PERSPECTIVE

LIM kinases in synaptic plasticity and their potential as therapeutic targets

LIM domain kinases (LIMKs), which modulate cytoskeletal dynamics, are found throughout the central nervous system. The synaptic junctions between neurons show structural alteration or plasticity during neurodevelopment, learning and memory formation, or after injury and disease; in some cases completely new connections form, while others are lost. This remodeling is under the control of the actin cytoskeleton and therefore is influenced by the activity of LIMKs (Scott and Olson, 2007). Recent reports discussed in this perspective shed light on the potential role of LIMK in hippocampal dendritic spines and photoreceptor presynaptic terminals and illustrate the importance of LIMK in nerve cell function.

LIM kinases are serine-threonine and tyrosine kinases whose family consists of two members, LIMK1 and LIMK2. The two proteins share 70% homology in their kinase domains, the site of regulatory interaction, but may have some distinct functions. Rho-associated coil containing kinases (ROCK1 and ROCK 2), p21 activated kinases (PAK1, PAK2, PAK3, PAK4), and myotonic dystrophy kinase-related Cdc-42 binding kinase, have been reported to phosphorylate and activate LIMKs. Meanwhile inactivation happens via phosphatases, such as slingshot 1 and chronophin (Scott and Olson, 2007).

A major function of the LIMKs is the control of cofilin, which is inactivated by phosphorylation at Ser3. The balance of phosphorylated and dephosphorylated cofilin is in turn one of the key modulators of actin filament assembly and disassembly. Thus, LIMKs play a key role in the organization of the actin cytoskeleton.

Cortical plasticity: Dendritic spines are small actin rich protrusions on the dendrites of neurons, which form excitatory synapses with other neurons. Alterations in dendritic spine morphology are dynamic and essential for maintaining normal synaptic transmission whereas perturbation in these structural mechanisms have been linked to altered brain/cognitive functions.

During long-term potentiation (LTP) and long-term depression (LTD), dendritic spines alter their shape to modify synaptic efficacy. These changes have been hypothesized to be the underlying cellular mechanism for learning and memory (Liu et al., 2017). Increases in dendritic spine size and number are linked to LTP, whereas shrinkage of the dendritic spine and decreases in the number of spines are connected to LTD (Lunardi et al., 2018). Late-phase LTP (L-LTP) is a form of long-lasting synaptic plasticity thought to be crucial for long-term memory (LTM). Data from LIMK1 knockout (KO) mice provided evidence that LIMK1 is essential for normal L-LTP and LTM formation (Todorovski et al., 2015). Lunardi et al. (2018) reported that intra-hippocampal administration of a LIMK inhibitor can interfere with contextual fear memory acquisition, consolidation, retrieval and reconsolidation, however memory extinction was not affected.

The mechanism of LIMK modulation in spines (**Figure 1A**) depends in part on modification by palmitoylation. George et al. (2015) demonstrated that the upstream kinase PAK, but not ROCK, is the key regulator of LIMK activation in the dendritic spines of hippocampal neurons. Dual palmitoylation of LIMK1 targets the kinase to spines and promotes the binding and activation by PAK3. They speculate that depalmitoylated LIMKs are inactive and localized mainly in the core of the spine, where actin filament disassembly is favored, and that the palmitoyl motif on LIMK is needed to bring the kinase close to the plasma membrane, in the so called juxtamembrane region where actin filament polymerization occurs. Experiments utilizing an shRNA knockdown to LIMK1 which allowed only a single palmitoylation event inhibited actin turnover and reduced the number of spines and synapses on hippocampal neurons (George et al., 2015).

Neuroligin (NLG-1), an important transmembrane protein involved in synapse development and function, was also linked to LIMK/cofilin-dependent actin remodeling. When neuroligin undergoes activity-dependent proteolytic cleavage, it releases its C-terminal domain (CTD) into the cytosol. CTD interacts with dendritic spine-associated Rap GTPase activating protein (SPAR) via its protein binding domain which subsequently activates LIMK (Liu et al., 2016). CTD-induced increase in phosphorylated-cofilin (p-cofilin) levels are eliminated in LIMK1/2 KO mice, indicating the connection between these two components. As mentioned above, modifying p-cofilin levels results in actin cytoskeleton rearrangement and thereby impacts both LTP and LTD. NLG-1 KO mice exhibit deteriorated hippocampal LTP, which is rescued by the overexpression of CTD. However, CTD overexposure had a blocking effect on LTD. This inhibitory effect did not work in LIMK1/2 double KO mice, adding an additional piece of evidence that LTD is mediated through the LIMK/cofilin pathway (Liu et al., 2016). Members of the p21 activated kinase (PAK) family are also implicated in modulation of LIMK1/cofilin phosphorylation during spine morphogenesis and neural plasticity. PAK 1, 2 and 3 have distinct expression patterns in the brain during development and deletion of the coding genes results in different phenotypes. $PAK2^{-/-}$ KO mice are embryonically lethal; $PAK1^{-/-}$, $PAK3^{-/-}$ and $PAK1^{-}/PAK3^{-}$ double KO mice are viable with preserved memory and social interactions. However, $PAK2^{+/-}$ mice have diminished spine density and insufficient LTP coupled with reduced phosphorylation of LIMK1 and cofilin and reduced actin polymerization in cortex and hippocampus (Wang et al., 2018). $PAK2^{+/-}$ mice also exhibit various autism-related behaviors. Consistent with these results, the authors found that PAK2 damaging mutations were significantly enriched in patients with autism-spectrum disorder.

Finally, deletion of the LIMK1 gene is associated with Williams-syndrome, a disease in which individuals are characterized by preserved short-term memory but heavily impaired visuospatial construction and LTM. Todorovski et al. (2015) created $LIMK1^{-/-}$and $LIMK1^{+/-}$ KO mice which exhibit features similar to humans suffering from Williams-syndrome: heavily impacted LTM with intact short-term memory. In these mice the L-LTP was impaired, but the early-phase long term potentiation (E-LTP) was unaltered. Although mechanisms for L-LTP are thought to be the attributed to synaptic mechanisms for LTM suggesting a role for LIMK in the formation of LTM, Todorovski et al. (2015) also provided evidence that LIMK might regulate L-LTP via a cofilin-independent pathway. Cyclic AMP response element-binding protein (CREB) is crucial for LTP and LTM, but not for E-LTP or short-term memory. They showed that LIMK1 is expressed and colocalized together with CREB in hippocampal neurons, and that plasticity-dependent activation of CREB is reduced in LIMK deficient mouse models. In line with this, manipulation of CREB itself, without cofilin, was enough to restore L-LTP and LTM in these animals supporting the cofilin independent pathway hypothesis. It is not clear how synaptic plasticity leads to LIMK1-mediated CREB activation. One plausible explanation is that activated LIMK in the spine translocates to the nucleus, where it binds to and activates CREB. Other possible scenarios are that LIMK might activate CREB via activating protein kinase C or mitogen-activated protein kinase (Todorovski et al., 2015).

A focus on microRNAs has led to an unusual therapeutic approach to LIMK-associated brain disease. MicroRNAs are small, endogenously expressed noncoding RNAs which regulate the expression of other RNAs by interacting with them. Elevated levels of miRNA134 have been found in the dendrites of hippocampal neurons in epileptic rats (Sun et al., 2017) and in a rodent model of ischemic stroke (Liu et al., 2017). miRNA134 is suspected to negatively regulate LIMK activation. This hypothesis is supported by the findings of Sun et al. (2017), where overexpression of miRNA134 in *in vitro* and *in vivo* status epilepticus models resulted in decreased LIMK RNA and protein levels. Additionally, the group reported that miRNA134 directly binds to the 3′ untranslated region of LIMK RNA. Moreover, by using Ant 134, a miRNA134 antagomir, the miRNA134 effects on LIMK and cofilin expression were reversed (Sun et al., 2017). In rat ischemic stroke, two weeks after middle cerebral artery occlusion, lesions on MRI are still present with minimal spontaneous recovery. In histological sections, the density of dendritic spines and number of synapses in hippocampal CA1 pyramidal cells are decreased. Western blots from these samples showed increased miRNA134 expression and diminished total and p-LIMK levels, indicating the connection between the two participants. In this experiment the effect of miRNA134 was ameliorated by electroacupuncture, suggesting that electroacupuncture blocks miRNA134 and might have therapeutic potential (Liu et al., 2017). Taken together, miRNA134 inhibition via electroacupuncture or a miRNA134 blocker has potential to alter LIMK1 signaling and restore synaptic function.

Retinal plasticity: Retina, as part of the central nervous system, also exhibits synaptic plasticity. Activity-dependent changes occur in the shape of the photoreceptor presynaptic terminal and in the composition of AMPA-type glutamate receptors in the inner retina; injury- or disease-induced changes include photoreceptor axonal retraction and sprouting (Lewis et al., 1998) and photoreceptor-induced glutamate receptor loss in postsynaptic bipolar cells followed by subsequent structural remodeling (Dunn, 2015).

The influence of LIMK in photoreceptor injury-induced structural remodeling is particular clear. While studying retinal detachment, Wang and Townes-Anderson (2015) observed that the active form of LIMK, phosphorylated-LIMK, which is present in photoreceptor terminals, increased after detachment in salamander. Others had previously shown that dramatic synaptic changes occur in rod cells after detachment (Lewis et al., 1998) and thus it was posited that activated LIMK plays a role in photoreceptor terminal plasticity. Isolated rod photoreceptors in culture exhibit axon retraction, followed by process growth at the basal (axon-bearing) region of the rod cells. Inhibition of LIMK's direct regulators, ROCK or PAK, reduced axon terminal retraction (Wang and Townes-Anderson, 2015). These results were consistent with the function of LIMK in regulating actin rearrangement. Direct inhibition of LIMK, by a pharmacological agent, also reduced structural changes in the rod photoreceptor axon terminal (**Figure 1B**). ROCK, PAK and LIMK inhibition also prevented the axonal sprouting, which is seen after surgical reattachment of the retina. Using a barbed-end assay to assess actin depolymerization and turnover, increased actin cytoskeleton rearrangement was observed in the axonal region after injury. Inhibition of ROCK or LIMK blocked such rearrangement and appeared to stabilize the actin filamentous network and thereby stabilize the presynaptic terminal (Wang et al., 2019).

The therapeutic potential of LIMK inhibition for the retina is due to the fact that such inhibition may preserve the photoreceptor-to-bipolar synapse. This is the first synapse in the visual pathway and without it there is no vision. Thus, preventing axon retraction, which results in synaptic disjunction, and axonal sprouting, which disrupts the neural circuitry, may preserve vision after

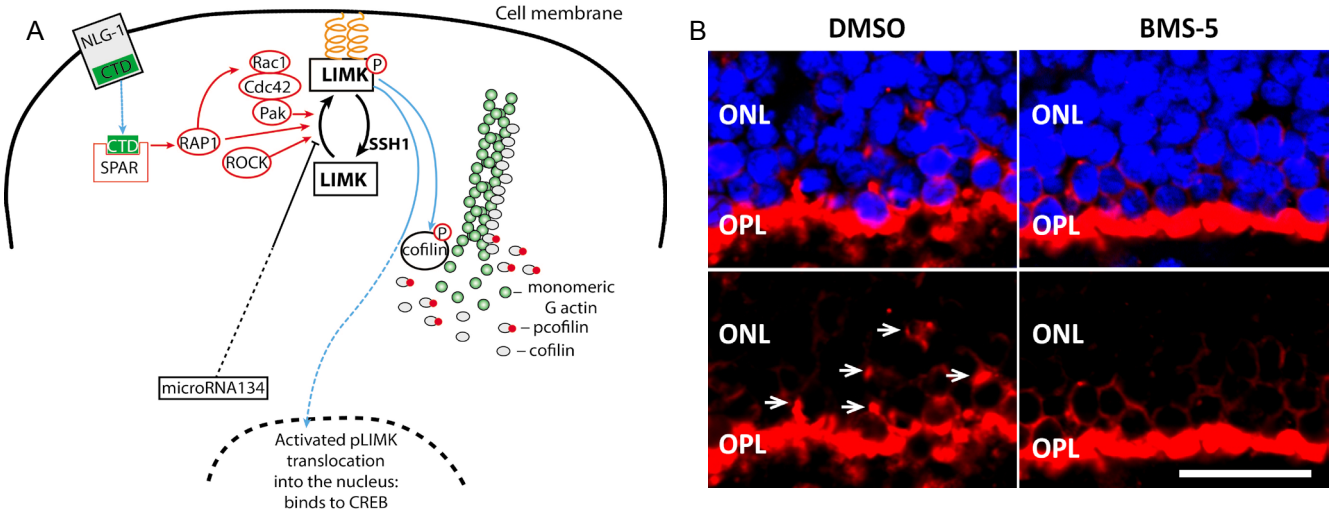

Figure 1 Lim kinases in synaptic plasticity.
(A) Diagram illustrating the regulation of LIMK in the dendritic spine. CREB: cAMP response element-binding protein; CTD: C-terminal domain of Neuroligin-1; LIMK: Lim kinase, shown with dual palmitoylation; Cdc42: cell division control protein 42 homolog; NLG-1: Neuroligin-1; P: phosphate; Pak: p21-activated kinases; pcofilin: phosphorylated cofilin; pLIMK: phosphorylated LIMK; Rac1: Ras-related C3 botulinum toxin substrate 1; RAP1: Ras-related protein 1; ROCK: Rho-associated protein kinase; SPAR: spine associated RAP GTPase activating protein; SSH1: protein phosphatase Slingshot homolog 1. (B) LIMK inhibition reduces photoreceptor axon retraction in detached porcine retina. Representative images of *in vitro* porcine retina 24 hours after detachment without (left) and with (right) treatment of a direct LIMK inhibitor BMS-5 (SYN-1024; Synkinase, San Diego, CA, USA). Rod photoreceptor synaptic terminals, at the outer plexiform layer (OPL), are labeled for synaptic vesicles with anti-SV2 antibody (red). Photoreceptor cell bodies make-up the outer nuclear layer (ONL); their nuclei are labelled with propidium iodide (blue). Left: Rod photoreceptor terminals (arrows) are among the photoreceptor cell bodies (ONL), demonstrating retraction after retinal detachment. Right: Inhibition of LIMK with BMS-5 demonstrates less SV2 in the ONL, indicating axon retraction is reduced. Scale bar: 10 µm. Adapted from Wang and Townes-Anderson (2015).

injury. This potential has been tested in the pig retina, which is a good model of human retina because of numerous similarities in structure and function. In pig retina, levels of phosphorylated cofilin, the downstream effector of LIMK, increased dramatically after retinal detachment, presumably promoted by LIMK activity. Inhibition of ROCK or LIMK reduced p-cofilin in the injured retina and significantly reduced axon retraction and thus synaptic disjunction (Wang and Townes-Anderson, 2015; Wang et al., 2019). Inhibition of LIMK therefore is suggested to stabilize the retinal circuitry following injury. A recent study demonstrated that inhibition of the ROCK/LIMK/cofilin pathway also improved functional visual recovery after retinal detachment (Townes-Anderson et al., 2019).

Synaptopathy, the dysfunction of synapses, is increasingly invoked as a cause for neurological disease. Autism-spectrum disorder, epilepsy, schizophrenia, Alzheimer's disease, Williams-Beuren syndrome, retinitis pigmentosa, have all shown synaptic disjunction or malfunction as part of their pathology. Injuries such as retinal detachment (described above), prolonged noise exposure, and traumatic brain injury also result in synaptic disjunction. Improved understanding of the role of LIMKs in normal and pathological synaptic plasticity may contribute to future therapies aimed to improve and preserve nervous system function.

In summary, LIMKs are present in all neurons that have so far been examined for it. They contribute to the structural changes which occur in dendritic spines with learning and memory as well as in morphological changes in synapses associated with injury and disease such as the axon retraction of rod cells. LIMKs are located downstream in the RhoA-ROCK pathway, a signaling pathway essential to control of the actin cytoskeleton. Although ROCK is a well-investigated target in neurodegenerative disease, LIMK has fewer substrates and thus controlling LIMK activity might result in positive effects similar to ROCK inhibition, but with potentially better efficacy and fewer side effects.

Éva Halász, Ellen Townes-Anderson, w eiwei w ang*
Department of Pharmacology, Physiology and Neuroscience, Graduate School of Biomedical Sciences, Rutgers, The State University of New Jersey, Newark, NJ, USA (Halász É, Townes-Anderson E)
Grousbeck Gene Therapy Center, Schepens Eye Research Institute and Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA (Wang W)
***Correspondence to:** *Weiwei Wang, PhD, weiwei_wang@meei.harvard.edu.*
***orcid:** *0000-0003-3518-9328 (Weiwei Wang)*
***Received:** *October 8, 2019*
***Peer review started:** *October 13, 2019*
***Accepted:** *December 2, 2019*
***Published online:** *January 28, 2020*

***doi:** *10.4103/1673-5374.274333*

References

Dunn FA (2015) Photoreceptor ablation initiates the immediate loss of glutamate receptors in postsynaptic bipolar cells in retina. J Neurosci 35:2423-2431.
George J, Soares C, Montersino A, Beique JC, Thomas GM (2015) Palmitoylation of LIM Kinase-1 ensures Spine-Specific actin polymerization and morphological plasticity. Elife 2015:1-25.
Lewis GP, Linberg KA, Fisher SK (1998) Neurite outgrowth from bipolar and horizontal cells after experimental retinal detachment. Investig Ophthalmol Vis Sci 39:424-434.
Liu A, Zhou Z, Dang R, Zhu Y, Qi J, He G, Leung C, Pak D, Jia Z, Xie W (2016) Neuroligin 1 regulates spines and synaptic plasticity via LIMK1/cofilin-mediated actin reorganization. J Cell Biol 212:449-463.
Liu W, Wu J, Huang J, Zhuo P, Lin Y, Wang L, Lin R, Chen L, Tao J (2017) Electroacupuncture regulates hippocampal synaptic plasticity via miR-134-mediated LIMK1 function in rats with ischemic stroke. Neural Plast 2017:9545646.
Lunardi P, Sachser RM, Sierra RO, Pedraza LK, Medina C, de la Fuente V, Romano A, Quillfeldt JA, de Oliveira Alvares L (2018) Effects of hippocampal LIMK inhibition on memory acquisition, consolidation, retrieval, reconsolidation, and extinction. Mol Neurobiol 55:958-967.
Scott RW, Olson MF (2007) LIM kinases: Function, regulation and association with human disease. J Mol Med 85:555-568.
Sun J, Gao X, Meng D, Xu Y, Wang X, Gu X, Guo M, Shao X, Yan H, Jiang C, Zheng Y (2017) Antagomirs targeting microRNA-134 increase Limk1 levels after experimental seizures in vitro and in vivo. Cell Physiol Biochem 43:636-643.
Todorovski Z, Asrar S, Liu J, Saw NMN, Joshi K, Cortez MA, Snead OC, Xie W, Jia Z (2015) LIMK1 regulates long-term memory and synaptic plasticity via the transcriptional factor CREB. Mol Cell Biol 35:1316-1328.
Townes-Anderson E, Halasz E, Sugino I, Zarbin MA (2019) Inhibition of ROCK activity improves morphological and functional outcomes of reattached retina. Abstract #2794. Presented at the Association for Research in Vision and Ophthalmology Annual Meeting, April 28-May 2, 2019, Vancouver, BC.
Wang W, Halasz E, Townes-Anderson E (2019) Actin dynamics, regulated by RhoA-LIMK-Cofilin signaling, mediates rod photoreceptor axonal retraction after injury. Investig Ophthalmol Vis Sci 60:2274-2285.
Wang W, Townes-Anderson E (2015) LIM Kinase, a newly identified regulator of presynaptic remodeling by rod photoreceptors after injury. Investig Ophthalmol Vis Sci 56:7847-7858.
Wang Y, Zeng C, Li J, Zhou Z, Ju X, Xia S, Li Y, Liu A, Teng H, Zhang K, Shi L, Bi C, Xie W, He X, Jia Z, Jiang Y, Cai T, Wu J, Xia K, Sun ZS (2018) PAK2 haploinsufficiency results in synaptic cytoskeleton impairment and autism-related behavior. Cell Rep 24:2029-2041.

C-Editors: Zhao M, Li JY; T-Editor: Jia Y