ABSTRACT

Loss of myopodin expression correlates with the transition from indolent to metastatic prostate cancer. However, the mechanisms underlying this correlation and the roles of myopodin in normal cell function have not been determined. Contradictory findings on whether myopodin suppresses or promotes prostate cancer cell migration, and the recent identification of several different myopodin isoforms further complicate our understanding of myopodin function. To address these deficits, I ectopically expressed the five different myopodin isoforms in PC3 prostate cancer cells. Transwell migration and invasion assays indicated myopodin isoforms alter the response of PC3 cells to different external stimuli, either increasing or decreasing cell migration depending on the stimulus while having little direct affect on cell invasion. Under the same external stimulus, myopodin isoforms differentially induced and colocalized with distinct actin structures in the cell body. Impairing formation of these myopodin-induced actin structures inhibited myopodin-stimulated cell migration. Subsequent studies revealed that myopodin expression increases RhoA activation, and the actin structures, tail retraction and enhanced cell migration associated with myopodin expression were all diminished by inhibiting the Rho/ROCK pathway. Although non-muscle myosin II (NM II) is a downstream effector of RhoA and is essential for cell migration, inhibiting NM II had no affect on myopodin-enhanced cell migration. Interfering with NM II activity did, however, inhibit the appearance of actin bundles in the cell body of myopodin-expressing cells. Timecourse analysis using serum-starvation to synchronize cells and inhibitors of Arp2/3 complexes and NM II indicated myopodin promotes Arp2/3-dependent lamellipodia formation and Arp2/3-independent actin bundle formation, and myopodin colocalizes with the actin fibers in these protrusions. The formation of lamellipodia and filopodia by myopodin does not require NM II-dependent FA maturation but myopodin does, either directly or indirectly, promote formation of FAs. Live cell imaging further showed the actin fibers generated in the protrusions are subsequently integrated into the stress fibers in the cell body by NM II contraction. Thus, myopodin enhances cell migration by stimulating actin bundle formation at the leading cell edge resulting in the formation of membrane protrusions and FA formation, and the prominent actin bundles formed within the cell body are an incidental effect of myosin contraction.