Sharpin: a novel Arp2/3 regulator during cell spreading?

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Abstract

Integrins are transmembrane receptors that mediate cell adhesion to the extracellular environment, which activates many intracellular signaling pathways. Deregulated integrin signaling has been implicated in many human diseases, including cancer. We identified Sharpin as an important integrin inhibitor (Rantala, Pouwels, et al., Nature Cell Biol. 2011). More recently, we showed (Pouwels et al., Cell Rep. 2013) that Sharpin controls lymphocyte migration and allows lymphocyte detachment during transmigration by inhibiting the lymphocyte-specific αLβ2-integrin. In addition, Sharpin has been shown to be overexpressed in cancer and to promote cancer progression, Sharpin knockout mice display a psoriasis-like phenotype with chronic inflammation and Sharpin also plays a key role in the oncogenic and proinflammatory NF-κB pathway.

The Arp2/3 complex catalyzes branching of actin filaments and regulates formation of lamellipodia, filopodia and actin stress fibers. It regulates a plethora of cellular functions that involve the actin cytoskeleton, such as cell migration, cell polarity, endocytosis and organelle movement. We have now identified several members of the Arp2/3 complex in a mass spectroscopy screen for Sharpin interactors. The interaction between Arp2/3 and Sharpin, which colocalize in lamellipodia, was confirmed using proximity ligation assays, fluorescence resonance energy transfer (FRET/FLIM) and co-immunoprecipitation. Interestingly, Arp2/3 activity is essential for this interaction as treatment with a specific inhibitor of Arp2/3 activity (CK666), which causes cells to round up due to lack of lamellipodia, abolished the Arp2/3-Sharpin interaction, which is fully rescued after CK666 washout. Importantly, cell spreading after CK666 washout was inhibited in cells lacking Sharpin, suggesting that Sharpin promotes Arp2/3 activity in lamellipodia. Spatial mapping of FRET between Arp2/3 and Sharpin showed that Arp2/3 and Sharpin mostly interact at the edges of these spreading cells. Together, these data identify Sharpin as a novel interactor of the Arp2/3 complex and suggest that Sharpin plays an important role in Arp2/3-mediated actin dynamics.