-regulation of tubulin tyrosine ligase that subsequently reduces α-tubulin detyrosination [149]
STMN1	Binds or releases tubulin dimers in a phosphorylation-dependent manner [154]	STMN1 level is increased in SMA-like mouse models [148]
MAP2	Promotes the assembly and stability of microtubules, and have overlapping functions with MAP1B [170–172]	In SMN-deficient motor neuron cells, the expression of MAP2 exhibits a down-regulation independent of MAP1B [150, 173]
TAU	Promotes the assembly and stability of microtubules [170]	The hyperphosphorylation of TAU mediated by cyclin-dependent kinase 5 degenerates motor neurons in SMA patients and mouse model [151]
EB3	Promotes the growth and stability of microtubules, and regulate the polarization of microtubules [179–181]	In SMN-depleted cells, EB3 exhibits a significant reduction in a MAP1B-dependent way [173]

The list of cytoskeleton-related proteins that were affected by SMA. PFN2: profilin-2; PLS3: plastin-3; MAP1B: microtubule-associated protein 1B; STMN1: Stathmin1; MAP2: microtubule-associated protein 2; TAU: tau protein; EB3: end-binding protein 3

inhibiting interaction between G-actin and PFN2 to a lesser extent [101, 102]. In neurons, ROCK interacts with PFN2 in a direct way, which is conducive to increase actin stability and exerts negative effects on neurite outgrowth [98, 103]. Besides, ROCK is capable of directly or indirectly phosphorylating myosin light chain phosphatase (MLCP) and cofilin which are involved in the regulation of actin cytoskeleton and neurite outgrowth [104–106]. However, as shown in PC12 cell with SMN deficiency, the binding of MLCP and cofilin to ROCK is reduced due to the competitive inhibition of PFN2 [88] (Fig. 3).

Overactivated ROCK and the changed phosphorylation of its downstream have important impacts on growth cones and neurite outgrowth in SMA. As an initial step, focal F-actin polymerization is an essential process for the onset of axon collaterals [107]. Extending filopodia and lamellipodia from the leading edge of growth cones is crucial in this process. ROCK prevents formation of filopodia and lamellipodia from pre-existing axonal F-actin patches [108]. Moreover, ROCK not only induces growth cone collapse directly to inhibit neurite outgrowth, but also serves as a mediator to affect some signaling processes promoting growth cone collapse [109]. Therefore, ROCK exerted negative regulation on the sprouting of neurites and phosphorylated PFN2 by ROCK is also believed to suppress branching, leading to a severe dysregulation of actin cytoskeleton in PC12 cell with SMN ablation [88, 103].

Under the stimulation of glutamate or electricity, PFN2 is enriched in the head of a dendritic spine and then promote stabilization of dendritic spine morphology via its PLP-binding domain [110]. Therefore, the reduction of PLP-binding capacity of PFN2 may be involved in synapse stripping in SMA PC12 cell model [88]. Moreover, increased available PFN2 and the enhanced ROCK pathway promoted actin rod formation in SMN-lacking cells [111], which induced synaptic loss by blocking axonal transport physically and by disrupting microtubule integrity, leading to the impaired integrity of motoneurons in SMA [111, 112]. Indeed, ROCK inhibitors ameliorated the defect of neurite outgrowth in SMA PC12 cell model [88]. Nevertheless, the inhibition of the RhoA pathway alone is inadequate to fully save neuronal outgrowth and differentiation in SMA mouse model [91]. This may be attributable to the increased availability of PFN2 in SMA also affecting other signaling pathways.

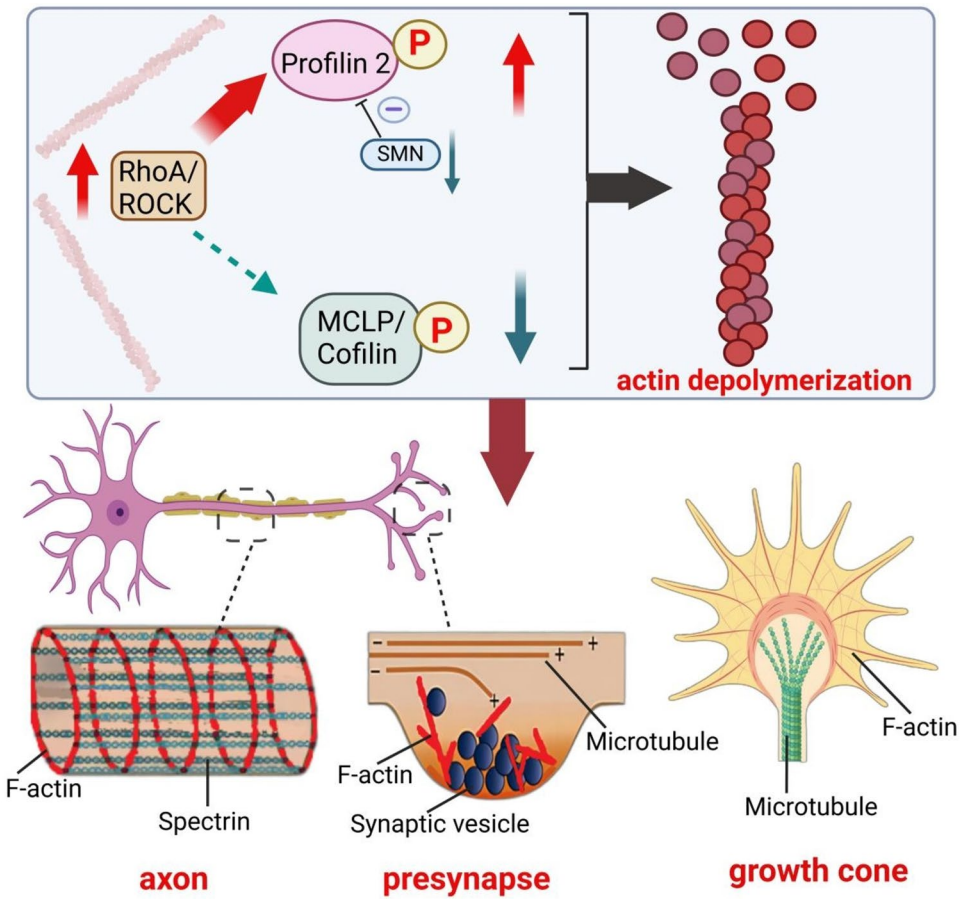

Fig. 3 SMN deficiency increases free profilin-2 that is available for combining with ROCK, and subsequently phosphorylated by ROCK. Due to the competitive inhibition, the phosphorylation of MLCP and cofilin is downregulated. Phosphorylated profilin-2 promotes depolymerization of actin filaments, contributing to imbalance of F/G-actin levels in motor neurons, blocked axonal transport, inhibited neurite outgrowth and synaptic dysfunction by influencing actin dynamics in axon, growth cone and synapses

PLS3, a potential protective modifier of SMA

The effect of PLS3 on SMA is controversial. In a study, high PLS3 expression was found in lymphoblastoid cells from asymptomatic female siblings sharing the same SMN genotype with their SMA-affected siblings, indicating that PLS3 may serve as a protective modifier of SMA with a calcium-binding and several actin-binding domains [113, 114]. However, other studies failed to find a relationship between PLS3 expression levels and SMA phenotype [115–117]. As shown in some studies, the effect of PLS3 on SMA is deemed gender-specific and age-dependent, since PLS3 transcript level exhibited a negative relationship with the severity of SMA only in post-pubertal female patients and was associated with *SMN2* copy number, gross motor function as well as clinical types [118, 119]. The strong colocalization of PLS3 and SMN was observed in granules throughout motor neuron axons [113], whereas some findings uncovered controversial results about PLS3 expression in SMA models. Ackermann et al. demonstrated that PLS3 did not change with reduction of SMN, suggesting that PLS3 plays a modifying role independent of SMN [120]. By contrast, PLS3 was decreased and dependent on SMN in zebrafish and mouse SMA models [91, 121, 122]. Therefore, overexpression of PLS3 exerts varied therapeutic effects in different SMA models. In zebrafish and intermediate SMA mouse models, overexpression of PLS3 rescued neurite length and axonal outgrowth deficiencies, and promoted survival and motor function [113, 120–123]. However, in severe SMA mice, overexpressed PLS3 did not exhibit a significant beneficial impact on this phenotype [124, 125]. One possible explanation is that PLS3 is able to alleviate the severity of SMA when SMN loss is moderate. Several studies found that human subjects with complete PLS3 protein loss display signs of osteoporosis instead of lower motoneuron degeneration, indicating that PLS3 may act as a protective modifier of SMA rather than an etiological factor [126, 127].

PLS3 can bind and bundle to actin filaments, and offset actin depolymerization activity of cofilin and PFN2 subsequently strengthening actin networks [128–130]. As a result, high expression of PLS3 can elevate F-actin levels in SMA, which rescues axon length and outgrowth defects [113, 131]. Overexpression of PLS3 increased axon input number, muscle fiber and endplate sizes in SMA mice, improving the neuromuscular transmission [120]. Moreover, increased PLS3 restored F-actin intensity, amount of presynapses and synaptic vesicles in SMA, thereby promoting synaptic function [120]. PLS3 restores the intensity and area of Piccolo which is related to F-actin dynamics [95], resulting in the rescue of active zones number in SMA mice. PLS3 also promoted organization of the readily releasable pool in vesicles and rescued vesicle release and electrophysiological defects [120]. In addition, PLS3 plays a pivotal role in cell endocytosis process based the fact that endocytosis of PLS3 KO yeast was impaired [132]. Endocytosis regulated by F-actin is indispensable for replenishing the recycling pool and influences the supply of vesicles to the release of neurotransmitters [133–136]. Overexpressed PLS3 can rescue endocytosis and synaptic vesicle recycling impaired by the depletion of SMN [122]. Moreover, PLS3, SMN and heterogeneous nuclear ribonucleoprotein (hnRNP) F/H act in the same complex that plays a vital role in endocytosis [137]. PLS3 is also important to a well-organized cytoskeleton associated with presynaptic compartment orchestration. The disturbed brain-derived neurotrophic factor/tropomyosin receptor kinase B (BDNF/TrkB)-signaling cascade due to the impaired localization of transmembrane proteins is important in the affected differentiation and maturation of SMA motor neurons [138–141]. PLS3 cooperating with actin-related protein 2/3 (Arp2/3) can improve the localization and cyclic adenosine monophosphate (cAMP)-induced translocation of TrkB in SMA, which strengthens BDNF/TrkB-signaling [141].

In SMA, calcium homeostasis was disturbed and overexpressed PLS3 rescued "cluster-like" formation of Cav2.2 and increased spontaneous calcium ion (Ca^{2+}) transients in motoneurons [141]. Indeed, overexpression of PLS3 without calcium-binding ability is not enough to make up for loss of SMN, while short of actin-binding domains in PLS3, remains capable of rescuing axon morphology [142]. Moreover, PLS3 cooperated with some proteins in a calcium-related way. For example, coronin 1C (CORO1C), as a F-actin-binding protein, can combine with PLS3 directly in a calcium-dependent way. Akin to PLS3, overexpressed CORO1C restores endocytosis in SMA cells by increasing F-actin content [122]. Moreover, calcineurin-like EF-hand protein 1 (CHP1) interacts with PLS3 which exists widely and abundantly at sites related to SMA including growth cones and neuromuscular junctions (NMJs). Together, the effect of PLS3 in SMA is through the cooperation of calcium influx and F-actin dynamics [122, 141, 143].

MAP, the bridge between SMA and microtubule dysfunction

Microtubule dynamics influencing neuronal functions are controlled by MAPs [144, 145], a set of proteins binding to microtubules and regulating their structures [146, 147]. Numerous studies claim that aberrant MAPs disturbing microtubule dynamics are involved in pathomechanism of SMA [148–153].

As a member of stathmin (STMN) phosphoprotein family, STMN 1 is one primary MAP related to SMA. STMN1 has C-terminal "STMN-like domain (SLD)" that can bind or release tubulin dimers in a phosphorylation-dependent manner, participating in regulation of microtubule dynamics [154]. STMN1 identified as a disease modifier for SMA and enhanced in SMN-depleted NSC34 cells and SMA-like mouse models. The aberrant up-regulation of STMN1 is correlated with the severity of SMA and is adverse to microtubule polymerization and mitochondrial transport towards axons [148]. Correspondingly, knockdown of STMN1 ameliorated the deficiencies of microtubule network formation, axonal outgrowth and mitochondrial transport in SMN-depleted NSC34 cells and SMA mouse model [148]. Further study uncovered that heterozygous rather than homozygous STMN1 KO mouse model rescued axonal microtubule density, motor function and NMJ maturation in SMA [61]. In reality, the reduction of STMN1 as a possible pathogenic modifier of motor neuron diseases was reported to be observed in vulnerable motor neurons [155]. By contrast, in intermediate SMA mouse model, overexpressed STMN1 recovered neuromuscular innervation and motor neuron preservation and improved lifespan, weight gain and the righting reflex by promoting microtubule turnover [156]. Contradiction between these findings may be caused by the differences in models. STMN1 is suggested to be a potential therapeutic target for SMA. Despite contributing to the improvement in SMA pathology, the reduction of STMN1 cannot rescue the survival of SMA mice [61]. The potential role of STMN1 on cytoskeleton of motor neuron in SMA needs further investigation.

Microtubule-associated protein 1B (MAP1B), as a member of MAP family, is also related to SMA. It mainly expresses in neurons and serves as a constituent of crossbridge between microtubules in neurons to axon growth, regeneration, growth cone pathfinding and neuronal migration [157–160]. Located on chromosome 5q13, *MAP1B* locus is close proximity to *SMN1* locus [161]. The human *MAP1B* gene displays close linkage to SMA mutations in a genetic linkage analysis [162]. Moreover, the spatial distribution of MAP1B nearly coincides with SMN granules in axons and presynaptic terminals [163]. These mapping data and colocalization of SMN and MAP1B suggest that MAP1B may be relevant to the pathomechanism of SMA.

Microtubule stability and dynamics are affected by acetylation and detyrosinated proteins [164–167]. In SMN-depleted cells, increased MAP1B up-regulated tubulin tyrosine ligase (TTL) activity that subsequently decreased α-tubulin detyrosination, impairing microtubule stability [149] (Fig. 4). Down-regulation of MAP1B rescued the aberrant levels of TTL and detyrosinated α-tubulin in SMN-depleted cells [149]. Moreover, damaged microtubule dynamics possibly affect axonal transport, which subsequently influences mitochondrial distribution along neurites in SMA. Decreased MAP1B also ameliorated mitochondrial distribution impaired in SMN-depleted cells, which suggests the restoration of axonal transport [149, 168, 169].

Although studies are relatively limited compared to STMN and MAP1B, several MAPs are also involved in the development of SMA. As a structural MAP abundantly expressed in neurons such as MAP1B and microtubule-associated protein 2 (MAP2) has overlapping functions with MAP1B in terms of neuronal migration and neurite growth, which results in a compensatory mechanism [170, 171]. MAP2 plays a significant role in microtubule nucleation and stabilization, which affects neurite outgrowth and axonal transport by regulating interactions between motor

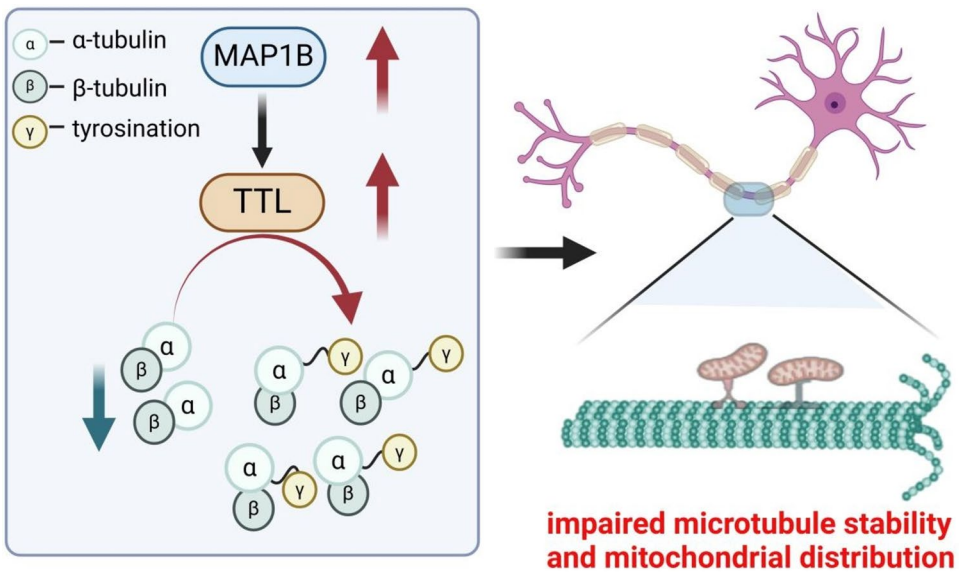

Fig. 4 In SMA, increased MAP1B up-regulates tubulin tyrosine ligase (TTL) activity which increases tyrosinated α-tubulin. The breakdown of α-tubulin tyrosination balance is harmful to microtubule stability. Moreover, damaged microtubule dynamics possibly affect axonal transport, influencing mitochondrial distribution along neurites

proteins and microtubules [170, 172]. In SMN-deficient motor neuron cells, the expression of MAP2 exhibits a down-regulation independent of MAP1B [150, 173]. In neurons, tau protein is another structural MAP affected by SMA. Similar to other MAP family members in the nervous system, tau maintains the stability of axonal microtubules [170]. In motor neurons of SMA mouse models and spinal cord of SMA patient, the hyperphosphorylation of tau mediated by cyclin-dependent kinase 5 contributes to the dissociation of MAPs from microtubule, subsequently leading to downregulated microtubule stability, impaired axonal transport and neuronal degeneration [151].

As end-binding proteins, plus-end-tracking proteins are regulated by major structural MAPs such as MAP1B, MAP2 and tau, which affects microtubule dynamics later [174–178]. Moreover, MAP1B and MAP2 interplay with microtubule end-binding proteins 1 and 3 (EB1 and EB3) and modulate their actions [174, 176, 178]. Unlike EB1 expressed ubiquitously, EB3 is mainly expressed in neurons and accumulates at the distal ends of freshly polymerized microtubules, and regulates growth of microtubules [179–181]. EB3 rather than EB1 exhibits a significant reduction in SMN-depleted cells. Furthermore, decreased MAP1B can elevate EB3 levels in SMN-depleted cells [173]. Located at both the cytoplasm and microtubule tips, EB3 interplays with proteins at microtubule tips like p150Glued and drebrin, and influences microtubule-dependent transport and interactions between F-actin and microtubules [182–185]. As shown in a study, the decline binding of EB3 to microtubule tips caused by overexpressed MAP1B, indicating that up-regulated MAP1B and decreased EB3, may cooperate in the impaired microtubule dynamics of SMA [176]. On top of that, comets, which are structures formed by agminated EB3 at microtubule ends, serve as markers for newly polymerized microtubules and are regulated by MAP1B and tau [174, 176, 179, 180]. They are up-regulated at proximal neurites in SMN-depleted cells, which indicates more growing microtubules at proximal neurites in SMA [173].

Neurofilament, a potential biomarker of SMA

Neurofilaments, as important structural proteins of neurons, are principally expressed in long axons [46]. As subunits of neurofilaments, neurofilament light chain and neurofilament heavy chain are common detection indicators. In severe SMA patients and mouse models, increased demyelinated axons were observed and degenerated rapidly postnatally, thereby resulting in release of neurofilament light chain [20]. Concentrations of neurofilaments in cerebral spinal fluid (CSF), serum, or plasma indicate neuronal damage and have been proposed as potential prognostic and treatment responsive biomarkers in several neurodegenerative diseases such as ALS, AD, PD and SMA [186].

As a potential biomarker of SMA, the scope of application of neurofilaments is controversial. Neurofilament light chain levels in serum and CSF showed strong correlation with motor function in a pediatric control cohort on SMA patients [187]. In a single-center pilot study, phosphorylated heavy chain and light chain neurofilaments in the CSF of SMA patients correlated with disease severity and activity, indicating that neurofilaments in CSF may serve as marker of neuronal loss and clinical outcome [188]. However, in some studies, although decreased after treatment with nusinersen, levels of neurofilaments and neurofilament light chain in the CSF of SMA type 3 patients did not exhibit correlation with motor functions, suggesting that neurofilaments may be insufficient to serve as an optimal surrogate treatment biomarker [189, 190].

In SMA infants, increased plasma neurofilaments levels correlate with age and several markers of disease severity including Hammersmith Infant Neurological Examination Section 2 motor milestones score, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders score, and peroneal and ulnar nerve compound muscle action potential amplitudes, inversely associated with SMN2 copy number [187, 191]. Moreover, in SMA infants and mouse model, levels of phosphorylated heavy chain and light chain neurofilaments in plasma declined rapidly after nusinersen treatment, suggesting their potential as a peripheral marker reflecting the pathological status of SMA [186, 187, 191, 192]. Nevertheless, SMA patients treated with onasemnogene abeparvovec monotherapy exhibited a significant rise in plasma neurofilaments levels, indicating that neurofilaments may be insufficient to function as the single marker to predict outcomes [186]. Further studies are needed to determine the role of neurofilaments in the pathomechanism of SMA.

Conclusion

Accumulated evidence suggests that cytoskeletal abnormalities may play an important role in motor neuron degeneration in progression of SMA. In SMA condition, cytoskeleton abnormalities induce growth cone formation, neurite extension and microtubule formation defects, thereby amplifying SMN-dependent cellular alterations. SMN deficiency dysregulated actin cytoskeleton by interfering with increased free PFN2 which led to an up-regulation of the ROCK pathway, contributing to neuronal damages such as inhibited neurite outgrowth, growth cone collapse and impaired axon pathfinding. PLS3 regulated calcium influx and F-actin dynamics to promote pathogenesis of SMA. The alterations of microtubule stability which affect axonal transport are

associated with the changes of tubulin modifications. The collaboration of several MAPs such as STMN1, MAP1B and tau contributes to downregulated microtubule stability, impaired axonal transport and subsequent neuronal degeneration. Existing clinical studies have shown that the level of neurofilaments in SMA can increase with the demyelination of axon and decrease after nusinersen treatment, suggesting the potential of neurofilaments to serve as prognostic and treatment responsive biomarkers for guiding therapeutic interventions.

The investigation into cytoskeleton holds significant potential for understanding the pathogenesis of SMA and other neurodegenerative disorders. However, the existing studies still have some limitations. First, the trends in the changes of cytoskeleton-associated proteins and influencing degrees of impaired cytoskeleton are controversial among various SMA models. Secondly, the causal link between SMN loss and cytoskeletal dysfunctions is unclear and needs further study. Third, investigations about the effect of cytoskeletal dysfunctions on SMA have focused on animal and cellular models. Although a series of clinical trials have suggested the potential of neurofilaments as biomarkers of SMA, the role of actin disturbance and microtubule instability in the pathomechanism of SMA needs to be further explored in clinical trials. Despite the constraints of current research, comprehending the fundamental mechanisms regulating cytoskeletal proteins is crucial for formulating fresh targeted therapies to treat SMA.

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Declarations

Conflicts of interest The authors declare that they have no competing interests.

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