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Layilin, a cell surface hyaluronan receptor, interacts with merlin and radixin

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Abstract

Layilin is a widely expressed integral membrane hyaluronan receptor, originally identified as a binding partner of talin located in membrane ruffles. We have identified merlin, the neurofibromatosis type 2 tumor suppressor protein and radixin, as other interactors with the carboxy-terminal domain of layilin. We show that the carboxy-terminal domain of layilin is capable of binding to the amino-terminal domain of radixin. An interdomain interaction between the amino- and the carboxy-terminal domains of radixin inhibits its ability to bind to layilin. In the presence of acidic phospholipids, the interdomain interaction of radixin is inhibited and layilin can bind to full-length radixin. In contrast, layilin binds both full-length and amino-terminal merlin-GST fusion proteins without a requirement for phospholipids. Furthermore, layilin antibody can immunoprecipitate merlin, confirming association in vivo between these two proteins, which also display similar subcellular localizations in ruffling membranes. No interaction was observed between layilin and ezrin or layilin and moesin. These findings expand the known binding partners of layilin to include other members of the talin/band 4.1/ERM (ezrin, radixin, and moesin) family of cytoskeletal–membrane linker molecules. This in turn suggests that layilin may mediate signals from extracellular matrix to the cell cytoskeleton via interaction with different intracellular binding partners and thereby be involved in the modulation of cortical structures in the cell.

Keywords: Layilin; Talin; Merlin; Radixin; ERM proteins; Hyaluronan receptor; Ruffles

Introduction

Key to determining the direction of a migrating cell is the definition of the front and back of the cell. This is achieved at least partly by interpreting signals at the plasma membrane, which lead to reorganization of the cytoskeleton. ERM (ezrin, radixin, and moesin) proteins are key regulators of cytoskeletal–plasma membrane interactions, especially in polarized cells. These proteins localize to dynamic actin structures such as microvilli, filopodia, lamellipodia, and membrane ruffles in different cultured cell lines [1–3]. Although ERM proteins are co-expressed in many types of cells, there exist differences in their subcellular localization [4]. Also, tissue- and cell-type-specific expression exists in whole organisms, since the distribution of ezrin and moesin is nearly mutually exclusive, as revealed by immunocytochemistry [1].

Abbreviations: ERM, ezrin, radixin, and moesin; FERM, four-point one, ezrin, radixin, and moesin; HRP, horseradish peroxidase; Ig, immunoglobulin; NF2, neurofibromatosis 2; ECM, extracellular matrix; GST, glutathione-*S*-transferase; PIP, phosphatidylinositol 4-phosphate sodium salt; PIP_2, phosphatidylinositol 4,5-bisphosphate; PC, phosphatidyl choline.

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Members of the band 4.1/ERM superfamily (band 4.1, talin, ezrin, radixin, moesin, and merlin) all share a homologous N-terminal domain, the FERM domain, that binds membrane proteins and links filamentous actin to receptors in the plasma membrane. This attachment is important for many different cellular functions including determination of cell shape, cell–substratum and cell–cell adhesion, and cell motility [5]. Functional redundancy in vertebrates has been suggested for ERM proteins since mice lacking moesin show no phenotypic change, not even in the expression level of other ERM proteins [6]. However, antisense suppression of radixin inhibits de novo adhesion and spreading of cells, while cells with a reduced amount of moesin adhere and spread normally. Similarly, cell–cell adhesion is reduced in cells lacking ezrin or radixin, but not in those lacking moesin. In the absence of all three proteins, thymoma cells lose microvilli and ruffles [7]. Some other studies have pointed to distinct functions of ERM proteins [4,8–11], suggesting that perhaps subtly different tissue-specific functions exist among the ERM protein family, members of which play a role in both establishing and maintaining adhesion.

Neurofibromatosis 2 (NF2) is a dominantly inherited disorder characterized by bilateral vestibular schwannomas and other tumors of the central nervous system [12]. The product of the *NF2* tumor suppressor gene was found to share a high degree of sequence similarity with the ERM family. The NF2 protein was named merlin (moesin, ezrin, radixin-like protein; [13]). Endogenous merlin localizes to leading and ruffling edges in fibroblasts and meningioma cells where it co-localizes with actin in motile regions but is not associated with stress fibers [3]. When overexpressed, merlin localizes to membrane ruffles as well as to other actin-rich structures such as microvilli and filopodia, thus resembling the ERM proteins [14]. Rat schwannoma cell lines over-expressing merlin show impaired cell motility, adhesion, and spreading, suggesting that merlin may function to maintain normal cytoskeletal organization [15].

As expected, on the basis of merlin's similarity to ERM proteins, these members of the band 4.1/ERM superfamily have overlapping functions (reviewed in [16,17]) although merlin also has distinct functions. This is exemplified by genetic analysis since loss of merlin function is lethal in mice and *Drosophila* [18,19]. Mice heterozygous for an *Nf2* mutation (mimicking human NF2 patients with one inherited mutated copy) are predisposed to a variety of highly metastatic tumors (mostly osteosarcomas and fibrosarcomas) leading to tumor formation in cells that still express ERM proteins [20]. If *Nf2*-heterozygous mice are crossed with *p53*-heterozygous mice, tumor types identical to those observed in *p53* heterozygotes alone show elevated rates of metastasis [20]. These in vivo results on the growth and motility-suppressing functions of merlin are opposite to the several lines of evidence that ERM proteins promote cell proliferation and motility [21]. Also, studies with the *Drosophila* homologue of merlin suggest that merlin has unique cellular functions which differ from those of other ERM family members [9].

Layilin is a transmembrane protein originally identified in a yeast two-hybrid screen as a ligand for talin, another member of the band 4.1/ERM superfamily [22]. Layilin is widely expressed in different cell types and, unlike integrins, a distinct family of talin ligands, co-localizes with talin in membrane ruffles where it is also recruited in cells induced to migrate. Layilin binds to talin's head FERM domain, indicating that it represents a membrane docking site for talin in ruffles while integrins anchor talin in focal contacts. Since layilin is not expressed in these more stable cell–matrix connections [23], talin, by binding to integrins and layilin, may be able to distinguish between the relatively static membrane–cytoskeletal connections in focal contacts and the highly dynamic membrane–actin linkages in ruffles. Recent studies have revealed that hyaluronan (but not other glycosaminoglycans such as heparin or chondroitin sulfate) is a layilin ligand and that layilin is a functional hyaluronan receptor capable of mediating cell adhesion [24]. Layilin does not contain a link domain, a common hyaluronan-binding module found in many ECM proteins and cell surface receptors [25,26]. However, layilin contains a C-type lectin domain [22], which may account for its ability to bind hyaluronan, since the three-dimensional structure of link domains is similar to that of C-type lectins [26].

Although layilin's exact function remains unclear, one attractive model is that layilin may mediate early interactions between spreading cells and the ECM by recruiting talin to ruffling edges to form transient connections which, after recruiting integrins, ultimately form focal contacts. This model is supported by layilin's ability to bind hyaluronan, a ubiquitous component of the extracellular matrix, and findings that the cytoplasmic domain of β1 integrins can bind to both head and rod domain of talin and the β1-binding site in talin's head domain overlaps with the layilin-binding site [27].

Given the sequence similarity between talin's N-terminal domain and the members of the ERM protein family and the observation that layilin and ERM proteins and merlin are all found in membrane ruffles, we have assessed the potential biochemical interactions between layilin and radixin/moesin/ezrin (members of the ERM family), as well as merlin, the product of the neurofibromatosis type 2 gene. In this paper, we report that both merlin and radixin associate with the intracellular domain of layilin. This expands the known range of layilin's binding partners beyond talin and suggests that there exist functional connections between layilin and various cytoskeletal–membrane linkers.

Materials and methods

Antibodies

For indirect immunofluorescence staining of NIH3T3 cells, 13H9 (anti-ERM) antibody was detected with