Biochemical and Biophysical Research Communications 558 (2021) 36–43

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Down-regulated in renal cell carcinoma 1 (DRR1) regulates axon outgrowth during hippocampal neuron development

Fangjin Lu [a], Lin Zhu [b], Bin Mu [c], Xiaoyu Jia [b], Jiao Wang [b], Ping Mu [b, *]

[a] Department of Pharmacology, Shenyang Medical College, Shenyang, Liaoning, PR China
[b] Department of Biochemistry and Molecular Biology, Shenyang Medical College, Shenyang, Liaoning, PR China
[c] Shanghai Zhaohui Pharmaceutical Co. Ltd., Shanghai, PR China

ARTICLE INFO

Article history:
Received 11 April 2021
Accepted 12 April 2021
Available online 22 April 2021

Keywords:
Down-regulated in renal cell carcinoma 1
Tropomodulin-2
Axon outgrowth

ABSTRACT

Down-regulated in renal cell carcinoma 1 (DRR1), a unique stress-induced protein, is highly expressed in the nervous system. This study investigated the roles of DRR1 in the brain by examining its expression pattern at different developmental stages of a rat brain and in cultured primary hippocampal neurons. High expression of *DRR1* was observed in all developmental stages of a rat brain and cultured primary hippocampal neurons. We then focused on the role of DRR1 in promoting neurite outgrowth during the early stage of hippocampal neuron development. Results showed that down-regulation of *DRR1* suppressed axon outgrowth. Mass spectrometry analysis revealed that tropomodulin-2 (Tmod2) is a novel binding partner of DRR1. Our results showed that both DRR1 and Tmod2 mediate axon formation during the early stage of hippocampal neuron development. Suppression of *TMOD2* expression rescued the abnormal axon outgrowth induced by *DRR1* knockdown during the early stage of hippocampal neuron development.

1. Introduction

Down-regulated in renal cell carcinoma 1 (*DRR1*), also known as the family with sequence similarity 107 member A (*FAM107A*) or Tohoku University cDNA clone A on chromosome 3 (*TU3A*), spans a 10 kilobase (kb) region on human chromosome 3p21.1 [1,2]. *DRR1* produces a 3.5 kb transcript, which encodes a protein with 144 amino acid residues. Four different transcript variants of *DRR1* have been identified [1,2]. Homology analysis showed that human *DRR1* is highly homologous to *DRR1* from *Mus musculas*, *Rattus norvegicus*, *Bos taurus*, *Gallus gallus domesticus*, *Xenopus laevis*, and *Pongo pygmaeus* [3]. The presence of a coiled-coil domain and a nuclear localization signal in the DRR1 protein suggests that it could bind to other proteins and play an indispensable role in nuclear activities [2,4]. Given the reduced expression in diverse types of carcinomas, *DRR1* was initially considered as a tumor suppressor [5–9]. *DRR1* is expressed in various tissues; the highest expression was reported in the nervous system [1,2]. Apart from the neural progenitor cells in the embryonic cortex [10,11], expression of *DRR1* has also been observed in the cerebral cortex, hippocampus CA3 region, lateral septum, and cerebellum of adult mice [12]. Several studies have demonstrated that abnormal *DRR1* expression is significantly associated with several nervous system disorders such as bipolar disorder, autism spectrum disorder, schizophrenia, social defeat stress, and temporal lobe epilepsy [13–17]. DRR1 has been implicated in neuron survival, neuronal migration, and synapse formation [18–20]. Recent studies have demonstrated that *DRR1* is a stress-related and glucocorticoid-responsive gene. It contains glucocorticoid receptor response elements, which are directly regulated by glucocorticoids at the transcription level [12,20]. Although the role of DRR1 in the nervous system has been acknowledged, the underlying mechanism remains largely unclear. Hence, we aimed to investigate the role and underlying mechanism of DRR1 in the brain and cultured primary hippocampal neurons in this study.

2. Material and methods

2.1. Animals

All animal experiments were performed following the Animal Care and Use Guidelines of Shenyang Medical College and the

* Corresponding author. Department of Biochemistry and Molecular Biology, Shenyang Medical College, Shenyang, 110034, Liaoning, PR China.
E-mail addresses: pingmu@symc.edu.cn, mpyjr1977@sina.com (P. Mu).

https://doi.org/10.1016/j.bbrc.2021.04.042
0006-291X/

experimental procedures were approved by the Institutional Animal Care and Use Committee of Shenyang Medical College (approval no. SYYXY-2019050801). Rats of both sexes were used for all experiments.

2.2. Plasmids

Non-targeting short hairpin RNA (shRNA) and specific shRNAs against rat *DRR1* and *TMOD2* were obtained from Origene (Rockville, MD, USA). Information on shRNAs is shown in Supplementary Table 1. CMV-DRR1-Venus was generated by flanking the polymerase chain reaction (PCR) fragments of human *DRR1* complementary DNA (cDNA) and then inserting it into CSII-CMV-Venus (RIKEN BioResource Center, Tsukuba, Japan) using *Not*I and *Bam*HI restriction sites. DRR1-glutathione S-transferase (GST) was generated by inserting mouse *DRR1* cDNA into pGEX-6P-2 vector (Amersham Biosciences MA, USA) using *Bam*HI and *Eco*RI restriction sites.

2.3. Primary hippocampal neuron culture and transfection

Primary hippocampal neurons were isolated from E18-day-old Sprague Dawley rat embryos. Hippocampi were isolated, cut into small pieces, and dissociated using the papain dissociation system (Worthington Biochemical Corporation, Lakewood, NJ, USA), according to the manufacturers' protocol. The tissues were incubated in papain solution (20 units/ml papain and 0.005% deoxyribonuclease) at 37 °C for 30 min with trituration using a 1 ml pipette every 10 min. The cloudy suspension was carefully collected in a new tube and centrifuged (1000 rpm) for 5 min at room temperature. The supernatant was discarded and the pellet was immediately resuspended in albumin ovomucoid inhibitor solution and centrifuged (1000 rpm) for 5 min at room temperature. The supernatant was discarded and the cell pellet was resuspended in the cell culture medium. Cells were counted and seeded on plates coated with poly-D-lysine (Sigma-Aldrich, St. Louis, MO, USA). Cells were cultured in Neurobasal medium (Invitrogen, Carlsbad, CA, USA) supplemented with 2% B-27 (Invitrogen) and 0.5 mM glutamine at 37 °C in a humidified chamber with 95% air and 5% carbon dioxide. Transfection experiment with shRNA was performed 6 h after cell seeding using FuGENE HD (Promega, Madison, USA).

2.4. Reverse transcription and quantitative real-time PCR

Different timed-pregnant Sprague Dawley rats (E12, E18) and ones with different postnatal days (P0, P7, P14, P21, and P56) were purchased from Changsheng Bio-Technology (Shenyang, China). Total RNA from the brain and cultured hippocampal neurons was extracted using ISOGEN (Nippon Gene, Tokyo, Japan). RNA extraction, reverse transcription (RT), and quantitative real-time PCR were carried out as previously described [18]. Primer details are shown in Supplementary Table 2.

2.5. Immunofluorescence staining

Immunofluorescence staining was performed as previously described [18]. Briefly, cells were washed thrice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS for 15 min at room temperature. The cells were then incubated with blocking solution (5% normal goat serum and 0.3% Triton X-100 in PBS) at room temperature for 1 h. The cells were then washed thrice with PBS and incubated with indicated primary antibodies in blocking solution for 1 h at room temperature. The cells were again washed thrice with PBS and incubated with appropriate secondary antibodies diluted in blocking solution for 1 h at room temperature. The slides were washed with PBS and mounted using the mounting medium (Vector Laboratories, CA, USA). Images were acquired using the Olympus BX41-33 microscope (Olympus, Tokyo, Japan). The following primary antibodies were used: anti-DRR1 (Wanleibio, Shenyang, China), anti-microtubule-associated protein 2 (MAP2) (Merck Millipore, Darmstadt, Germany), anti-tau (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-TMOD2 (Sigma-Aldrich).

2.6. GST pull down assay

DRR1-GST and control plasmid pGEX-6P-2 were transformed into *Escherichia coli* BL21 for protein expression. The recombinant proteins were collected by lysing the cells with lysis buffer (50 mM Tris, 150 mM sodium chloride, 1% Triton X-100, 1% protease inhibitor, 1 mM phenylmethylsulfonyl fluoride). The GST pull down experiment was performed using 1 ml Pierce™ Glutathione Spin Columns (Thermo Fisher, Waltham, MA, USA) according to the manufacturers' protocol. The protein complex was eluted with 5 mM reduced glutathione. The eluate was collected and purified using the AKTA-FPLC protein purification system (GE Healthcare, Chicago, IL, USA). The purified protein was then visualized by sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie staining. The eluate was finally analyzed using the *LTQ* Orbitrap XL *mass* spectrometry system (Thermo Fisher).

2.7. Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were performed as previously described [4]. Briefly, cell lysates were incubated with the indicated antibody at 4 °C for 4 h, mixed with protein G Sepharose (GE Healthcare), and then incubated for another 2 h. The mixture was centrifuged (15000 rpm) at 4 °C for 10 min and washed thrice with cold PBS. After the last wash, the sample buffer was added to the pellet and the sample was boiled for 5 min. The solution was again centrifuged (15000 rpm) at 4 °C for 10 min and the supernatant was collected for Western blot analysis. The following antibodies were used for immunoprecipitation and immunoblotting: anti-green fluorescent protein antibody (anti-GFP; MBL, Nagoya, Japan) and anti-hemagglutinin antibody (anti-HA; Sigma-Aldrich).

2.8. Statistical analysis

Statistical analysis was performed using the GraphPad 5.0 software (GraphPad Software, San Diego, CA, USA). Comparison between the two groups was performed using the Student's *t*-test and data are presented as mean ± standard deviation. The differences were considered statistically significant for p-values < 0.05.

3. Results

3.1. DRR1 expression in rat brain and cultured primary hippocampal neurons

Quantitative real-time PCR analysis of RNA isolated from rat brains at different developmental stages (E12, E18, P0, P7, P14, P21, and P56) showed that *DRR1* was expressed in the E12 rat brain, a very early stage of brain development. Expression of *DRR1* gradually increased from E18 and continued to increase until adulthood (Fig. 1A).

Next, we cultured primary hippocampal neurons in vitro. The cultured primary hippocampal neurons exhibit different morphology on different culture days, which represent five different developmental stages [21] (Fig. 1B). We checked the expression of *DRR1* in these cultured neurons at different

developmental stages using real-time PCR (Fig. 1C). Results revealed that *DRR1* was expressed at stage I of primary hippocampal neuron development and the expression increased steadily until stage V. These results were consistent with those obtained from rat brain analysis. Taken together, these results indicate that *DRR1* is expressed very early during neuronal development and the expression is maintained until maturation, implying that DRR1 might play important roles at all neuronal developmental stages.

3.2. DRR1 regulates axon outgrowth during the early stage of hippocampal neuron development

The actin cytoskeleton regulates several neuronal processes such as dendrite development, spine formation, and synapse function [22]. Actin-related proteins regulate actin dynamics and play a critical role in the development and functioning of the brain [23]. As an actin binding protein, DRR1 plays a significant role in spine formation and synaptic function. However, the role of DRR1 during the early stage of neuron development has not been studied. We examined the localization of DRR1 in cultured stage IV primary hippocampal neurons and found that DRR1 is localized in both MAP2-positive dendrites and tau-positive axons (Fig. 2A). To assess the role of DRR1 during the early stage of neuron development, we transfected *DRR1* shRNA-GFP and control shRNA-GFP into cultured primary hippocampal neurons (6 h after seeding) and examined neurite formation after four days (Fig. 2B). Compared to the control group, *DRR1* shRNA transfected neurons showed a reduction in the length of axon (Fig. 2B and C). No significant difference was found in the length of dendrites between *DRR1* shRNA-GFP group and control shRNA-GFP group (Fig. 2D). These results indicate that DRR1 plays an important role in axon outgrowth during neuron development.

3.3. Tmod2 is a binding partner of DRR1

Sequence analysis showed that DRR1 protein contains a coiled-coil domain [2], which might allow it to interact with other proteins. To screen for the binding partners of DRR1, we performed GST pull down experiments followed by mass spectrometry analysis. Several candidate binding partners of DRR1 were screened in the mouse brain lysates. After excluding the known binding partner-actin, Tmod2 exhibited the highest score in the data of mass spectrometry analysis (Supplementary Table 3).

To validate the results of GST pull down experiments, *DRR1*-Venus and *TMOD2*-HA were co-expressed in HEK293T cells and immunoprecipitation was performed with the indicated antibodies. Results confirmed that DRR1 binds to Tmod2 (Fig. 3A). We then examined the expression profile of *TMOD2* in the rat brain and cultured primary hippocampal neurons. Similar to *DRR1*, *TMOD2* was expressed at all developmental stages of the rat brain and cultured primary hippocampal neurons. However, in contrast to *DRR1*, *TMOD2* expression was relatively stable from E18 to

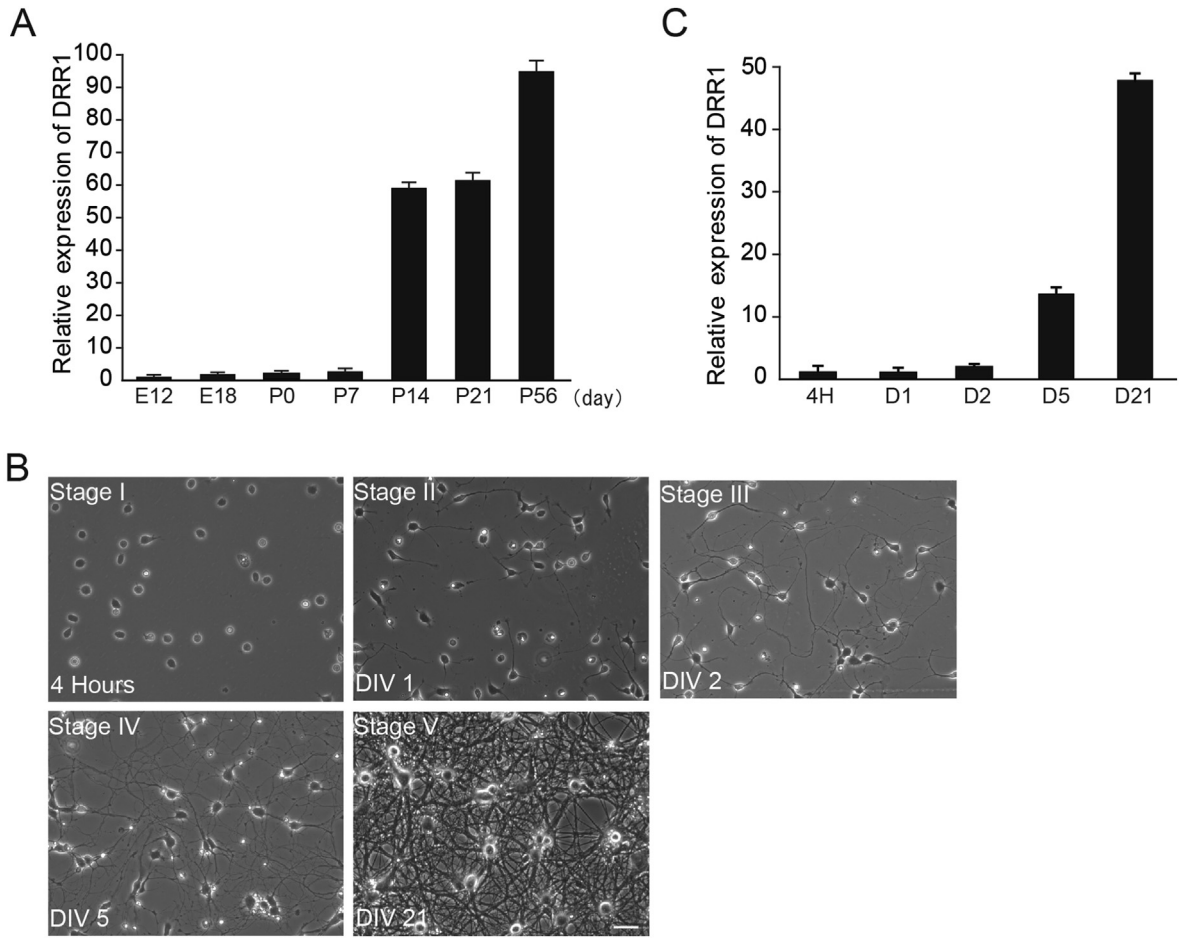

Fig. 1. *DRR1* expression in rat brain and cultured primary hippocampal neurons. (A) mRNA level of *DRR1* in different developmental stages of rat brain was examined by real-time PCR. n = 3. (B) Phase contrast images of cultured primary hippocampal neurons in different developmental stages. DIV: day in vitro. Scale bar: 50 μm. (C) mRNA level of DRR1 in cultured primary hippocampal neurons at different developmental stages was examined by real-time PCR. n = 3.

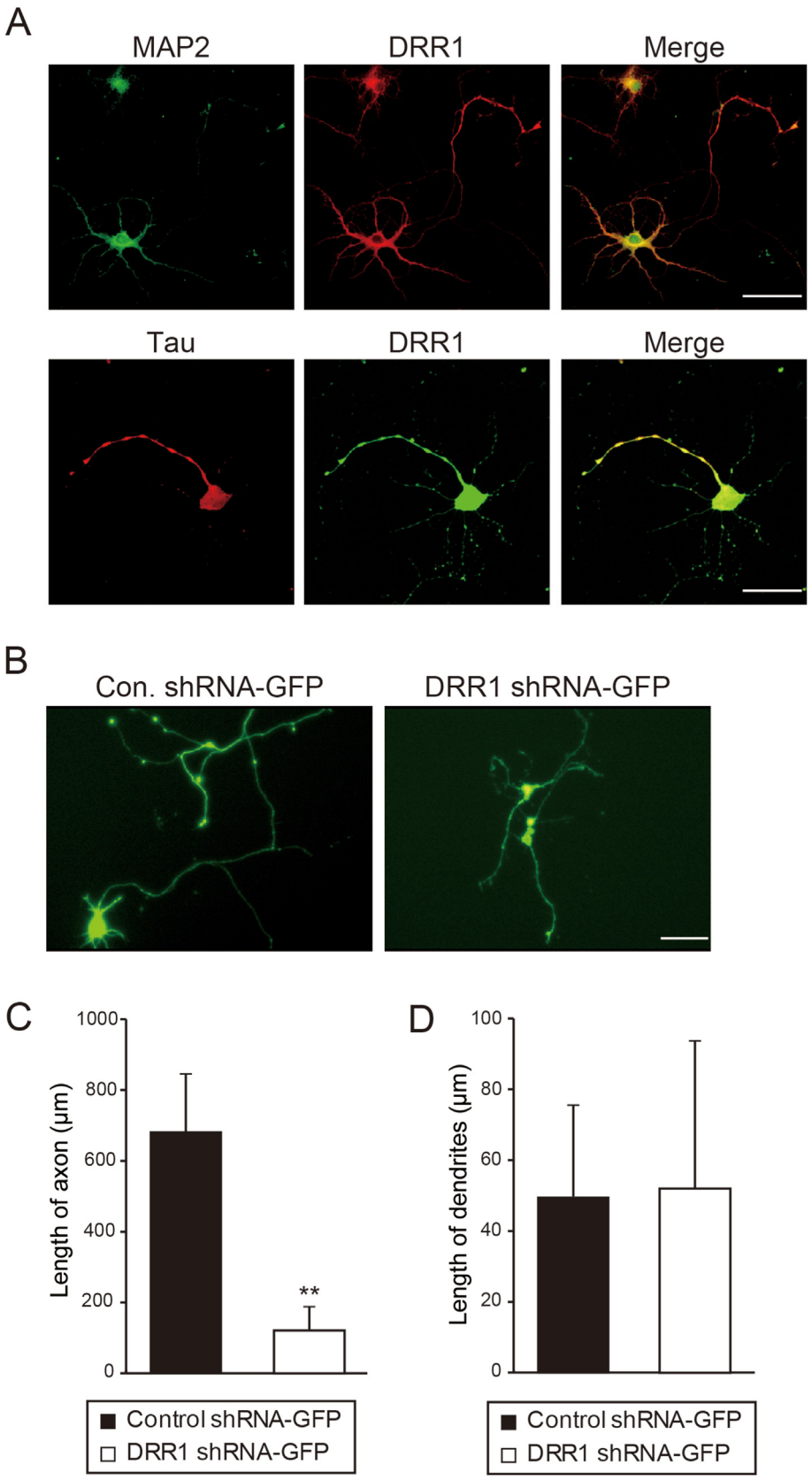

Fig. 2. Down-regulation of *DRR1* expression suppressed axon formation in the early developmental stage of cultured primary hippocampal neurons. (A) Microscopic analysis of DRR1 (red) and microtubule-associated protein 2 (MAP2, green) in cultured primary hippocampal neurons (upper panel). Microscopic analysis of DRR1 (green) and tau (red) in cultured primary hippocampal neurons (lower panel). Fluorescence images were taken on day 3 after hippocampal neuron seeding. Scale bar: 50 μm. (B) Control shRNA-GFP or *DRR1* shRNA-GFP was transfected into primary hippocampal neurons 6 h after seeding. Fluorescence images were taken on day 4 after transfection. Scale bar: 50 μm. (C) Length of axon was examined in 5 hippocampal neurons treated with control shRNA-GFP or *DRR1* shRNA-GFP. $^{**}p < 0.01$, versus control shRNA. (D) Length of dendrites was examined in 5 hippocampal neurons treated with control shRNA-GFP or *DRR1* shRNA-GFP. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

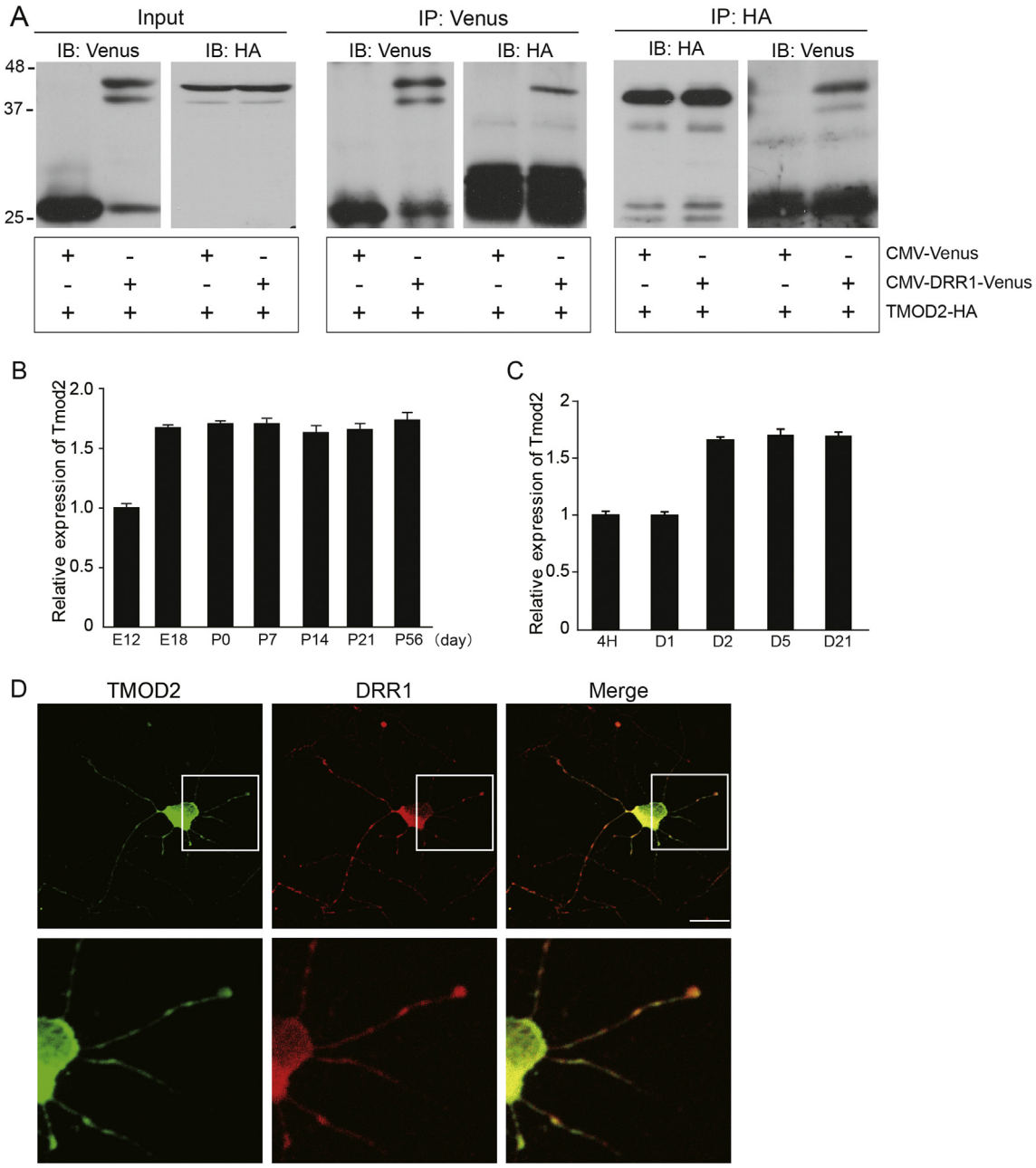

Fig. 3. Tmod2 is a binding partner of DRR1. (A) HEK293T cells transfected with CMV-Venus, CMV-*DRR1*-Venus, and pCDNA3.1-*TMOD2*-HA were lysed using Tris buffer and subjected to immunoprecipitation and western blotting with the indicated antibodies. (B) mRNA level of *TMOD2* in different developmental stages of rat brain was examined by real-time PCR. n = 3. (C) mRNA level of *TMOD2* in cultured primary hippocampal neurons at different developmental stages was examined by real-time PCR. n = 3. (D) Microscopic analysis of endogenous DRR1 (red) and endogenous TMOD2 (green) in primary hippocampal neurons. The lower panel shows enlarged images of the upper panel. Fluorescence images were taken on day 3 after seeding. Scale bar: 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

adulthood in the rat brain (Fig. 3B) and from stage III to stage V in cultured primary hippocampal neurons (Fig. 3C). Immunofluorescence staining of cultured stage IV primary hippocampal neurons demonstrated co-expression of *DRR1* and *TMOD2* (Fig. 3D).

3.4. Suppression of TMOD2 interrupts with the effect of DRR1 shRNA in cultured primary hippocampal neurons

To investigate the relationship between DRR1 and Tmod2 in cultured primary hippocampal neurons, cells were treated with *TMOD2* shRNA-red fluorescent protein (RFP) 6 h after cell seeding. Four days after transfection, *TMOD2* shRNA-RFP-treated cells showed a slight increase in the length of axon compared to that in the control group (Fig. 4A and B). No significant difference was found in the length of dendrites between *TMOD2* shRNA-RFP group and control shRNA-RFP group (Fig. 4C).

To assess the role of DRR1 and Tmod2 in regulating axon outgrowth, *DRR1* shRNA-GFP and *TMOD2* shRNA-RFP were co-transfected into primary hippocampal neurons. Four days after transfection, the length of axon was measured. Cells treated with *DRR1* shRNA-GFP and control shRNA-RFP showed a reduction in the length of axon when compared to that of the control shRNA-GFP

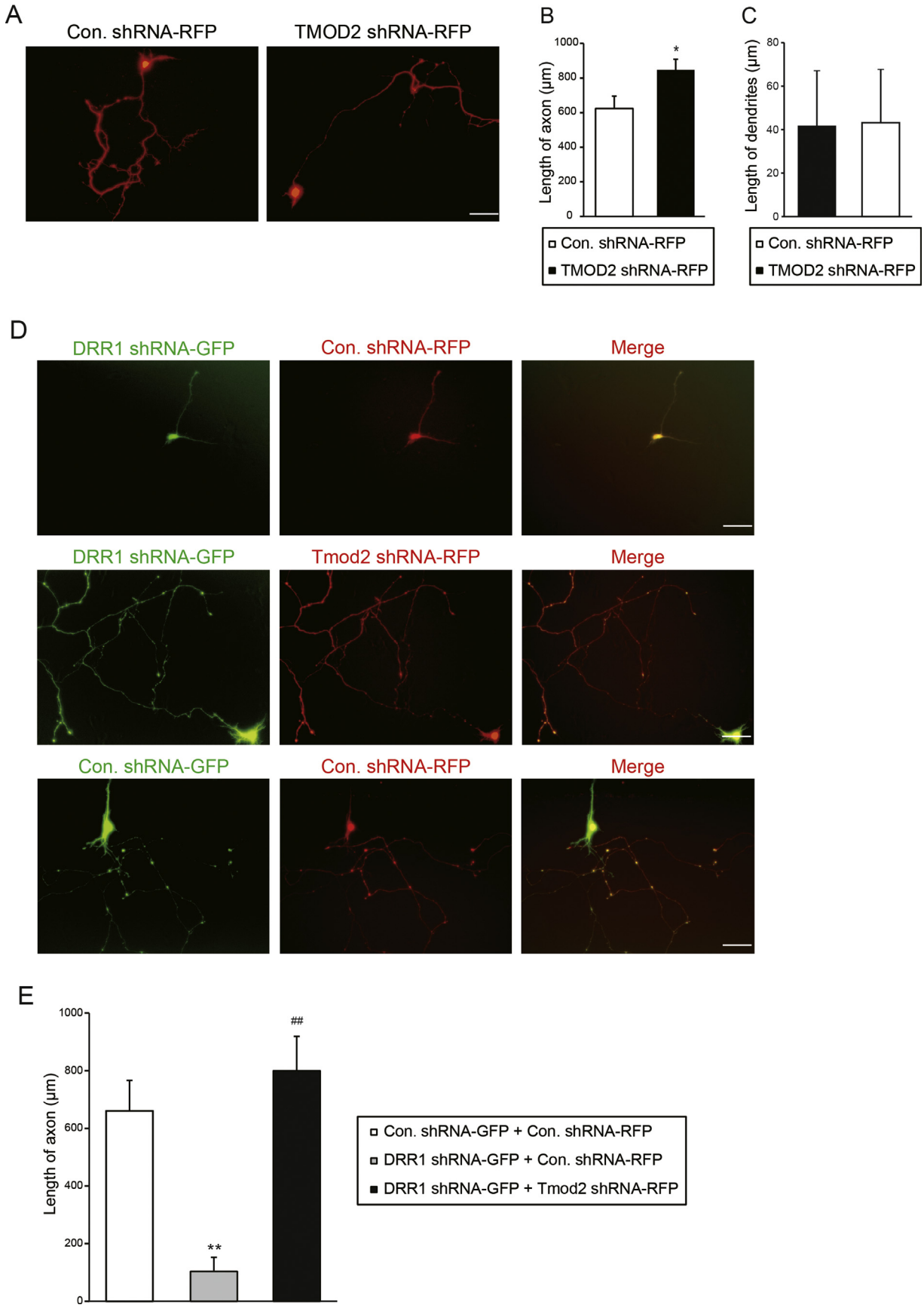

Fig. 4. Down-regulation of *TMOD2* rescues the *DRR1* shRNA-induced abnormal axon outgrowth. (A) Control shRNA-RFP or *TMOD2* shRNA-RFP was transfected into cultured primary hippocampal neurons 6 h after seeding. Fluorescence images were taken on day 4 after transfection. Scale bar: 50 μm. (B) The length of axon was examined in 5 hippocampal neurons treated with control shRNA-RFP or *TMOD2* shRNA-RFP. $^{*}p < 0.05$ versus control shRNA-RFP. (C) The length of dendrites was examined in 5 hippocampal neurons treated with control shRNA-RFP or *TMOD2* shRNA-RFP. (D) Cultured primary hippocampal neurons were transfected with the indicated shRNA 6 h after seeding. Fluorescence images were taken on day 4 after transfection. Scale bar: 50 μm. (E) The length of axon was examined in 5 hippocampal neurons treated with the indicated shRNA. $^{**}p < 0.01$ versus control shRNA-GFP and control shRNA-RFP group. $^{\#\#}p < 0.01$ versus *DRR1* shRNA-GFP and control shRNA-RFP group.

and control shRNA-RFP groups. However, in contrast to the control shRNA treated cells, the ones treated with both *DRR1* shRNA-GFP and *TMOD2* shRNA-RFP showed similar length of axon (Fig. 4D and E).

4. Discussion

Several studies reported multiple roles of DRR1 in the nervous system. In this study, a series of experiments were performed to examine the expression profile and functions of endogenous DRR1 in cultured primary hippocampal neurons. DRR1 expression was reported in both early and late developmental stages of hippocampal neurons, indicating the involvement of DRR1 in several biological processes such as early neurite development, neurite maintenance, and synapse formation. Previous studies demonstrated that DRR1 regulates protrusion density and shape of the synapse by affecting actin dynamics in the presynapse [20]. We specifically focused on the function of DRR1 during early neurite development. Results showed that in cultured primary hippocampal neurons, down-regulation of *DRR1* suppresses axon outgrowth in the early developmental stage.

Actin cytoskeleton is crucial for multiple neuronal functions such as neuronal differentiation and outgrowth, cell morphology maintenance, and synaptic function [22–24]. Studies reported that dysfunctional actin dynamics results in several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and polyglutamine repeat diseases [25,26]. In the present study, we found that DRR1 regulates early axon outgrowth in hippocampal neurons. DRR1 is an actin-related protein, which promotes F-actin bundling and affects synapse formation and long-term potentiation [20]. Considering the role of actin in neurite outgrowth, it is anticipated that DRR1 may affect axon outgrowth by mediating actin dynamics.

To uncover the underlying mechanism, we performed the GST pull down experiment to screen for DRR1-interacting proteins. Mass spectrometry analysis identified Tmod2 as the candidate DRR1-interacting protein. Subsequent experiments confirmed the interaction between DRR1 and Tmod2, and showed the co-localization of these proteins in the neurites of cultured primary hippocampal neurons.

Tmod is a family of actin-binding proteins comprising four members (Tmod1, Tmod2, Tmod3, and Tmod4) [27]. Tmod1 and Tmod3 show high expression in various tissues, while Tmod2 and Tmod4 are specifically expressed in the nervous system and skeletal muscles, respectively [27]. Tmods regulate actin dynamics by capping the pointed end of actin filaments [28]. Tmod2 has been shown to regulate neurite extension and spine density in neurons [29].

Previous studies have shown that overexpression of *DRR1* results in decreased spine density [20], while overexpression of *TMOD2* increases the spine density [29]. Given that DRR1 and Tmod2 are actin-binding proteins and are associated with regulation of F-actin dynamics, we hypothesized that DRR1 and Tmod2 may affect actin dynamics differently. Our results showed opposite effects of DRR1 and Tmod2 knockdown on early axon formation in hippocampal neurons; down-regulation of *DRR1* suppresses axon outgrowth, while down-regulation of *TMOD2* promotes axon outgrowth. These results indicate the differential regulation of actin dynamics by DRR1 and Tmod2. Additionally, experiments also demonstrated that down-regulation of *TMOD2* rescues the abnormal axon formation induced by *DRR1* shRNA, suggesting that DRR1 in association with Tmod2 regulates axon formation by affecting the actin dynamics. However, further studies are needed to understand the role of DRR1 and Tmod2 in regulating actin dynamics in hippocampal neurons, and the function of DRR1-actin-Tmod2 complex during hippocampal neuron development.

Declaration of competing interest

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

Acknowledgments

This work was financially supported by the Liaoning Province "Xingliao talent" program in China (XLYC1807116), Liaoning Provincial Natural Science Foundation of China (20180550491, 2019-ZD-0323), and Shenyang Medical College, Shenyang, China (20184053). The funds were not utilized in the study design; data collection, analysis, and interpretation of data; writing of the report; and decision making to submit the article for publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2021.04.042.

References

[1] T. Yamato, K. Orikasa, S. Fukushige, et al., Isolation and characterization of the novel gene, TU3A, in a commonly deleted region on 3p14.3→p14.2 in renal cell carcinoma, Cytogenet, Cell Genet 87 (1999) 291–295, https://doi.org/10.1159/000015452.

[2] L. Wang, J. Darling, J.S. Zhang, et al., Loss of expression of the DRR1 gene at chromosomal segment 3p21.1 in renal cell carcinoma, Genes Chromosomes Cancer 27 (2000) 1–10, https://doi.org/10.1002/(SICI)1098-2264(200001)27:1<1::AID-GCC1>3.0.CO;2-6.

[3] X.Y. Zhao, S.F. Liang, S.H. Yao, et al., Identification and preliminary function study of Xenopus laevis DRR1 gene, Biochem. Biophys. Res. Commun. 361 (2007) 74–78, https://doi.org/10.1016/j.bbrc.2007.06.158.

[4] P. Mu, T. Akashi, F. Lu, et al., A novel nuclear complex of DRR1, F-actin and COMMD1 involved in NF-κB degradation and cell growth suppression in neuroblastoma, Oncogene 36 (2017) 5745–5756, https://doi.org/10.1038/onc.2017.181.

[5] I.D. Kholodnyuk, S. Kozireva, M. Kost-Alimova, et al., Down regulation of 3p genes, LTF, SLC38A3 and DRR1, upon growth of human chromosome 3-mouse fibrosarcoma hybrids in severe combined immunodeficiency mice, Int. J. Canc. 119 (2006) 99–107, https://doi.org/10.1002/ijc.21794.

[6] A. Lawrie, S. Han, A. Sud, et al., Combined linkage and association analysis of classical Hodgkin lymphoma, Oncotarget 9 (2018) 20377–20385, https://doi.org/10.18632/oncotarget.24872.

[7] J. Van Den Boom, M. Wolter, B. Blaschke, et al., Identification of novel genes associated with astrocytoma progression using suppression subtractive hybridization and real-time reverse transcription-polymerase chain reaction, Int. J. Canc. 119 (2006) 2330–2338, https://doi.org/10.1002/ijc.22108.

[8] D. Pastuszak-Lewandoska, K.H. Czarnecka, M. Migdalska-Sęk, et al., Decreased FAM107A expression in patients with non-small cell lung cancer, Adv. Exp. Med. Biol. 852 (2015) 39–48, https://doi.org/10.1007/5584_2014_109.

[9] S. Udali, P. Guarini, A. Ruzzenente, et al., DNA methylation and gene expression profiles show novel regulatory pathways in hepatocellular carcinoma, Clin. Epigenet. 7 (2015) 43, https://doi.org/10.1186/s13148-015-0077-1.

[10] A.A. Pollen, T.J. Nowakowski, J. Chen, et al., Molecular identity of human outer radial glia during cortical development, Cell 163 (2015) 55–67, https://doi.org/10.1016/j.cell.2015.09.004.

[11] X. Zhou, S. Zhong, H. Peng, et al., Cellular and molecular properties of neural progenitors in the developing mammalian hypothalamus, Nat. Commun. 11 (2020) 4063, https://doi.org/10.1038/s41467-020-17890-2.

[12] M. Masana, S. Westerholz, A. Kretzschmar, et al., Expression and glucocorticoid-dependent regulation of the stress-inducible protein DRR1 in the mouse adult brain, Brain Struct. Funct. 223 (2018) 4039–4052, https://doi.org/10.1007/s00429-018-1737-7.

[13] A.M. Stankiewicz, J. Goscik, A.H. Swiergiel, et al., Social stress increases expression of hemoglobin genes in mouse prefrontal cortex, BMC Neurosci. 15 (2014) 130, https://doi.org/10.1186/s12868-014-0130-6.

[14] T. Jene, N.C. Gassen, V. Opitz, et al., Temporal profiling of an acute stress-induced behavioral phenotype in mice and role of hippocampal DRR1, Psychoneuroendocrinology 91 (2018) 149–158, https://doi.org/10.1016/j.psyneuen.2018.03.004.

[15] M. Masana, Y.A. Su, C. Liebl, et al., The stress-inducible actin-interacting protein DRR1 shapes social behavior, Psychoneuroendocrinology 48 (2014) 98–110, https://doi.org/10.1016/j.psyneuen.2014.06.009.

[16] L. Shao, M.P. Vawter, Shared gene expression alterations in schizophrenia and bipolar disorder, Biol. Psychiatr. 64 (2008) 89–97, https://doi.org/10.1016/j.biopsych.2007.11.010.

[17] A. Quartier, L. Chatrousse, C. Redin, et al., Genes and pathways regulated by androgens in human neural cells, potential candidates for the male excess in autism spectrum disorder, Biol. Psychiatr. 84 (2018) 239–252, https://doi.org/10.1016/j.biopsych.2018.01.002.
[18] Y. Asano, S. Kishida, P. Mu, et al., DRR1 is expressed in the developing nervous system and downregulated during neuroblastoma carcinogenesis, Biochem. Biophys. Res. Commun. 394 (2010) 829–835, https://doi.org/10.1016/j.bbrc.2010.03.085.
[19] H. Nakajima, K. Koizumi, Family with sequence similarity 107: a family of stress responsive small proteins with diverse functions in cancer and the nervous system (Review), Biomed. Rep. 2 (2014) 321–325, https://doi.org/10.3892/br.2014.243.
[20] M.V. Schmidt, J.P. Schülke, C. Liebl, et al., Tumor suppressor down-regulated in renal cell carcinoma 1 (DRR1) is a stress-induced actin bundling factor that modulates synaptic efficacy and cognition, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 17213–17218, https://doi.org/10.1073/pnas.1103318108.
[21] A.P. Barnes, F. Polleux, Establishment of axon-dendrite polarity in developing neurons, Annu. Rev. Neurosci. 32 (2009) 347–381, https://doi.org/10.1146/annurev.neuro.31.060407.125536.
[22] C.K. Frese, M. Mikhaylova, R. Stucchi, et al., Quantitative map of proteome dynamics during neuronal differentiation, Cell Rep. 18 (2017) 1527–1542, https://doi.org/10.1016/j.celrep.2017.01.025.
[23] K.C. Flynn, F. Hellal, D. Neukirchen, et al., ADF/cofilin-mediated actin retrograde flow directs neurite formation in the developing brain, Neuron 76 (2012) 1091–1107, https://doi.org/10.1016/j.neuron.2012.09.038.
[24] L.A. Cingolani, Y. Goda, Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy, Nat. Rev. Neurosci. 9 (2008) 344–356, https://doi.org/10.1038/nrn2373.
[25] Z. Yan, E. Kim, D. Datta, et al., Synaptic actin dysregulation, a convergent mechanism of mental disorders? J. Neurosci. 36 (2016) 11411–11417, https://doi.org/10.1523/JNEUROSCI.2360-16.2016.
[26] R. Kumari, Y. Jiu, P.J. Carman, et al., Tropomodulins control the balance between protrusive and contractile structures by stabilizing actin-tropomyosin filaments, Curr. Biol. 30 (2020) 767–778, https://doi.org/10.1016/j.cub.2019.12.049, e5.
[27] S. Yamashiro, D.S. Gokhin, S. Kimura, et al., Tropomodulins: pointed-end capping proteins that regulate actin filament architecture in diverse cell types, Cytoskeleton 69 (2012) 337–370, https://doi.org/10.1002/cm.21031.
[28] O.F. Omotade, Y. Rui, W. Lei, et al., Tropomodulin isoform-specific regulation of dendrite development and synapse formation, J. Neurosci. 38 (2018) 10271–10285, https://doi.org/10.1523/JNEUROSCI.3325-17.2018.
[29] Z. Hu, D. Yu, Q.H. Gu, et al., MiR-191 and miR-135 are required for long-lasting spine remodelling associated with synaptic long-term depression, Nat. Commun. 5 (2014) 3263, https://doi.org/10.1038/ncomms4263.