Int J Clin Exp Med 2015;8(12):22337-22344
www.ijcem.com /ISSN:1940-5901/IJCEM0015483

Original Article

CRMPs colocalize and interact with cytoskeleton in hippocampal neurons

Yuhao Yang[1,2*], Bo Zhao[2*], Zhisheng Ji[1,2], Guowei Zhang[1], Jifeng Zhang[2], Sumei Li[2], Guoqing Guo[2], Hongsheng Lin[1]

[1]Department of Orthopedics, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China; [2]Department of Anatomy, Medical College of Jinan University, Guangzhou 510630, China. [*]Equal contributors.

Received September 2, 2015; Accepted December 5, 2015; Epub December 15, 2015; Published December 30, 2015

Abstract: CRMP family proteins (CRMPs) are widely expressed in the developing neurons, mediating a variety of fundamental functions such as growth cone guidance, neuronal polarity and axon elongation. However, whether all the CRMP proteins interact with cytoskeleton remains unknown. In this study, we found that in cultured hippocampal neurons, CRMPs mainly colocalized with tubulin and actin network in neurites. In growth cones, CRMPs colocalized with tubulinmainly in the central (C-) domain and transition zone (T-zone), less in the peripheral (P-) domain and colocalized with actin in all the C-domain, T-zone and P-domain. The correlation efficiency of CRMPs between actin was significantly higher than that between tubulin, especially in growth cones. We successfully constructed GST-CRMPs plasmids, expressed and purified the GST-CRMP proteins. By GST-pulldown assay, all the CRMP family proteins were found to beinteracted with cytoskeleton proteins. Taken together, we revealed that CRMPs were colocalized with cytoskeleton in hippocampal neurons, especially in growth cones. CRMPs can interact with both tubulin and actin, thus mediating neuronal development.

Keywords: CRMP-5, actin, tubulin, cytoskeleton, growth cone, neurite growth, hippocampal neuron

Introduction

Growth cone is only active structure to guide the formation and outgrowth of neurites [1]. Precise navigation by a neuronal growth cone requires the modulation of the growth cone's responsiveness to spatial and temporal stimulation of guidance cues [2]. Neurite outgrowth and branching is the result of multiple cascades of signaling transductions, the remodeling of microtubules and actin filaments within the growth cone is especially critical for these processes. Microtubules and actin filaments are abundantly presented in growth cones, where actin filaments mainly distributed in the peripheral (P-) domain and microtubules in the central (C-) domain. When neurite extending, actin in the P-domain firstly senses the external growth signals deciding the growth cone to collapse or grow. Then dynamic microtubules in C-domain sense the changes of actin network to decide whether tubulin insertion into P-domain or not [3]. However, the mechanism of how microtubules communicate with actin remains to be further illustrated. According the literature, there are two potential mechanisms of interaction between microtubules and actin, regulatory and structural [4]. Regulatory interactions says microtubules and actin indirectly regulate each other, but by their effect on signaling cascades [5-7]. Structural interactions says the two systems are directly linked by a variety of structural proteins, such as microtubule-associated proteins (MAPs) [8], +TIPs [9, 10] or spectraplakins [11, 12].

Collapsin response mediator proteins (CRMPs) are a family of microtubule related proteins, consisting five cytosolic proteins (CRMP1-5) which are expressed in developing and adult nervous systems [13-15], functioning in cell migration, differentiation, neurite extension, axon regeneration and some other cellular processes [16, 17]. The target structure of CRMPs is the cytoskeleton, and some CRMP isoforms were reported to regulate actin [18]. However, there is no direct evidence telling whether CRMPs interact tubulin and actin simultane-

Table 1. CRMPs primers and the restriction sites

Gene	Primer		Restriction enzyme
CRMP1	forward	5'-AGGTCGACATGTCTCATCAGGGGAAG-3'	SalI
	reverse	5'-ATGCGGCCGCACCGAGGCTGGTGATGTT-3'	NotI
CRMP2	forward	5'-ATTCGAATTCGCCACCATGTCTTATCAGGGGAAGAAA-3'	EcoRI
	reverse	5'-ATTCGCGGCCGCTTAGCCCAGGCTGGTGATGTT-3'	NotI
CRMP3	forward	5'-ATTCGAATTCATGTCCTTCCAAGGCAAGAAGAGC-3'	EcoRI
	reverse	5'-ATTCGCGGCCGCCTAAGAAAGTGAAGTGATGTT-3'	NotI
CRMP4	forward	5'-ATGTCGACATGTCCTACCAGGGCAAG-3'	SalI
	reverse	5'-TTGCGGCCGCACTCAGGGATGTGATGTT-3'	NotI
CRMP5	forward	5'-AGGTCGACATGCTTGCCAATTCAGCC-3'	SalI
	reverse	5'-TTGCGGCCGCCCAAATACCGCTCGACCT-3'	NotI

ously. Here in the current study, we tried to determine whether CRMPs interacted with tubulin and actin thus to regulate cytoskeleton coordination. We observed the colocalization of CRMPs with tubule and actin in neurite and the growth cone, then we constructed GST-CRMPs plasmids, expressed and purified the GST-CRMP proteins to reveal their interaction with cytoskeleton *in vitro* by GST-pulldown assay.

Materials and methods

Animals

The experiments were carried out on 1-day-old pups of Sprague-Dawley rats. All animal procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Jinan University Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize the suffering and number of animals used.

Agents

TRIzol reagent and SuperScript VILO cDNA Synthesis Kit were from Invitrogen, Life Technologies (Carlsbad, CA). Plasmid miniprep kit, DNA purification kit, NotI, EcoRI and SaLI restriction endonucleases, DNA polymerases for PCR reaction and T4 DNA ligase were purchased from Takara (Otsu, Japan). DMEM/F12, Neurobasal medium, B27 supplement, Ara-C and FBS were purchased from Gibco (Carlsbad, CA, USA). Glutathione-Sepharose beads and Protein A/G were from TransGen Biotech (China). Rabbit anti-CRMPs antibody (Santa Cruz, CA). Rabbit anti-GAPDH, anti-Actin and mouse anti-Tubulin were from Abcam (Cambridge, UK). Anti-rabbit or anti-mouse secondary antibody conjugated to Alexa Fluor 488/555 were from Molecular Probes (Invitrogen, Eugene, OR, USA).

Cell culture

Hippocampi were dissected from postnatal rat pups, and dissociated hippocampal neurons were obtained using 0.125% trypsin and plated at a density of 1×10^4 cells/cm^2 onto poly-D-lysine-coated glass coverslips. Cultures were maintained in Neurobasal-A medium containing 2% B27 and 0.5 mM glutamine supplement at 37°C in a 5% CO_2 humidified incubator. One-half of the culture media was replaced every 3 days.

Immunofluorescence

Hippocampal neurons were grown on coverslips (Fisher, Newark, DE, USA) and processed for immunofluorescence according to the standard protocol described previously [19]. Cells were fixed with 4% (w/v) paraformaldehyde (Sigma, St. Louis, MO) for 5 min at room temperature and permeabilized with 0.1% Triton X-100 in PBS for 20 min. The cells were blocked in 3% normal donkey serum in TBS + 0.1% Triton X-100 for 1 h at room temperature and incubated with rabbit anti-CRMPs antibody (Santa Cruz) and mouse anti-Actin/Tubulin (Abcam) at 4°C overnight. The cells were washed 3 times for 10 min with PBS + 0.1% Tween20, and incubated with monoclonal donkey anti-rabbit IgG Dylight 549 (Jackson ImmunoResearch) or monoclonal donkey anti-mouse IgG Dylight 488 (Jackson ImmunoResearch) for 2 h at room temperature. After three washes, cells were mounted on glass slides with Fluoro Gel II containing DAPI (EMS, Hatfield, PA). Microscopy and image analysis were carried out using the same optical slice thickness for every channel using a confocal microscope (LSM 710; Carl Zeiss, Germany). The colocalization efficiency was calculated by confocal software.

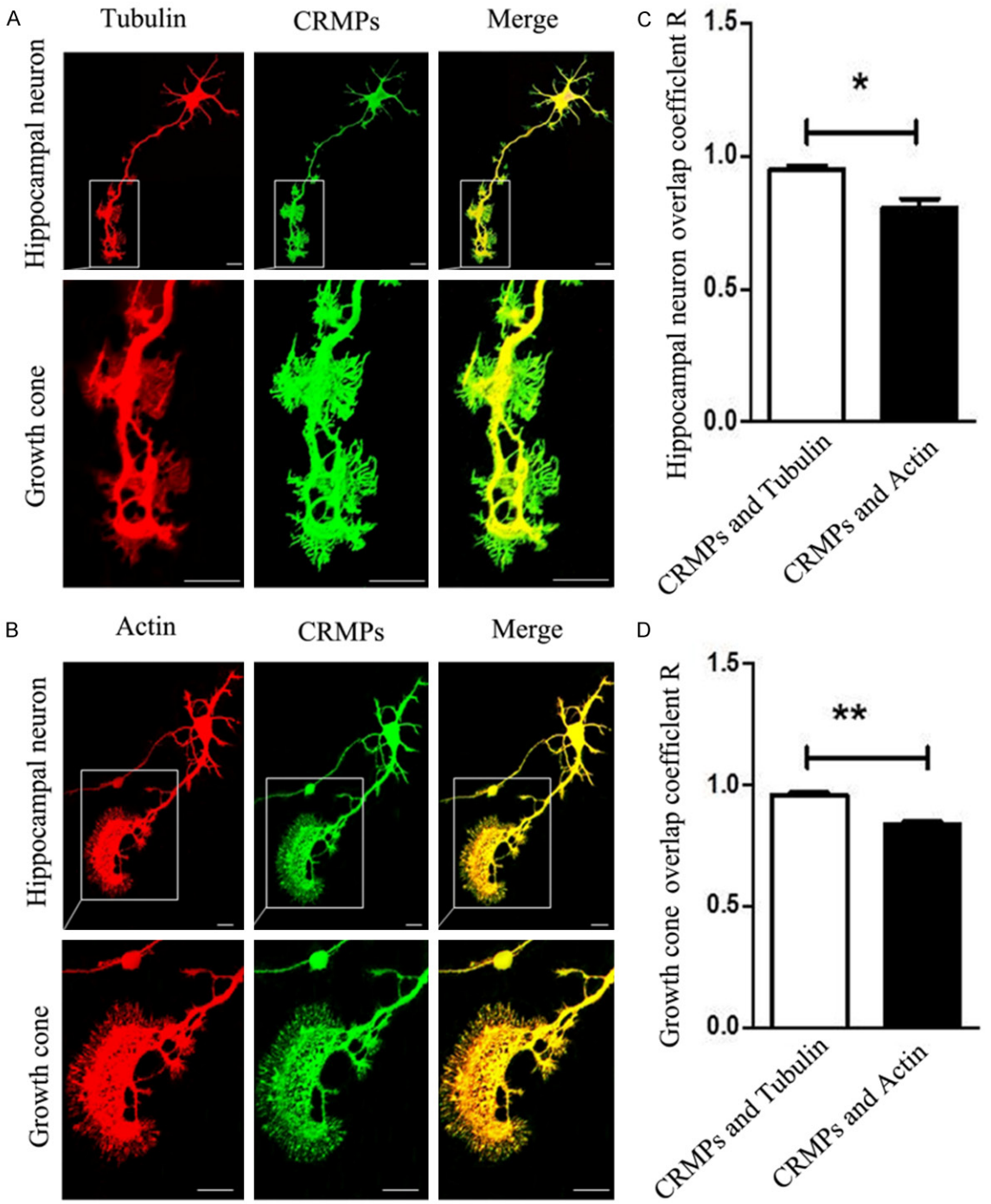

Figure 1. CRMPs colocalizes with tubulin and actin in hippocampal neurons. A. Anti-total CRMPs and anti-tubulin antibodies were used to detect endogenous proteins in the neurite and growth cone of hippocampus neurons. B. Anti-CRMPs and anti-actin antibodies were used to detect endogenous proteins in the growth cone of hippocampus neurons. C. Statistical data of overlap co-efficient value between CRMPs and tubulin/Actin in whole cell criteria were shown as Mean ± SEM, n=3; *, denotes $P<0.05$. D. Statistical data of overlap co-efficient value between CRMPs and tubulin/Actin, in growth cone criteria. Mean ± SEM, n=3; **, denotes $P<0.01$. Scale bar, 10 μm.

Plasmids and constructs

The cDNA encoding full-length rat CRMPs were achieved by using the PCR-based method accordingly [20]. pGEX-5X-3 were nicely given from Dr. Yuan Chen at Sun Yat-sen University. CRMP RNAs were cloned from rat brain tissue and reversed into cDNA. CRMPs encoding

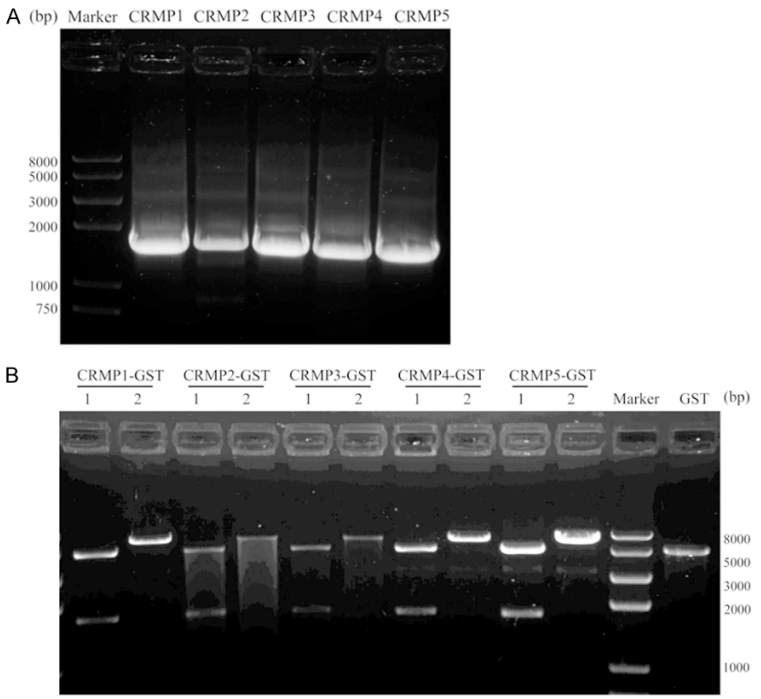

Figure 2. Consturction of GST-CRMPs expression plasmids. A. The gel electrophoresis results of CRMP gene fragments. B. Restriction enzyme digest the recombinant plasmids of CRMPs-GST. 1, the recombinant plasmid of CRMPs-GST by double enzyme digestion; 2, the recombinant plasmid of CRMPs-GST by single enzyme digestion; Lane Marker, DNA standard molecular mass; Lane GST, GST vector single enzyme digestion.

genes were purchased by RT-PCR by using the primer (**Table 1**). Then CRMPs were subcloned into the pGEX-5X-3 vector. All constructs were verified by sequencing.

Recombinant proteins expression and GST pulldown assay

GST fusion CRMPs proteins expression and pulldown assays were performed as previously described [21]. Briefly, GST-CRMPs constructs were transformed into the BL21 (DE3) strain of *Escherichia coli* (Invitrogen, Grand Island, NY). Production of fusion proteins was induced by incubation with 0.2 mmol/L isopropyl-1-thio-b-d-galac-topyranoside for 6 h at 20°C. Bacteria were spun down and resuspended with a cocktail of protease inhibitors (Merck, Whitehouse Station, NJ). The cell suspension was treated with 0.1% lysozyme followed by 0.5% deoxycholic acid on ice for 20 min. After sonication, the cell debris was removed by centrifugation (15,000 g for 30 min). The supernatant, with 1% Triton X-100, was used for the purification of the GST fusion proteins using glutathione-Sepharose beads.

Western blotting

Western blot analysis was performed as previously described [21]. Briefly, lysates were separated using SDS-PAGE and electrophoretically transferred to a polyvinylidene difluoride membrane. Membranes were blocked in Tris-buffered saline with 5% milk and 0.05% Tween and probed with primary antibodies at 4°C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibodies (Jackson ImmunoResearch, West Grove, PA) and visualized using the ECL reagents.

Statistical analysis

Data are presented as the mean ± SEM. Significant differences were assessed with one-way ANOVA followed by Bonferroni or Tamhane*post hoc* tests. $P<0.05$ was considered to be statistically significant.

Results

CRMPs colocalize with tubulin and actin in hippocampal neurons

In order to determine the relationship of CRMPs with cytoskeleton, cultured hippocampal neurons at DIV 3 were immunostained with total CRMP antibody, tubulin and actin antibody. As shown in **Figure 1A**, tubulin distributed in cytosol, neurite backbone and branches; in the growth cone, tubulin mainly expressed in C-domain, less expressed in T-zone and P-domain. Actin distributed in neuronal cytosol, neurites and branches and more in growth cones; in the growth cone, the expression of actin in C-and P- domain is more than that in the transition (T-) zone (**Figure 1B**). CRMPs expressed mainly in neurites, branches and growth cones, less in cytosol and nuclei (**Figure 1A**, **1B**). When CRMPs merged with tubulin, strong colocalizations were seen in neurites, branches and C-domains in the growth cone

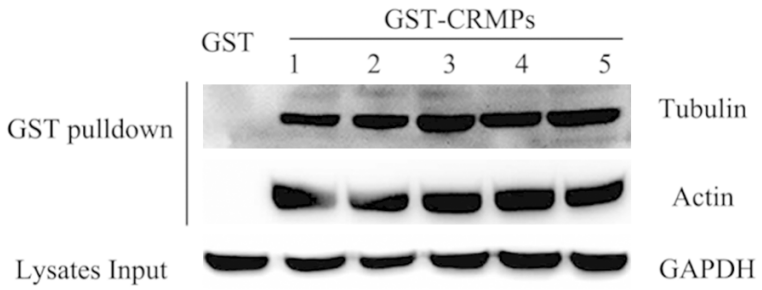

Figure 3. CRMPs interact with cytoskeleton *in vitro*. Bacterial recombinant GST-CRMPs were purified and were subjected to GST-pulldown with growth cone extracts from rat brain. Then the pulldown sediments were subjected to western blot with antibodies of tubulin and actin, of GAPDH as lysate input control. Thisresult is representative of three separate experiments with similar results.

(**Figure 1A**, yellow signal); when CRMPs merged with actin, there is a same pattern as to tubulin (**Figure 1B**, yellow signal). When counting the whole cell, the overlap co-efficiency value of CRMPs with tubulin or actin was 88±2% and 97±3% (**Figure 1C**); when counting only the growth cone area, the overlap co-efficiency value of CRMPs with tubulin or actin was 84±2% and 96%±3% (**Figure 1D**). The overlap value of CRMPs between actin was significantly higher than that with tubulin in both cell and growth cone criteria. These results suggest that CRMPs colocalize with cytoskeleton protein in hippocampal neurons.

The construction of GST-CRMP recombinant plasmids

In order to reveal the interaction of CRMPs with cytoskeleton, we constructed GST-CRMP expression plasmids. From rat brain tissue lysates, coding sequences of CRMP1-5 about 1700 bp were amplified by RT-PCR from the cDNA library (**Figure 2A**). Then CRMP genes were restricted and inserted into the pGEX-5X-3 vector. The constructed GST-CRMPs were confirmed by re-digestion. 6600 bp fragments were resulted from single restriction; 4900 bp and 1700 bp fragments were resulted from double restriction, where 1700 bp fragments were CRMPs genes (**Figure 2B**). The selected GST-CRMP plasmids were then further confirmed by gene sequencing. These data suggest the successful construction of GST-CRMPs.

CRMPs interact with cytoskeleton proteins in vitro

When purified the GST-CRMP proteins, we baited them with rat brain lysates to perform GST pulldown assay. The sediments were immune-blotted with tubulin and actin antibodies, to reveal the interaction between CRMPs and cytoskeleton. As shown in **Figure 3**, GST protein cannot bind tubulin and actin, acting as negative control; but tubulin and actin signals can be detected in the pulldown sediments of all the GST-CRMPs. CRMP1-5 showed nearly equal abilities to interact with tubulin and actin. The GAPDH control indicated equivalent loading for the various growth cone lysates. These data suggest that CRMPs interact with the cytoskeleton *in vitro*.

Discussion

In this study, we demonstrated that CRMPs colocalized with cytoskeleton in neurite and growth cone and CRMPs interacted with tubulin and actin *in vitro* by GST-pulldown assay. CRMPs may be the junctions between microtubules and actin to regulate cytoskeleton dynamics during axonal guidance and elongation.

Neurite extension and guidance are processes of cytoskeleton remodeling [22]. The enlarged growth cone in the distal end of an axon senses the external growth signal, transforming the stimulation to cytoskeleton to adjust the grow direction [23]. Filopodium, which are fulfilled with actin filaments, are the forefront of a growth cone to detect the surrounding guidance cues and the first structure to adjust the growth cone [24]. Microtubule is another critical factor deciding the extension direction of a neurite [25]. Microtubules mainly distribute in the C-domain and T-zone of the growth cone. The arrangement of microtubule is directional that is the assembly end always pointing to the distal end to confer the extension of a neurite [26]. CRMP is a class of microtubule associated proteins, which can promote the assembly of tubulin to regulate neurite outgrowth, axon elongation and cell migration [27, 28]. Our results showed that CRMPs colocalized with tubulin in the proximal branching points. CRMPs are related to neurite branch, so the expression in branching points is high, which is consistent with previous reports [29]. Also, CRMPs colocalized with tubulin mainly in the C-domain,

less in the T-zone and P-domain. This may due to the low expression of tubulin in the P-domain, except dynamic microtubules [30]. CRMPs not only colocalize with tubulin but also with actin, especially in growth cone at C-domain, T-zone and P-domain. CRMPs distributed at the edge of lamellipodia and filopodia [31], which is consistent with the distribution of actin. CRMPs colocalized with actin and tubulin in the C-domain and T-zone, suggesting that CRMPs not only participate in the assembly of tubulin, but also interact with the cytoskeleton to coordinate the cytoskeleton movement, which may be contribute to mediating axon elongation and guidance [25, 32].

Accordingly, some CRMP isoforms can interact with tubulin and some with actin, separately reported. CRMP2 can bind tubulin to promote the assembly of microtubules [33]; CRMP4 interacts with actin to regulate neurite growth [34]; CRMP5 interacts with tubulin regulating growth cone and axon development in hippocampal neurons [21, 35]. On the other side, the CRMP family shares high sequence homology. CRMP1-4 share nearly 75% homology and CRMP5 shares about 50% homology with other CRMPs [36]. So we suspect that all the CRMPs may interact with tubulin and actin, which was confirmed in the current study.

Neurite outgrowth and the formation of branches are regulated by extracellular signals, such as Sema, Netrin and NGF [37, 38]. By activating their receptors in the membrane, these signals adjust cytoskeleton dynamics to regulate neurite extension and guidance [39]. However, the communication pattern between microtubules and actin is not so clearly. According to previous reports, CRMPs can alter the polymerization of actin under cell membrane [31]. In the peripheral domain, actin polymerized into microfilaments which are transported to the central domain by axonal retrograde. This process on one hand stops the intrusion of microtubule into P-domain, on the other hand breaks microtubule to expose the plus end, changing the direction of microtubule assembly to make growth cone steer or form new neurite branch [40]. Our data suggest that CRMPs may function as structural regulator between microtubules and actin. CRMPs are also closely related to dendrite development, for example, genetic knockdown of CRMP3 induced the impairment of dendrite formation [41]. Whether and how CRMPs mediated cytoskeleton interaction modulate dendritic development remain to be further illustrated.

In summary, the current study reveals the colocalization and interaction of CRMPs with cytoskeleton in neurites and growth cones. CRMPs may function as a structural regulator between microtubules and actin, coordinating cytoskeleton dynamic to mediate growth cone and axon development. These findings provide new insights for the understanding of brain development.

Acknowledgements

The authors declare no conflict of interests. This work was supported by the National Natural Science Foundation of China (31170941), the Natural Science Foundation of Guangdong Province (S2013010014191 and 2014A030313357) and the Fundamental Research Funds for the Central Universities (21615423).

Disclosure of conflict of interest

None.

Address correspondence to: Hongsheng Lin, Department of Orthopedics, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China. E-mail: tlinhsh@jnu.edu.cn; linhongsheng_lhs@163.com; Guoqing Guo, Department of Anatomy, Medical College of Jinan University, Guangzhou 510630, China. E-mail: tgqguo@yahoo.com

References

[1] Dent EW, Gupton SL and Gertler FB. The growth cone cytoskeleton in axon outgrowth and guidance. Cold Spring Harb Perspect Biol 2011; 3.

[2] Gallo G and Letourneau P. Axon guidance: proteins turnover in turning growth cones. Curr Biol 2002; 12: R560-2.

[3] Geraldo S and Gordon-Weeks PR. Cytoskeletal dynamics in growth-cone steering. J Cell Sci 2009; 122: 3595-604.

[4] Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 2003; 5: 599-609.

[5] Ren XD, Kiosses WB and Schwartz MA. Regulation of the small GTP-binding protein Rho by

cell adhesion and the cytoskeleton. EMBO J 1999; 18: 578-85.
[6] Waterman-Storer CM, Worthylake RA, Liu BP, Burridge K, Salmon ED. Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. Nat Cell Biol 1999; 1: 45-50.
[7] Watabe-Uchida M, Govek EE and Van Aelst L. Regulators of Rho GTPases in neuronal development. J Neurosci 2006; 26: 10633-5.
[8] Cueille N, Blanc CT, Popa-Nita S, Kasas S, Catsicas S, Dietler G, Riederer BM. Characterization of MAP1B heavy chain interaction with actin. Brain Res Bull 2007; 71: 610-8.
[9] Watanabe T, Noritake J and Kaibuchi K. Roles of IQGAP1 in cell polarization and migration. Novartis Found Symp 2005; 269: 92-101; discussion 101-5, 223-30.
[10] Komarova Y, Lansbergen G, Galjart N, Grosveld F, Borisy GG, Akhmanova A. EB1 and EB3 control CLIP dissociation from the ends of growing microtubules. Mol Biol Cell 2005; 16: 5334-45.
[11] Suozzi KC, Wu X and Fuchs E. Spectraplakins: master orchestrators of cytoskeletal dynamics. J Cell Biol 2012; 197: 465-75.
[12] Kodama A, Lechler T and Fuchs E. Coordinating cytoskeletal tracks to polarize cellular movements. J Cell Biol 2004; 167: 203-7.
[13] Minturn JE, Fryer HJ, Geschwind DH, Hockfield S. TOAD-64, a gene expressed early in neuronal differentiation in the rat, is related to unc-33, a C. elegans gene involved in axon outgrowth. J Neurosci 1995; 15: 6757-66.
[14] Fukada M, Watakabe I, Yuasa-Kawada J, Kawachi H, Kuroiwa A, Matsuda Y, Noda M. Molecular characterization of CRMP5, a novel member of the collapsin response mediator protein family. J Biol Chem 2000; 275: 37957-65.
[15] Yuasa-Kawada J, Suzuki R, Kano F, Ohkawara T, Murata M, Noda M. Axonal morphogenesis controlled by antagonistic roles of two CRMP subtypes in microtubule organization. Eur J Neurosci 2003; 17: 2329-43.
[16] Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. Cell 2005; 120: 137-49.
[17] Ip JP, Fu AK and Ip NY. CRMP2: Functional Roles in Neural Development and Therapeutic Potential in Neurological Diseases. Neuroscientist 2014; 20: 589-98.
[18] Rosslenbroich V, Dai L, Baader SL, Noegel AA, Gieselmann V, Kappler J. Collapsin response mediator protein-4 regulates F-actin bundling. Exp Cell Res 2005; 310: 434-44.
[19] Tan M, Cha C, Ye Y, Zhang J, Li S, Wu F, Gong S, Guo G. CRMP4 and CRMP2 Interact to Coordinate Cytoskeleton Dynamics, Regulating Growth Cone Development and Axon Elongation. Neural Plast 2015; 2015: 947423.
[20] Seki T. Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents. J Neurosci Res 2002; 70: 327-34.
[21] Ji Z, Tan M, Gao Y, Zhang J, Gong X, Guo G, Lin H. CRMP-5 interacts with tubulin to promote growth cone development in neurons. Int J Clin Exp Med 2014; 7: 67-75.
[22] Higgins S, Lee JS, Ha L, Lim JY. Inducing neurite outgrowth by mechanical cell stretch. Biores Open Access 2013; 2: 212-6.
[23] Vitriol EA and Zheng JQ. Growth cone travel in space and time: the cellular ensemble of cytoskeleton, adhesion, and membrane. Neuron 2012; 73: 1068-81.
[24] Tucker KL, Meyer M and Barde YA. Neurotrophins are required for nerve growth during development. Nat Neurosci 2001; 4: 29-37.
[25] Conde C and Caceres A. Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci 2009; 10: 319-32.
[26] Piper M, Lee AC, van Horck FP, McNeilly H, Lu TB, Harris WA, Holt CE. Differential requirement of F-actin and microtubule cytoskeleton in cue-induced local protein synthesis in axonal growth cones. Neural Dev 2015; 10: 3.
[27] Quach TT, Honnorat J, Kolattukudy PE, Khanna R, Duchemin AM. CRMPs: critical molecules for neurite morphogenesis and neuropsychiatric diseases. Mol Psychiatry 2015; 20: 1037-45.
[28] Charrier E, Reibel S, Rogemond V, Aguera M, Thomasset N, Honnorat J. Collapsin response mediator proteins (CRMPs): involvement in nervous system development and adult neurodegenerative disorders. Mol Neurobiol 2003; 28: 51-64.
[29] Brot S, Smaoune H, Youssef-Issa M, Malleval C, Benetollo C, Besançon R, Auger C, Moradi-Améli M, Honnorat J. Collapsin response-mediator protein 5 (CRMP5) phosphorylation at threonine 516 regulates neurite outgrowth inhibition. Eur J Neurosci 2014; 40: 3010-20.
[30] Rajnicek AM, Foubister LE and McCaig CD. Growth cone steering by a physiological electric field requires dynamic microtubules, microfilaments and Rac-mediated filopodial asymmetry. J Cell Sci 2006; 119: 1736-45.
[31] Tang Y, Ye Z, Wei Y, Lin C, Wang Y, Qin C. Vertebrate paralogous CRMPs in nervous system: evolutionary, structural, and functional interplay. J Mol Neurosci 2015; 55: 324-34.
[32] Lowery LA and Van Vactor D. The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 2009; 10: 332-43.
[33] Arimura N, Ménager C, Kawano Y, Yoshimura T, Kawabata S, Hattori A, Fukata Y, Amano M,

Goshima Y, Inagaki M, Morone N, Usukura J, Kaibuchi K. Phosphorylation by Rho kinase regulates CRMP-2 activity in growth cones. Mol Cell Biol 2005; 25: 9973-84.

[34] Khazaei MR, Girouard MP, Alchini R, Ong Tone S, Shimada T, Bechstedt S, Cowan M, Guillet D, Wiseman PW, Brouhard G, Cloutier JF, Fournier AE. Collapsin response mediator protein 4 regulates growth cone dynamics through the actin and microtubule cytoskeleton. J Biol Chem 2014; 289: 30133-43.

[35] Brot S, Auger C, Bentata R, Rogemond V, Ménigoz S, Chounlamountri N, Girard-Egrot A, Honnorat J, Moradi-Améli M. Collapsin response mediator protein 5 (CRMP5) induces mitophagy, thereby regulating mitochondrion numbers in dendrites. J Biol Chem 2014; 289: 2261-76.

[36] Zhang Z, Ottens AK, Sadasivan S, Kobeissy FH, Fang T, Hayes RL, Wang KK. Calpain-mediated collapsin response mediator protein-1, -2, and -4 proteolysis after neurotoxic and traumatic brain injury. J Neurotrauma 2007; 24: 460-72.

[37] Kaselis A, Treinys R, Vosyliūtė R, Šatkauskas S. DRG axon elongation and growth cone collapse rate induced by Sema3A are differently dependent on NGF concentration. Cell Mol Neurobiol 2014; 34: 289-96.

[38] Yamagishi S, Yamada K, Sawada M, Nakano S, Mori N, Sawamoto K, Sato K. Netrin-5 is highly expressed in neurogenic regions of the adult brain. Front Cell Neurosci 2015; 9: 146.

[39] Yan Y, Cui H, Guo C, Wei J, Huang Y, Li L, Qin Q. Singapore grouper iridovirus-encoded semaphorin homologue (SGIV-sema) contributes to viral replication, cytoskeleton reorganization and inhibition of cellular immune responses. J Gen Virol 2014; 95: 1144-55.

[40] Mattila PK and Lappalainen P. Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 2008; 9: 446-54.

[41] Quach TT, Wilson SM, Rogemond V, Chounlamountri N, Kolattukudy PE, Martinez S, Khanna M, Belin MF, Khanna R, Honnorat J, Duchemin AM. Mapping CRMP3 domains involved in dendrite morphogenesis and voltage-gated calcium channel regulation. J Cell Sci 2013; 126: 4262-73.