JCB Journal of Cell Biology

SPOTLIGHT

Plastin 3 rescues BDNF-TrkB signaling in spinal muscular atrophy

Arren Ramsey[1] and Eric J. Huang[1,2]

In this issue, Hennlein and colleagues (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202204113) show that F-actin–bundling protein Plastin 3 is drastically reduced in motor neurons with spinal muscular atrophy, whereas virus-mediated overexpression of Plastin 3 restores actin cytoskeleton and promotes BDNF–TrkB signaling in the growth cones of spinal motor neurons.

Spinal muscular atrophy (SMA) is a common motor neuron disease in children and young adults caused by mutations in the gene *survival of motor neuron 1* (*SMN1*; 1, 2). Deficiency in SMN1 leads to immature and degenerative motor neurons in the spinal cord, with motor neurons exhibiting features of decreased growth cone size, shorter axons, and disrupted clustering of voltage-gated channel $Ca_v2.2$ (3). Previous work has shown that Plastin 3 (PLS3) can increase F-actin level and rescue the axonogenesis phenotypes in the motor neurons of SMA mice (4). Furthermore, deficiency in Tropomyosin receptor kinase B (TrkB), the receptor for brain-derived neurotrophic factor (BDNF), in motor neurons phenocopies the impaired differentiation and Ca^{2+} transient phenotypes in SMA motor neurons (5). However, the exact cause for the impaired BDNF–TrkB signaling in SMA remains unclear.

Reporting in this issue of *JCB*, Hennlein and colleagues began with the observation that TrkB was reduced in the neuromuscular junction (NMJ) of *Transversus abdominis anterior* (TVA) muscle in the *SMNΔ7* SMA model mice, as well as in the growth cones of cultured primary motor neurons from the embryos of another SMA model mouse line, *Smn*$^{-/-}$;*SMN2* (6). However, Western blot analysis using whole-cell lysates did not show a difference in total TrkB protein levels, suggesting the impaired TrkB signaling was likely due to mislocalization, rather than reduced expression of TrkB receptors. As TrkB is a kinase stimulated by BDNF (7), the authors treated the TVA NMJ and primary motor neurons with BDNF. While control motor neurons showed an increase in phosphorylatedTrkB (p-TrkB), *SMNΔ7* and *Smn*$^{-/-}$;*SMN2* motor neurons exhibited no change. Furthermore, treatment of control motor neurons with the actin polymerization inhibitor Cytochalasin D (CytoD) prior to BDNF treatment resulted in impaired p-TrkB levels similar to that observed in SMA motor neurons. To determine whether recycling of TrkB is defective in SMA motor neurons, the authors labeled TrkB on the cell surface of live motor neurons and showed that treatment with BDNF triggered endocytosis of TrkB in both control and SMA motoneurons. However, treatment with cyclic AMP (cAMP) stimulated the return of labeled TrkB to the surface in control motor neurons, but not in SMA motor neurons. Similar TrkB recycling phenotypes were observed when control motor neurons were treated with CytoD. Together, these results suggest that cytoskeletal defects may account for the TrkB recycling phenotypes in SMA motor neurons.

To uncover the mechanism of impaired TrkB signaling in SMA motor neurons, the authors conducted transcriptomic analyses using total RNA from the somatodendridic and axonal compartments of SMA motor neurons, which were separated using compartmentalized microfluidic chambers. This approach identified multiple actin-related genes downregulated in the axonal compartment, including *Pls3* and *Arpc1b*, which encode PLS3 and Arp2/3 that regulate actin filament bundling and branching, respectively (8–10). To characterize the role of PLS3 in motor neurons, the authors used a shRNA approach (sh*Pls3*) to knock down the endogenous PLS3. Indeed, knocking down PLS3 in control motor neurons recapitulated key phenotypes in SMA motor neurons, including impaired actin movement in the growth cones and reduced BDNF- and cAMP-induced TrkB activation and recruitment (Fig. 1). Structural illumination microscopy (SIM) revealed that sh*Pls3*-treated control motor neurons exhibited a significant reduction in the formation of $Ca_v2.2$ "cluster-like" accumulations and Ca^{2+} transients in the axons, similar to SMA motor neurons. Conversely, overexpression of PLS3 via lentivirus-mediated transduction of human PLS3 (LV-h*PLS3*) rescued several key phenotypes in SMA motor neurons, including BDNF-mediated TrkB activation, cAMP-induced TrkB recruitment, and $Ca_v2.2$ cluster-like formations. Additionally, the authors used SIM to

[1]Department of Pathology, University of California, San Francisco, San Francisco, CA, USA; [2]Pathology Service 113B, San Francisco Veterans Administrations Health Care System, San Francisco, CA, USA.

Correspondence to Eric J. Huang: eric.huang2@ucsf.edu.

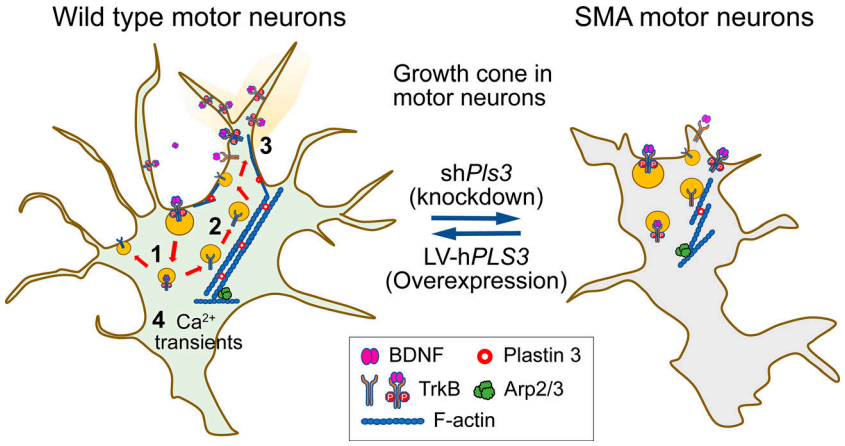

Figure 1. **PLS3 regulates growth cone's response to BDNF.** In wild-type spinal motor neurons, F-actin–bundling protein PLS3 and actin branching protein Arp2/3 cooperatively regulate a balanced actin cytoskeleton to support TrkB receptor recycling (1), recruitment along F-actin (2), and signal amplification (3), and Ca^{2+} transients along growth cones. In contrast, PLS3 and Arp2/3 are significantly downregulated in the axons of motor neurons in SMA, leading to a drastic reduction in BDNF–TrkB signaling.

examine the role of Arp2/3 in TrkB recycling in the growth cones of motor neurons. This approach showed that PLS3 was distributed all over the growth cones with enrichment in the F-actin–rich protrusions, whereas Arp2/3 was mainly located in the core of growth cones (Fig. 1). Interestingly, knocking down PLS3 significantly reduced Arp2/3 in the growth cones, whereas overexpression of PLS3 restored Arp2/3 signal to the control level in SMA motor neurons. Furthermore, treatment with CK-666, an Arp2/3 inhibitor, prevented the recruitment of TrkB and abolished the effect of LV-h*PLS3* in rescuing SMA motor neurons. Finally, the authors showed that the level of profilin, which regulates actin polymerization, was reduced in SMA motor neurons and that overexpression of PLS3 restored BDNF-induced phosphorylation of profilin (Fig. 1). These results support that PLS3 and Arp2/3 work cooperatively to maintain a functional and balanced cytoskeleton network capable of mediating ligand-induced translocation of TrkB to the cell surface. To expand their in vitro findings, the authors injected AAV9-h*PLS3* into *SMNΔ7* mice. This approach led to an increased post/presynaptic ratio in the NMJ and restored TrkB levels. Ex vivo transplants of the TVA muscle showed an increase in p-TrkB after stimulation with BDNF and an increase in $Ca_v2.1$ cluster-like formations. Altogether, these results elegantly demonstrate a novel role of PLS3 in maintaining proper F-actin cytoskeleton dynamics to support the correct alignment of transmembrane proteins TrkB and $Ca_v2.1/2$ in motor neurons. Such alignment is essential for proper motor neuron differentiation and maturation.

While this study firmly establishes PLS3 as a key player in orchestrating several phenotypes in SMA motor neurons, future studies are needed to determine how PLS3 regulates TrkB recycling, transport, and signal amplification. Nonetheless, the results from this study introduce the idea that restoring PLS3 expression in motor neurons could be a possible therapeutic approach for ameliorating the pathogenic effects of SMA. Indeed, although the antisense oligonucleotide (ASO) approach has been shown to be effective in restoring neuromuscular functions in infants and children with SMA, ∼40% of patients show no improvement (11, 12). These results raise the intriguing possibility that the combination of ASO and restoration of PLS3 may improve the therapeutic efficacy. Finally, as dysregulation of BDNF–TrkB signaling plays a crucial role in many other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, it is possible that PLS3 may have broader therapeutic implications for these diseases, though further work will be necessary to confirm this.

Acknowledgments

Funding provided by National Institutes of Health grants P01 NS083518, RF1 NS128908, R01 AA027074, R01 AG057462, and R01 AG068290, Veterans Affairs Merit Review Award I01 BX001108, and the Bluefield Project to Cure FTD (E.J. Huang).

References

1. Lefebvre, S., et al. 1995. *Cell*. https://doi.org/10.1016/0092-8674(95)90460-3
2. Lefebvre, S., et al. 1997. *Nat. Genet.* https://doi.org/10.1038/ng0797-265
3. Jablonka, S., et al. 2007. *J. Cell Biol.* https://doi.org/10.1083/jcb.200703187
4. Oprea, G.E., et al. 2008. *Science*. https://doi.org/10.1126/science.1155085
5. Dombert, B., et al. 2017. *Front. Mol. Neurosci.* https://doi.org/10.3389/fnmol.2017.00346
6. Hennlein, L., et al. 2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202204113
7. Patapoutian, A., and L.F. Reichardt. 2001. *Curr. Opin. Neurobiol.* https://doi.org/10.1016/S0959-4388(00)00208-7
8. Karpova, T.S., et al. 1995. *J. Cell Biol.* https://doi.org/10.1083/jcb.131.6.1483
9. Shinomiya, H. 2012. *Int. J. Cell Biol.* https://doi.org/10.1155/2012/213492
10. Welch, M.D., et al. 1997. *J. Cell Biol.* https://doi.org/10.1083/jcb.138.2.375
11. Finkel, R.S., et al. 2017. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa1702752
12. Mercuri, E., et al. 2018. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa1710504