Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

During Tangential Migration, SDF1 Lends the Cytoskeleton a Guiding Hand

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Review of Lysko et al.

The cerebral cortex is a laminar structure that is composed of two major neuronal populations: glutamatergic projection neurons and GABAergic interneurons. Projection neurons are born in the ventricular zone and migrate radially into the cortical plate, with the youngest neurons continuing their migration to the most superficial cortical layers. In contrast, interneurons are born in the medial ganglionic eminence (MGE), which means they must first undergo tangential migration toward the dorsal telencephalon before radially migrating into the cortical plate (Nadarajah and Parnavelas, 2002). Tangential migration is maintained, in part, by the chemokine stromal cell-derived factor 1 (SDF1). SDF1 is a secreted molecule that is expressed along the major path of interneuron tangential migration, where it acts on migrating interneurons via its two primary receptors, CXCR4 and CXCR7 (Stumm et al., 2003). When the SDF1 signal is low interneurons cease tangential migration and invade the cortical plate.

Neuron migration is a complex cellular process that can be divided into three steps. The migrating neuron establishes a leading process (LP) that explores the environment for attractant and/or repulsive molecules. As the LP tracks along the migratory path, the nucleus translocates into the LP (nucleokinesis). After nucleokinesis, the trailing process retracts, leading to the net movement of the cell along the migratory path. In a previous study, Lysko et al. (2011) demonstrated that SDF1 maintains tangential interneuron migration by reducing branching frequency of the LP. They proposed that, by limiting LP branching, SDF1 makes the interneuron less likely to explore the cortical plate and receive strong cortical plate attractant signals.

In a recent follow-up study published in *The Journal of Neuroscience*, Lysko et al. (2014) explored the mechanism by which SDF1 limits LP branching. Using MGE explant cultures embedded within a 3D extracellular matrix-like environment, the authors demonstrated that SDF1 reduces branch frequency by decreasing branch lifetime, rather than by blocking branch initiation. Reduced branch lifetime reflects an inability to grow and/or stabilize branches, and cytoskeletal dynamics play a prominent role in branch stabilization. Therefore, Lysko et al. (2014) asked: does SDF1 regulate LP branching by altering cytoskeletal dynamics?

First, the authors investigated SDF1's effect on the filamentous actin (F-actin) network. In control explants, the LP of migrating interneurons contained a broad wedge of F-actin. However, in SDF1-treated explants, F-actin consolidated into a narrow strip at the tip of the LP, indicating that F-actin branching was defective. The authors then demonstrated that SDF1 increased calpain-mediated degradation of the F-actin branching molecule cortactin, leading to reduced cortactin and branched F-actin at the LP tip. Finally, Lysko et al. (2014) established a functional connection between SDF1 signaling, cortactin-mediated actin branching, and branching along the LP. They showed that cortactin overexpression was sufficient to increase LP branching and, further, that cortactin overexpression blocked SDF1-mediated reduction in branch frequency.

The LP of migrating interneurons contains microtubules that diverge as they extend into secondary LP branches and the LP tip. Upon SDF1 treatment, LP microtubules consolidated into a thin bundle, which was unable to invade and stabilize off-angle LP branches. To determine whether microtubule consolidation resulted from increased microtubule depolymerization, the authors partially depolymerized microtubules with a low dose of nocodazole and asked whether SDF1 has an additive effect on branch reduction. Individual application of low-dose nocodazole or SDF1 reduced branching, while cotreatment with nocodazole and SDF1 did not cause a further reduction in branching, leading the authors to speculate that SDF1 stabilizes the microtubule network against nocodazole-induced depolymerization. However, this interpretation is complicated by prior studies demonstrating that substoichiometric (nanomolar) concentrations of nocodazole actually stabilize

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microtubules in cultured cells (Vasquez et al., 1997). Microtubule stability is enhanced by microtubule associated proteins (MAPs), such as doublecortin (DCX), which increase microtubule bundling. Therefore, the authors tested whether SDF1 stabilizes microtubules by influencing MAPs within the LP. They identified DCX as a promising candidate downstream of SDF1 by showing that that DCX localized to the LP, DCX overexpression reduced LP branching, and DCX knock-down increased LP branching. Importantly, DCX RNAi prevented SDF1 from reducing LP branching, indicating that DCX mediates SDF1's ability to increase microtubule stability.

Based on the above data, Lysko et al. (2014) put forth a model whereby SDF1 coordinately regulates both actin and microtubules in a manner that causes migrating interneurons to form a stable, unbranched leading process. SDF1 signaling increases calpain-mediated cortactin degradation, which reduces F-actin branch stability and limits LP membrane protrusion and the formation of off-angle branches. Moreover, SDF1 increases the microtubule bundling activity of DCX, which increases microtubule stability and decreases microtubule invasion of off-angle branches. SDF1 exists as a gradient along the migration pathway. Therefore, when SDF1 is present, the LP tracks linearly until SDF1 levels drop below some threshold, at which point off-angle branches stabilize, allowing exploration of the cortical plate.

The relationship between SDF1 and calpain activity appears to be quite complex. SDF1-mediated calpain activation affects leading process branching in the same way as pharmacological calpain inhibition (Lysko et al., 2014). The authors speculate that calpain inhibition increases F-actin to such an extent that leading process branching becomes impaired. However, in other systems, calpain inhibition has been shown to stabilize microtubules (Hoang et al., 2010), which, as the authors argue, restricts microtubule support of off-angle branches. Therefore, rather than excessive F-actin accumulation in the leading process tip inhibiting branching, broad calpain inhibition may actually stabilize microtubules in migrating MGE interneurons. Regardless, calpain's ultimate effect on the cytoskeletal network may be dictated by calpain's local proteolytic activity. Although the calpain substrate spectrin did not display polarized cleavage within migrating interneurons, it would be interesting to analyze the localization of calpain-mediated proteolysis of cortactin in the absence and presence of SDF1. Lysko et al. (2014) showed that DCX is required for SDF1-mediated reduction of leading process branching, which argues that DCX-induced microtubule bundling is downstream of SDF1. However, at the level of fixed-cell light microscopy it is difficult to distinguish microtubule bundling from microtubule loss, because both would reduce the tubulin signal within a given area. As the ultra-structural analysis of microtubules *in vivo* progresses (Bouchet-Marquis et al., 2007), it will be interesting to observe microtubules within the LP tip of actively migrating neurons. In addition, it was recently discovered that microtubules can branch from preexisting microtubules in a γ-tubulin-dependent manner (Petry et al., 2013). Since the leading process of migrating neurons harbors the centrosome (Higginbotham and Gleeson, 2007), which is rich in γ-tubulin, it would be interesting to know whether SDF1 signaling limits microtubule branching by feeding into this newly discovered cellular process. Lysko et al. (2014) present their findings on SDF1's regulation of the actin and microtubule networks as two complementary effects: cortactin-mediated actin dynamics are distinct from DCX-mediated microtubule consolidation. However, emerging evidence has implicated the septin protein family as a dual regulator capable of coordinating actin and microtubule dynamics. For example, in sensory neurons, collateral axon branching requires SEPT6-mediated interaction with cortactin to initiate filopodia extension and SEPT7-mediated interaction with microtubules to stabilize the filopodia (Hu et al., 2012). In addition, SEPT4 and SEPT14 interact to maintain the leading process of radially migrating neurons within the cortical plate (Shinoda et al., 2010). These observations raise the exciting possibility that SDF1 could directly couple actin dynamics to microtubule dynamics via septins. Therefore, it would be interesting to test whether SDF1 modulates the localization of septin family members to the leading process tip.

In conclusion, Lysko et al. (2014) present compelling evidence that tangentially migrating interneurons transduce SDF1 into a signal that coordinately regulates the actin and the microtubule cytoskeletal networks. The authors provide substantial mechanistic insight into the process that interneurons use while navigating their route to the cerebral cortex. In doing so, they have opened new doors, allowing cellular neurobiologists to investigate the nuanced relationship between extracellular guidance cues and the dynamic cytoskeleton.

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