Cell motility, adhesion and actin cytoskeletal rearrangements occur upon integrin-engagement to the extracellular matrix and activation of the small family of Rho GTPases, RhoA, Rac1 and Cdc42. The activity of the GTPases is regulated through associations with guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine dissociation inhibitors (GDIs). Recent studies have demonstrated a critical role for actin-binding proteins, such as ezrin, radixin and moesin (ERM), in modulating the activity of small GTPases through their direct associations with GEFs, GAPs and GDIs. Dematin, an actin binding and bundling phospho-protein was first identified and characterized from the erythrocyte membrane, and has recently been implicated in regulating cell motility, adhesion and morphology by suppressing RhoA activation in mouse embryonic fibroblasts. Although the precise mechanism of RhoA suppression by dematin is unclear, several plausible and hypothetical models can be invoked. Dematin may bind and inhibit GEF activity, form an inactive complex with GDI-RhoA-GDP, or enhance GAP function. Dematin is the first actin-binding protein identified from the erythrocyte membrane that participates in GTPase signaling, and its broad expression suggests a conserved function in multiple tissues.

Cell adhesion and motility are mediated through activation of integrin receptors and the family of small Rho GTPases. Engagement of integrin receptors to the extracellular matrix leads to the activation of multiple kinase pathways (i.e., FAK, Src), inducing the assembly of the focal adhesion complex and actin/myosin contraction. Furthermore, activation of the receptor tyrosine kinases (i.e., insulin receptor) or G-protein coupled receptors (i.e., LPA receptor), leads to downstream signaling events that also trigger multiple kinase pathways that regulate the protrusive and contractile actin/myosin dynamics. These adhesion-dependent or receptor-driven signaling cascades ultimately result in the activation of the small family of Rho GTPases: Cdc42, Rac1 and RhoA, key regulators of actin cytoskeleton assembly. The activation of these GTPases induces lamellipodia (Rac1), filopodia (Cdc42), actin stress fiber formation (RhoA) and focal adhesion complex formation (Rac1 and RhoA). Nascent focal adhesion complex formation within the lamellipodia is a result of Rac1 activation; however, mature focal adhesions and actin cytoskeletal rearrangements are a direct consequence of RhoA activation.

The regulation of RhoA activity occurs through its interactions with guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine dissociation inhibitors (GDIs) (reviewed in refs. 4-6). The regulatory intricacies that govern RhoA association with GEFs, GAPs and GDIs remain poorly understood. However, it is now well accepted that actin-binding proteins participate and play a significant role in regulating the functional activity of RhoA through their direct association with RhoGEFs, GDIs and RacGAPs (Table 1). Dematin, an actin binding protein, has been recently identified as a novel suppressor of RhoA activation; however, the precise mechanism of this function remains unknown.

Dematin, previously known as erythrocyte membrane protein band 4.9, is a member of the villin family of headpiece-containing

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**Table 1** List of actin-binding proteins that are known to directly bind to GEFs, GAPs or GDIs and regulate RhoA activity

| GEF/GAP/GDI | Act binding protein | Ref. |
|------------|------------------|-----|
| GEFs       | dbl              | Radixin | 31  |
|            | Trio             | TARA   | 32  |
|            | Lfc              | Spinophilin | 33  |
|            | Lfc              | Neurobin | 33  |
| GAPs       | CdgAP            | Actopaxin | 19  |
|            | Rgd1             | Vrp1   | 20  |
| GDIs       | RhoGDI           | Ezrin/Radixin/Moesin | 21 |
|            | RhoGDI           | Merlin | 34  |

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Regulatory models of RhoA suppression by dematin

Dematin was first isolated and characterized from the mature erythrocyte membrane, where it functions to maintain erythrocyte shape and membrane structural integrity via a novel linkage at the actin-spectrin junctional complex through glucose transporter-1 (GLUT1) in a species specific manner. Despite its wide-spread expression, relatively little is known about the biological function of this actin-binding protein in non-erythroid cells. Previous studies have shown that the human dematin gene (EPB4.9) maps to 8p21.1, a chromosomal region that is frequently deleted in prostate cancers. Interestingly, it was demonstrated that a sub-set of metastatic prostate tumors show a loss of heterozygosity of the dematin gene. Furthermore, it was demonstrated that in PC-3 cells, a prostate cancer cell line, the overexpression of the dematin gene was able to revert the oncogenic morphology (cell rounding) to a normal prostate epithelial morphology (microvillar and cytoplasmic extensions), thus suggesting a possible role for dematin in modulating these cellular processes. To determine the in vivo function of dematin, a dematin headpiece-null mouse (HPKO) model was generated in our laboratory, lacking the c-terminal actin binding headpiece domain. Consequently, the HPKO model expresses a truncated variant of dematin containing the N-terminal “core-domain.” Hematological analysis of the HPKO erythrocytes revealed evidence of membrane fragility, spherocytosis and mild hemolytic anemia. Since the loss of the dematin actin-binding headpiece resulted in morphological defects in the erythrocyte, we extended these studies to investigate if these defects would manifest in non-erythroid cells. Isolated mouse embryonic fibroblasts from HPKO mice display abnormal cell morphology, motility and adhesion, presumably resulting from RhoA hyperactivation and subsequent phosphorylation of downstream signaling molecules, such as focal adhesion kinase (FAK) and myosin light chain (MLC). These data suggest that dematin acts upstream of RhoA perhaps by associating with one of the known regulators of RhoA activation: GEFs, GAPs and GDIs (Fig. 1).

Figure 1. Hypothetical models of dematin mediated regulation of RhoA signaling. (A) Dematin has been shown to bind the DH domain of RasGRF2, but does not modulate Rac1 or Ras activation through RasGRF2. In several yeast-2-hybrid RasGRF2 clones, an insert from the GEFD2 domain of the RhoA GEF, Trio, was identified. It is possible that dematin may bind to and inhibits the RhoA GEF activity on Trio. (B) Dematin may complex with GDI and inactive RhoA-GDP, by tethering GDI to the actin cytoskeleton. The release of dematin from the cytoskeleton results in RhoA-GDP release and activation. (C) Dematin may also act to spatially localize RhoGAP to enhance the activity on RhoA-GTP, which in turn results in RhoA suppression.
or Ras. Moreover, the yeast-2 hybrid results revealed that several of the isolated RasGRF2 clones contained an insert from the GEFD2 domain of Trio, a RhoA GEF. It is therefore plausible, that in vivo, dematin associates with Trio, and inhibits RhoA activation, similar to TRIPalpha, the first known inhibitor of a RhoA GEF, which specifically blocks the Trio GEFD2-exchange activity of RhoA. The significance of the postulated in vivo dematin interactions with Ras-GRF2 and TrioRhoGEF has not been established, but taken together; this model may provide a mechanistic link between dematin and RhoA (Fig. 1).

RhoGAPs catalyze the hydrolysis of the active GTP-bound state of RhoA to the inactive GDP-bound form through intrinsic GTPase activity. Although there is no indication that dematin binds to a RhoGAP, it is possible that dematin behaves similarly to actopaxin and VRP, actin-binding proteins that provide spatial and temporal regulation of RhoGAP function, and consequently RhoA inhibition. In addition to the regulation of RhoA through GEFs and GAPs, the actin-binding proteins, ezrin, radixin and moesin (ERMs) are known to sequester the guanine dissociation inhibitor, GDI, from RhoGDP. The tethering of GDI to the actin cytoskeleton reduces GDI activity, resulting in an increase in RhoA activation. Furthermore, recent studies have shown that PKA phosphorylation of GDI results in an increase in the association between GDI and RhoA-GDP, thus resulting in a decrease in RhoA activity. Interestingly, PKA phosphorylates and inhibits dematin’s actin-bundling activity by inducing a conformational change in the dematin-actin-binding headpiece domain. It is possible that in the absence of PKA, dematin robustly interacts with GDI resulting in a stronger and tighter linkage to the actin cytoskeleton; thus in turn resulting in an increase in RhoA activation. Phosphorylation of dematin by PKA may result in the release of GDI from dematin and the actin cytoskeleton and causing subsequent suppression of RhoA activity. It is also possible that dematin retains inactive Rho-GDP in the cytosol, through an association with RhoGDI and the actin cytoskeleton. RhoA activation would occur when the dematin-RhoGDI-RhoA-GDP complex dissociates from the cytoskeleton via intracellular signaling events (Fig. 1).

In addition to the aforementioned mechanisms of RhoA regulation through GEFs, GAPs and GDIs, it is also possible that dematin participates in the signaling cascade several steps upstream of RhoA activation. Dematin’s interaction with GLUT1, and with the scaffolding protein 14-3-3ζ may provide alternative models to investigate the mechanism of dematin-mediated suppression of RhoA. Since dematin interacts with GLUT1, it is possible that dematin mediates GLUT1 trafficking to the plasma membrane. In the absence of dematin, GLUT1 trafficking may be altered, thus resulting in abnormal glucose uptake. Metabolic defects have significant effects on intracellular signaling, which manifest itself in a variety of phenotypes, such as altered cell morphology, motility and adhesion.

Proteomic analysis, as well as seven consensus 14-3-3 binding motifs, suggests that dematin may interact in vivo with the scaffolding protein, 14-3-3ζ. Recent evidence has shown that PI3-Kinase/Akt activation induces the association of an ankyrin repeat domain-containing protein, KANK, with 14-3-3ζ, which in turn results in RhoA activation. The mechanism by which KANK negatively regulates 14-3-3ζ-activation of RhoA is unknown. However, it has been reported that the RhoGEF, AKAP-Lbc, is inhibited by anchoring PKA to 14-3-3ζ. It is thus possible that dematin exists in a similar complex to suppress RhoA activation.

The unexpected finding that dematin functions as a suppressor of RhoA activity has its significance as being the first protein isolated from the erythrocyte that has been functionally linked to a small GTPase and regulates its activity. There is a significant amount of RhoA in the human erythrocytes, and it is possible that other cytoskeletal components of the erythrocyte membrane are also able to module small Rho-GTPases in vivo. Recent evidence has implicated the small GTPase, Rac1 and Rac2 in modulating the deformability of the erythrocyte membrane and Rac GTPases together with mDia2 regulate enucleation in mammalian erythroblasts. Although the precise mechanism of these processes is not yet clear, it raises the possibility that the erythrocyte membrane yet again serves as a paradigm for elucidating fundamental biochemical processes beyond the field of red cell biology. Future studies on the dematin-RhoA signaling pathway will be directed toward elucidating the mechanism by which dematin is able to suppress RhoA activation in relevant cell types.

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