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Cytoskeleton and CRMPs in Neuronal Morphogenesis and Neurological Diseases: Potential Targets for New Therapies

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Abstract

Cytoskeletal proteins, and the molecules that control their assembly/disassembly, regulate neurite and spine growth and retraction, which are necessary for normal brain function. The neuron begins as a spherical shape, then lamellipodia and filopodia form nascent neurites which differentiate into dendrites and axons. These processes, also involved in neuronal plasticity, require changes in the dynamics of the cytoskeleton proteins and their intracellular binding partners, including collapsin-response mediator proteins (CRMPs). Abnormal changes in CRMP signaling induce structural/ functional abnormalities in neurons which are characteristics of various neurological disorders. Modulation of these pathways may represent unexplored areas for treatment of these diseases.

Keywords: CRMP; Cytoskeleton; Neuritogenesis

Introduction

Neurons integrate and transmit information to allow a living animal to dynamically adapt to environmental changes over time from development to aging. Through the process of neurite specification, arborization, branching, outgrowth, and pruning of inappropriate dendritic and/or axonal branches, an individual neuron interacts efficiently with multiple targets. The neurite development and arborization process requires specific changes in the dynamics of the cytoskeleton, particularly tubulin and actin, and their intracellular binding partners including CRMPs. Regulatory mechanisms maintain the neurite arborization structure by balancing plasticity versus stability. Accordingly, direct changes in the intracellular signaling pathways that affect cytoskeletal dynamics or changes secondary to the increase or decrease of neurotrophic factors and permissive/inhibitory guidance cues may induce structural abnormalities in neurons. These abnormalities can alter information processing, and neuronal network formation/maintenance and connections, which are characteristic of many neurological disorders. CRMPs bind to cytoskeletal proteins and regulate neurite growth, maintenance and plasticity. CRMP activity is regulated at the transcription level by splice variants lacking specific binding sites, and post-translationnally by phosphorylation by various kinases that affects their binding affinity to their effectors. Interestingly, many neurological disorders with abnormalities in cytoskeletal structure/organization are associated with CRMPs dysfunction. The primary intent of this review is to define the functional roles of CRMPs and their cytoskeletal binding partners in neurite formation and maintenance as they relate to their possible involvement in several

neurological disorders characterized by structural abnormalities of neurites. Recent investigations on the modulation of cytoskeletal proteins and CRMPs' activity suggest new potential targets for therapeutic intervention in these diseases.

Cytoskeleton and CRMPs Dynamics in Neuronal Morphogenesis

Although neurons must be able to change their shape during different phases of development to achieve specific functions, in adults, despite the great diversity of morphology between neuronal types, most vertebrate nerve cells exhibit distinctive dendrite morphology and specific dendritic/axonal arbor/ramification depending on their location. The axono-dendritic arbor which influences the number of synaptic inputs that each neuron can integrate emerges as a convergent product of specific pattern of growth, branching and retraction and is differentially regulated at multiple points, including the control of the number of primary branches and their mode and frequency of branching. The cellular cytoskeleton and molecules regulating its dynamics mediate these changes [1,2]. Accordingly, disruption of the activity of these proteins may lead to or be associated with various neurological disorders.

Lamellipodia formation, neuritogenesis and dendrite morphogenesis

Cytoskeleton:Establishment of lamellipodia is considered a principal means of initiating primary neurites formation. Lamellipodia are thin protrusions of membrane roughly 140 nm long surrounding the cell. They contain a stretched meshwork and bundles of branched F-actin

filaments having their barbed ends directed towards the cell membrane. F-actin assembles from ATP-bound G-actin monomers and requires many actin-regulating proteins such as cofilin, ADF, MARCKS, PIP2, N-WASP, MAPs, Arp2/3, Ena/Vasp, profilin and filamin [3-6]. Over time, lamellipodia undergo segmentation. The segmented areas in which actin subunit addition/filament elongation occurs gradually extend away from the leading edge to allow these structures to form filopodia, critical elements for neurite formation. The specific repertoires of proteins that orchestrate this complex sequence of morphological events, including their engagement in a spatio-temporal manner to organize different actin isoforms, microtubule arrays –and their reciprocal interaction through MAPs– and the molecular mechanisms of the protrusion of the leading edge membrane remain largely unknown. Furthermore, recent studies provide evidence that there are multiple signaling pathways/molecular mechanisms that can initiate filopodia formation or its retraction even in the same cell [7]. Despite the variability of dendrite arbors, dendrites share some fundamental characteristics: there is abundant evidence that their development is determined by various intrinsic/extrinsic codes. During development, neurons likely encounter similar environment factors. Intrinsic pathways (Ras, RhoA, Rac, Cdc42, Tiam-1, CaMKII, GSK3β, mTOR) within each neuron control the cellular interpretation of the extrinsic cues to allow neurons to generate different patterns of dendrite development [8-10]. In addition, various intrinsic transcription factors (Foxo6, Neurogenin, NeuroD, CREB, CREST, Sp4, MEP2A) may also operate with different extrinsic chemo-attractive and chemo-repulsive cues such as semaphorins, ephrin, Slit, reelin, VGCC, neurotrophins, extracellular matrix, to facilitate the tiling/self-avoidance mechanisms (Dscam; Stop Signal Notch1), to tailor the dendritic shape in response to the new environment or various stimuli, and importantly to maintain an equilibrium between dendrite dynamics and stabilization for each distinct dendrite arbor morphology [11,12]. Ultimately, all these processes are intimately tied to their effect on the proper dynamic of cytoskeletal proteins through the Rho family of GTPases.

CRMPs: CRMPs represent a family of proteins that binds to cytoskeleton proteins and are involved in the regulation of dendrite and axon growth and retraction. In addition to neuron cell cultures, gene-targeted inactivation in rodents has enabled elucidation of unique functions of individual CRMPs in different cell types *in vivo*. In culture, neurons from *Crmp3-/-* knockout mice cannot break the cellular symmetry to extend neurites and establish neuronal polarity, demonstrating that CRMP3 is essential in the early stage of neurite initiation and dendrite formation [13]. Consistent with this idea, CRMP3 over-expression promotes important lamellipodia formation and significantly increases dendritic number, length and branching per neuron [14]. In contrast, CRMP5 depletion by RNAi enhances the length and number of neurites while its over-expression induces mitophagy and reduces dendrite length without affecting primary dendrite formation. Further, over-expression of a truncated CRMP5 isoform devoid of the tubulin-binding domain has no effect on dendrite length or formation, demonstrating the critical role of tubulin-binding domain in the neurite inhibitory outgrowth of CRMP5 [15]. CRMP2 has been reported to enhance dendrite lengths. CRMP5 and CRMP2 both interact with the tubulin complex and study on the combinatorial functions of both CRMP2-CRMP5 led to the finding that the neurite outgrowth-promoting function of CRMP2 is totally abolished by CRMP5, which acts as a dominant negative signal [15]. Increasing evidence supports CRMP4 involvement in regulation of dendritic outgrowth. In *Crmp4-/-* mice, the distance from the soma

to the first bifurcation point in the apical dendrites of CA1 pyramidal neurons is significantly decreased, confirming an inhibitory role of CRMP4 on dendrite bifurcation and branching [16]. CRMP1 is mainly localized in dendrites of various brain structures and its temporal light–mediated inactivation promotes retraction of lamellipodia followed by retardation of neurite formation. In addition, *Crmp1-/-* mice display abnormal GAP43/PSD95/MAP2/Golgi staining in the CA1 area of hippocampus, consistent with structural dendritic alteration [17,18].

Axonogenesis

Cytoskeleton:During brain development, neurons display a dazzling diversity of distinct axonal phenotypes identified by their complexity of axonal arbor structures with distinct branching management (basket cell axons vs parallel fibers of cerebellum) and diameters (myelinated vs unmyelinated fibers). Axon elongation is ultimately achieved through microtubule assembly and extension, actin reorganization and membrane genesis at the leading edge of growth cones. It has been generally assumed that the axon-dendrite polarity involves polarized transport of various cargoes, complex arrays of microtubule orientation -axons contain microtubules oriented uniformly plus-end distal while dendrites have microtubules in a mixed orientation through dynein function-, and distinct repertoires of ECM/structural/ signaling proteins. For example, 1) the multipolar cells might receive extrinsic factors (TAG-1) from the surrounding microenvironment to establish polarity and differentiate into bipolar cells, 2) down regulation of MAP2 inhibits dendrite formation whereas down regulation of Tau1 inhibits axon formation. After the neuron polarizes, the axon may extend a long distance to reach its targets and form pre-synaptic structures and the kinase GSK-3β is the main intracytoplasmic-integrating signal that translates the extracellular codes into controlled axonal extension and guidance through various substrates, including CRMP2, APC, Tau, Stathmin, MAP1b, Katanin, KIF2C, myosin2 and CLAPS1/2 [19-21]. Another significant difference in axonal phenotype is the proximal axon characterized by the axonal initial segment (AIS) which is a microtubule-based filter that retains Tau in the axon while Spectrin and Ankyrin B form the basis of the plasma membrane barrier [22].

CRMPs: It has been generally assumed that specification in axon or dendrite involves specific proteins. However, several molecules such as CRMP2 can affect both dendrites (as discussed above) and axons. Overexpression of CRMP2 in hippocampal neurons induces supernumerary axons, axon elongation, and increases axonal branching while its inhibition with a dominant negative protein suppresses the formation of primary axon [23]. CRMP2 can also convert existing dendrites into axon. Mechanisms for the regulation of the axonal growth may involve changes in microtubule polymerization, participation in the Sra-1/WAVE/kinesin cargo complex and modulation of vesicle/membrane trafficking/remodeling through interaction with Numb, depending upon specific post-translational modifications [24,25]. CRMP2 is an effector of secreted neurotrophic and neurite collapsing factors. BDNF and NT3, by inhibiting CRMP2 phosphorylation by GSK-3β, induce axonal growth and branching activity while Sema-3A, by increasing CRMP2 phosphorylation via GSK-3β, induces axonal retraction [26]. Ectopic expression of the short-isoform of CRMP4 also enhances axonal extension whereas it dephosphorylated, long-isoform mediates MAI-induced neurite outgrowth inhibition via actin-dependent processes [27]. These observations suggest that CRMP2 phosphorylation levels and CRMP4

isoform ratio represent possible mechanisms of regulation of axonal structure/ function.

Immunocytochemical and mutant protein studies of CRMP1 detected a reduction in axonal extension induced by NT3 in neurons from dorsal root ganglia [28]. However, axonal length from cerebellar explants cultures of *Crmp1-/-* mice are similar to wild type [18]. In contrast to their role in dendritogenesis, CRMP3 and CRMP5 deletion or overexpression yields no apparent effect on axon morphogenesis in cultured hippocampal CA1 neurons and cultured cerebellar explants. Considering the multifaceted phenotypes of CRMPs-deficient mice from different contexts, it is not surprising that these proteins are found altered in several neurological disorders [2].

## Neurological Diseases: A Focus on CRMPs and Cytoskeleton

Alzheimer's disease (AD)

The cellular hallmark of AD is dendritic dystrophy, including reduction of dendrite length/complexity, and fragmentation and alteration of spine density/shape. The molecular hallmark is the defect and collapse in cytoskeletal proteins which represents the seminal event described as senile plaques, neurofibrillary tangles (NFTs) and cofilin pathology: Tau in promoting tubulin polymerization plays a critical role in tubulin dynamic. Tau's aberrant hyperphosphorylation by GSK-3β/Cdk5 alters its ability to bind microtubules resulting in their disassembly; actin and cofilin may also be involved [29]. Alzheimer patients present a decline in cognitive ability beginning with the loss of short term memory. The hippocampus -with prominently reduced dendritic complexity- is one of the earliest and most vulnerable brain regions affected by the disease [30]. Interestingly, increased phosphorylated CRMP2 has been detected in the soluble fraction of AD-affected brain tissue but not in other types of dementia [31]. The hippocampal synaptic dysfunction and memory impairments of AD are modeled in AD transgenic mice with neuritic senile plaques (β-amyloid) and NFTs, supporting the neuropathological concept of a 'dendritic-dysconnectivity' syndrome [32]. In transgenic AD mouse models, phosphorylated CRMP2 is increased in hippocampus well before plaque and tangle formation suggesting that it is an early event in the pathogenesis of the disease [33]. Although the relationship between pathophysiological mechanisms and AD symptoms remains to be clarified, these findings position phosphorylated CRMP2 as a potential biomarker for AD early detection. Of note, in the 6-hydroxydopamine (6-OHDA) model of Parkinson disease, which is associated with alteration in microtubule integrity, CRMP2 is up-regulated in brain regions including the corpus callosum, an area where CRMP2 is normally not present [34].

Prion diseases

In the sporadic/familial Creutzfeld-Jacob disease and other prion diseases, cellular alpha-helix rich prion protein (PrP^c) is converted to the beta-structure-rich insoluble abnormal protease-resistant conformer (PrP^{Sc}) [35]. These diseases are characterized by dendritic dystrophy followed by neuronal loss, gliosis and spongiform changes in the brain leading to dementia/ataxia. *In vitro* experimental procedures have shown a direct interaction between PrP and α/β-isoform of tubulin. PrP has been reported to disassemble the microtubules cytoskeleton (Figure 1C and 1B) leading to a loss of neurites and

necrotic cell death. A specific increase in CRMP4 expression and dendritic degeneration are the two earliest morphological features detectable in mouse models of prion disease before clinical symptoms are evident [36,37]. In the late stage of the disease, the amount of a truncated CRMP2 (CRMP2-ΔC), which lacks the sequence from Ser-518 to the C-terminus) is increased significantly [38]. Consistent with these evidences, we observed that the cytotoxic peptide PrP$^{106\text{-}126}$ induces dendrite dystrophy in cultured neurons as well as an alteration of CRMP2 expression and a profound disturbance of CRMP2 electrophoretic pattern (Figure 1A). This effect was prevented by inhibition of calpain, suggesting that a calpain-mediated alteration of CRMP2 may result in disruption of microtubules, leading to pathogenic effects (Figure 1A and 1D).
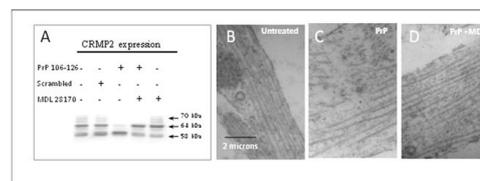
Figure 1: Changes in CRMP2 and microtubules after treatment with prion peptide: Association of CRMP2 calpain-mediated cleavage with cytoskeletal perturbation in PrP106-126 treated neurons. The balance between the different forms of CRMP2 was drastically changed in neurons treated with PrP106-126 (A lane 3) but not in neurons treated with scrambled peptide (A lane 2), the upper bands being reduced concomitantly with a marked increase of the 58 kDa product analyzed by western blot. Transmission electron micrograph studies show that the change in CRMP2 induced by PrP106-126 is associated with microtubule network disorganization (C) compared to control (B). Inhibition of calpain by MDL 28170 not only prevents the change in CRMP2 (A lane 3) but also rescues neurons from microtubule breakdown (D). Thus in prion disease, a calpain-mediated alteration of CRMP2 may result in disruption of microtubules, leading to pathogenic effects.
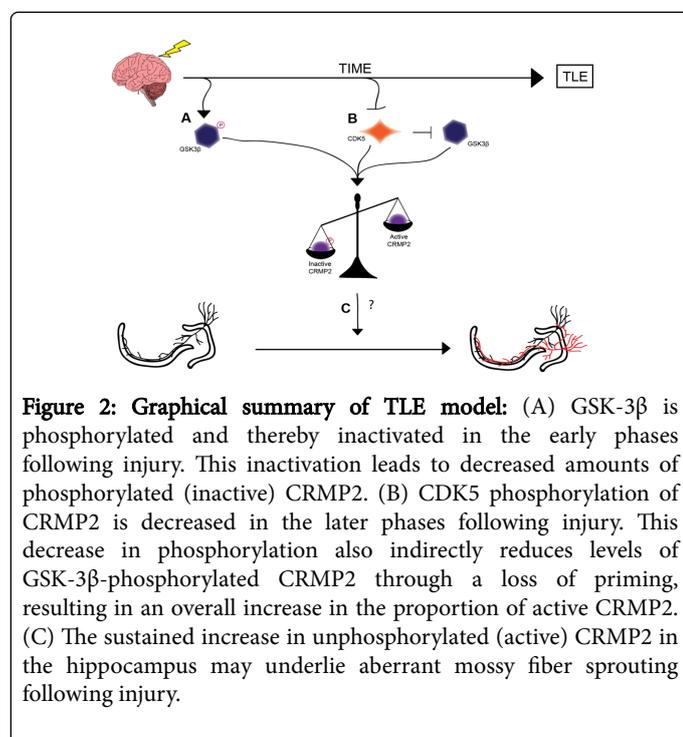
Amyotrophic lateral sclerosis (ALS)

ALS is a multifaceted neurodegenerative disease influenced by varied genetic (mutation of SOD1; single nucleotide polymorphism (SNPs) of C9ORF72)/epigenetic (neurotoxin from cyanobacteria; pesticides) factors affecting upper and lower motor neurons [39]. Other cellular and molecular experiments have identified defects in growth factors, axonal transport or defect in RNA processing. Data from clinical and animal studies support the idea that ALS is a distal "dying-back" axonopathy that occurs at very early stage at the neuromuscular synapses prior to symptom onset, with important disturbance of microtubules and actin structure and impaired axonal transport [40,41]. In this scenario and as CRMP2 links the cytoskeleton microtubule to the molecular motors, it is tempting to suggest that CRMP2 activity may also be part of the development of the presymptomatic stages of ALS. Moreover, the stable tubule only polypeptide (STOP), various guidance cues including the repulsive axon Sema3A and its downstream effectors CRMPs, are linked to the pathophysiology of ALS [42]. It has been shown in animal models that expression of the long variant of CRMP4 is increased in a subpopulation of lumbar motor neurons in SOD1 mice starting at presymptomatic stages, and its over-expression in cultured

motoneurons leads to inhibition of neurite outgrowth followed by cell death.

Epilepsy

Next generation sequencing has led to the identification of an array of epilepsy genes [43]. Various animal and human tissue studies suggest that epilepsy development involves reorganization and sprouting of hippocampal mossy fibers, and changes in cytoskeleton dynamics, calcium signaling and CRMP expression [44-46]. The nexus between CRMP2 and epilepsy was recently highlighted by recent studies on posttraumatic epilepsy, the development of temporal lobe epilepsy (TLE) following traumatic brain injury, which accounts for 20% of symptomatic epilepsies. Reorganization of mossy fibers within the hippocampus is a common pathological finding of TLE and likely in combination with other mitigating factors contributes to epileptogenesis. During TLE, mossy fibers innervate the inner molecular layer of the hippocampus where they synapse onto the dendrites of other dentate granule cells, leading to the formation of recurrent excitatory circuits. Although the exact role of CRMP2 in mossy fiber sprouting has not yet been determined and a causal relationship has yet to be drawn between mossy fiber sprouting and epileptogenesis, it is possible that the loss of GSK-3β phosphorylation immediately following injury contributes to the induction of mossy fiber sprouting while the loss of priming by CDK5 in later phases contributes to its maintenance. It is of great interest that these mechanistically distinct events culminate in a similar end-point: an increase in the amount of active CRMP2 (Figure 2). At the very least, mossy fiber sprouting is linked to the exacerbation of the progression of the disease as well as the manifestation of its symptoms. The involvement of CRMP2 in such processes, however, remains untested.

Figure 2: Graphical summary of TLE model: (A) GSK-3β is phosphorylated and thereby inactivated in the early phases following injury. This inactivation leads to decreased amounts of phosphorylated (inactive) CRMP2. (B) CDK5 phosphorylation of CRMP2 is decreased in the later phases following injury. This decrease in phosphorylation also indirectly reduces levels of GSK-3β-phosphorylated CRMP2 through a loss of priming, resulting in an overall increase in the proportion of active CRMP2. (C) The sustained increase in unphosphorylated (active) CRMP2 in the hippocampus may underlie aberrant mossy fiber sprouting following injury.

Paraneoplastic neurological disorders (PND)

The neuronal damage of PNDs is caused through a remote immune-effect of the tumor on the nervous system. Auto-antibodies against CRMP5 (also called anti-CV2 or anti-CV2/CRMP5) have been described in a subgroup of PND [47]. Patients with these antibodies frequently show peripheral sensorimotor neuropathy, cerebellar ataxia, limbic encephalitis, and chorea. The identification of CRMP5 as the main target of anti-CV2 antibodies in PND patients is important for understanding the pathophysiology of the disease [48]. Indeed, Crmp5-/- mice mimic PND neurologic pathology and symptoms resulting from damage of the peripheral and central neurons, and exhibit peripheral neuropathy with abnormal motor conduction velocities, abnormal polyaxonal ensheathment, abnormal Schwann cell structure, as well as altered Purkinje dendrites in cerebellum with abnormal limb-clasping reflex and abnormal long term depression, suggesting that the various symptoms in PND patients can be linked to the CRMP5 protein [49].

Autism spectrum disorders (ASD)

A consistent feature of neurons in ASD patients is abnormal dendritic structure with alterations in dendritic spine morphology. The discovery of single gene mutations of several genes involved in cytoskeletal proteins (MECP2 involved in the regulation of neuronal α-tubulin expression, SHANK3 which has a pivotal role in the formation and maturation of spine via an actin-dependent mechanism, the neurexin gene family- and cytoskeleton dynamics –LIMK1, PAK3, ARFGEF6, 9), copy number variations, chromosome abnormalities, changes in protein expression (altered neuritogenesis mediated by altered cellular PrP^c levels), and epidemiological twin studies suggest that genetic abnormalities play important role in ASD pathogenesis [50-52]. Of note, it has been found that AUTS2 (the Autism susceptibility candidate 2 gene) induces lamellipodia but suppress filopodia formation. On the other hand, the findings of the presence of maternal autoantibodies directed against fetal brain tissues together with the passively transferred human IgG from mother brain-reactive antibodies studies provide strong evidence that maternally derived antibodies could lead to neurodevelopmental alterations in a subgroup of ASD. In this context, it has been shown that maternal autoantibodies reactive to cypin, CRMP1 and CRMP2 confer an increased risk for autism [53].

Neuropathic pain (NP)

Associated with damage/trauma to central or peripheral nervous system caused by a primary injury or by numerous diseases, NP may be related to abnormal sprouting and spine remodeling that lead to neuron hyperexcitability and decreased in pain threshold. Three points are important to emphasize. First, proteomic studies identify that changes in cytoskeletal tubulin3, calcium channels, CRMP2 and MAP2 are associated with NP in animal models. Second, CRMP5 expression is altered in injured and regenerating stages of sciatic nerve. Third, N-type VGCC (CaV2.2) are genetically and clinically validated targets for pain management, and uncoupling these proteins from CRMP2, with which they bind, is anti-nociceptive in various models of neuropathic pain [54-56]. Thus, the indirect change of CaV2.2 by regulating its interaction with CRMP2 may afford better safety and efficacy profile than currently available CaV2.2-selective blockers which have many untoward side effects [57].

Therapeutic Perspectives

It is clear that the cytoskeleton and CRMPs play a critical role in maintaining neuronal phenotype and activity and their alteration affect the axono-dendritic arbor –which is a major site of histopathologic alterations in many neurological diseases. Alterations in synaptic structure with increased connectivity may impact epilepsy, neuropathic pain, and autism while decreased function may be related to neurodegenerative disorders such as AD and prion disease or lead to muscle atrophy and fasciculation as in ALS. Few available treatments in humans are regulating CRMPs and cytoskeletal proteins, and targeting them specifically is explored in various animal models.

Actin as a potential biological therapeutic target

Cumulative clinical and histopathological evidence suggests that memory loss correlates better with synapse/spine loss than with plaques or tangles formation in AD. Genetic studies further demonstrate a significant causal link between spine density/structure and the cognitive deficit via a change in drebrin – a dendritic spine actin-regulating protein, and fractin – a caspase-cleaved fragment of actin, and the fractin/actin ratio. That actin is a possible therapeutic target can be surmised from the following observations: 1) docosahexaenoic acid (DHA), an essential fatty acid which prevents caspase cleavage of actin, decreases the likelihood of developing AD and 2) urokinase-type plasminogen activator (uPA), which shifts the actin pool from G-actin to F-actin, induces the re-emergence of filopodia and spines during the recovery phase of brain injury [58,59].

### Microtubule as potential biological therapeutic target

Alteration in microtubule spatio-temporal regulation by their molecular binding partners (MBP) has negative consequence on axonal transport and neuronal function, which recently lead to proposing microtubules/MBPs as promising therapeutic targets for treatment of nervous system disorders and injury [60,61]. Accordingly, recent studies demonstrate that microtubule-stabilizing agents used as anticancer drugs can yield beneficial outcomes in promoting axonal regeneration and ameliorating the pathogenic symptoms in mice models of neurodegenerative disorders: low doses of paclitaxol promote serotonergic axon regeneration, reduce macrophage infiltration and decrease scar formation at spinal cord injury sites while epothilone improves cognition of tau-transgenic mice models [62,63]. These findings indicate that microtubule and MBPs may offer effective targets to control multiple pathways to produce better outcome in brain disorders treatment [64].

CRMPs as potential biological therapeutic targets

Under various physiological and pathological conditions during neuron development, CRMPs are critical signal transducers and intracytoplasmic regulators involved in structuring cytoskeletal proteins activity and dynamic. Accordingly, CRMPs hold considerable promise as therapeutic agents. Indeed, a 15 amino acid CRMP-2-derived peptide (designated as tat-CBD3) that disrupts the CaV2.2/CRMP2 interaction has proved to be anti-nociceptive in preclinical animal models of inflammation and neuropathic pain [54-57,65]. AAV-encoded CBD3 delivered to rat peripheral sensory neurons through DRG injection provides sustained relief (> 6 weeks) of NP [66]. The positive effect and relative lack of toxicity of the AAV-targeted expression of CBD3 peptide in this animal model suggest that CBD3 may be a valuable approach, not only for exploring the role of

presynaptic VGCCs and long-term modulation of neurotransmission, but to treat chronic NP and other disorders [67].

In neurons in culture, it was found that PrP106-126 induced dystrophic changes in dendrites, an effect prevented by Taxol treatment that interferes with the normal breakdown of microtubules (Figure 3) and by overexpression of CRMP3 [14]. In patients with prion pathology, the extensive neuronal loss associated with the spongiform degeneration affects the basal ganglia and the cerebral and cerebellar cortex whereas the CRMP3-rich hippocampal formation is protected until the final stage of disease [68,69]. The high levels of CRMP3 expression in hippocampus may explain, at least in part, the relative protection of this structure from the disease, and suggests that CRMP3 may prove useful in the prevention of altered dendritic structure *in vivo*. Similarly, the fact that CRMP2 expression and/or phosphorylation/activity are altered in Alzheimer's disease raises the attractive hypothesis that the protein may be a viable therapeutic target [70]. In an animal model of AD, suppression of CRMP2 phosphorylation is associated with amelioration of β-amyloid-induced cognitive dysfunction and hippocampal axon degeneration [70].
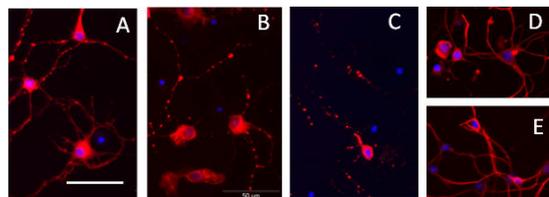
Figure 3: Effects of microtubule stabilizing agents on cells treated with prion peptide: Primary neurons (β-tubulin positive) subjected to different treatments: untreated cells (A), PrP106-126 80 μM for 2 h (B), PrP106-126 80 μM for 24 h (C), PrP106-126 80 μM and Taxol (a microtubule-stabilizing agent) 10 μM for 24 h (D), or Taxol 10 μM for 24 h (E). All micrographs are represented with the same total magnification. White bar: 50 microns.

There is no efficient therapy for ALS patient, and new therapeutic targets need to be explored [71]. The observations of increased CRMP4 in animal models of ALS is further strengthened with adeno-associated virus (AAV)-mediated overexpression of CRMP4 in motoneurons *in vivo* that leads to significant muscle denervation and reduction in motoneuron numbers [72]. Altogether, these findings strongly suggest that CRMP4 may be an early effector candidate in the SOD1 mouse model of ALS and controlling its activity may ameliorate ALS symptoms.

Finally, S-lacosamide ((S)-LCM) -the enantiomer of Vimpat, an antiepileptic drug in clinical use- inhibits CRMP2 phosphorylation without off target effects but with functional consequence such as blocking calcium influx via inhibition of VGCCs [73]. The extent of mossy fiber sprouting, a hallmark of temporal lobe epilepsy (TLE) in rats that received (S)-LCM following a controlled cortical impact injury, is markedly decreased compared to controls [74]. This suggests that (S)-LCM, a small molecule, may be a novel tool with potential therapeutic applications not only for epilepsy but other neuropathologies (Figure 4).
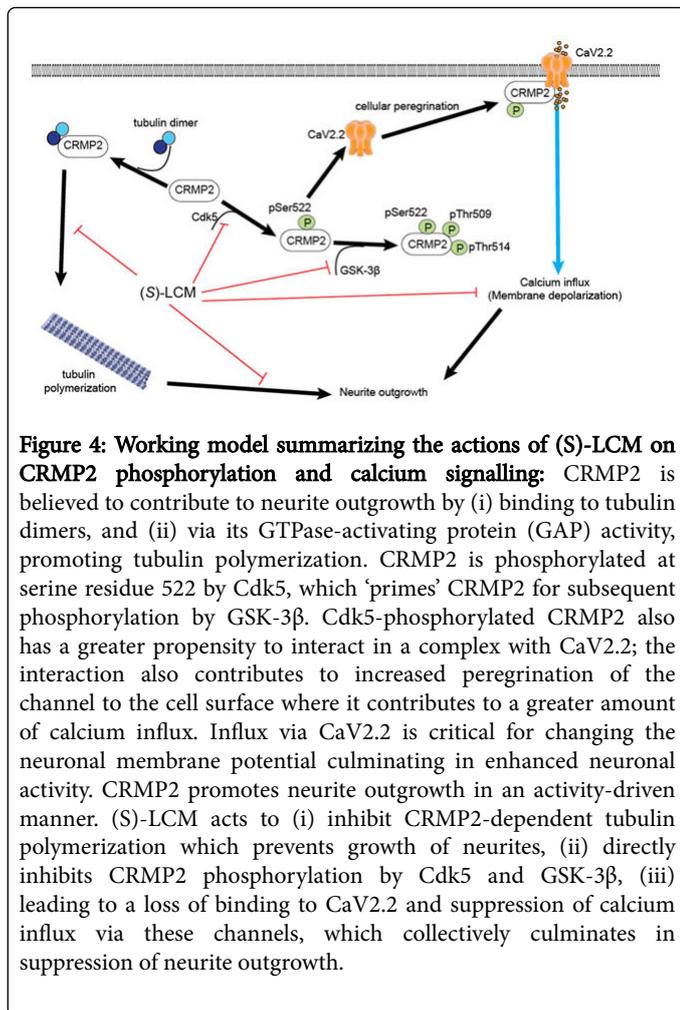
Figure 4: Working model summarizing the actions of (S)-LCM on CRMP2 phosphorylation and calcium signalling: CRMP2 is believed to contribute to neurite outgrowth by (i) binding to tubulin dimers, and (ii) via its GTPase-activating protein (GAP) activity, promoting tubulin polymerization. CRMP2 is phosphorylated at serine residue 522 by Cdk5, which 'primes' CRMP2 for subsequent phosphorylation by GSK-3β. Cdk5-phosphorylated CRMP2 also has a greater propensity to interact in a complex with CaV2.2; the interaction also contributes to increased peregrination of the channel to the cell surface where it contributes to a greater amount of calcium influx. Influx via CaV2.2 is critical for changing the neuronal membrane potential culminating in enhanced neuronal activity. CRMP2 promotes neurite outgrowth in an activity-driven manner. (S)-LCM acts to (i) inhibit CRMP2-dependent tubulin polymerization which prevents growth of neurites, (ii) directly inhibits CRMP2 phosphorylation by Cdk5 and GSK-3β, (iii) leading to a loss of binding to CaV2.2 and suppression of calcium influx via these channels, which collectively culminates in suppression of neurite outgrowth.

Conclusion

The CRMPs signaling system is a critical pathway that regulates neurite initiation, axono-dendritic growth/branching and retraction during neuronal maturation and plasticity and consequently affects neuronal connectivity/activity and behavioral responses. In agreement with this role, it is apparent that the cytoskeleton and CRMPs interactions may also be involved in neurological disorders associated with dendrite or axon dystrophy, atrophy or hypertrophy. Although presently the biochemical, pharmacological and electrophysiological data originate mostly from *in vitro* experimental models or from *in vivo* genetically modified mice and more rarely from patients samples, the proposed concept is compelling and worthy of continued research. Additional post-mortem-CRMPs-directed immunohistopathology observations, especially at different disease stages, and probing for specific CRMP splice variants and un/phoshorylated isoforms are needed to confirm the critical role of these proteins in brain diseases while SNP linkage, GWAS and meta-analyses of the polygenic score profile may reveal complexity in CRMP genetic contribution. Causality cannot be inferred from the human data but *in vitro* and *in vivo* animal model suggest possible mechanisms. Other molecules involved in cytoskeletal assembly are also likely to be associated with these diseases. However, the fact that CRMPs directly bind to tubulin to regulate assembly and disassembly of microtubules place them at the downstream end of the signaling pathways initiated by extracellular or intracellular factors controlling structural changes of neurites and spines. This can explain their association with various diseases and make them a perfect target for intervention in disorders associated with alterations of neurite structure. Since CRMPs activity is regulated by phosphorylation, kinase inhibitors represent a first way to investigate pharmacological modification of their activity. Small molecules increasing or decreasing the binding of CRMPs to their effectors represent another more specific strategy. Finally, unraveling the finer molecular details of how these different molecules interact under physiological and pathological conditions to regulate the CRMPs phosphorylation/cytoskeletal dynamics [75,76], associated with genomic studies may aid in the design of combinatorial therapies defying these devastating neurological diseases within the framework of personalized pharmacogenetical therapy.

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