Role of the actin-binding protein profilin1 in radial migration and glial cell adhesion of granule neurons in the cerebellum

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Abbreviations: G-actin, actin monomer; actin filament; VASP, vasodilator-stimulated phosphoprotein; Mena, mouse homolog of Drosophila enabled; EVL, Ena/VASP-like; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP-associated verprolin homologous protein; mDia, mammalian diaphanous; CGN, cerebellar granule neurons; BrdU, 5-bromo-2'-deoxyuridine; CaMKIV, Ca2+/calmodulin-dependent protein kinase IV; VGluT2, vesicular glutamate transporter 2; MDLS, Miller-Dieker lissencephaly syndrome

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Profilins are small G-actin-binding proteins essential for cytoskeletal dynamics. Of the four mammalian profilin isoforms, profilin1 shows a broad expression pattern, profilin2 is abundant in the brain, and profilin3 and profilin4 are restricted to the testis. In vitro studies on cancer and epithelial cell lines suggested a role for profilins in cell migration and cell-cell adhesion. Genetic studies in mice revealed the importance of profilin1 in neuronal migration, while profilin2 has apparently acquired a specific function in synaptic physiology. We recently reported a mouse mutant line lacking profilin1 in the brain; animals display impaired neuronal migration. We found that during cerebellar development, profilin1 is specifically required for radial migration and glial cell adhesion of granule neurons. Profilin1 mutants showed cerebellar hypoplasia and aberrant organization of cerebellar cortex layers, with ectopically arranged granule neurons. In this commentary, we briefly introduce the profilin family and summarize the current knowledge on profilin activity in cell migration and adhesion. Employing cerebellar granule cells as a model, we shed some light on the mechanisms by which profilin1 may control radial migration and glial cell adhesion. Finally, a potential implication of profilin1 in human developmental neuro-pathies is discussed.

The Profilin Family

More than 30 years ago, profilin was purified from calf spleen and identified as an actin-binding protein.¹ Later, profilin was found in all cell types and species investigated, including yeast, plants, amoeba, flies or mammals.² Four mammalian profilin isoforms have been identified, including the broadly expressed profilin1, the neuron-specific profilin2 and the more recently discovered, and only poorly described, testis-specific profilin3 and profilin4.³⁴⁵ Although the profilin family members and the profilins from different species share relatively little sequence homologies, the structure and function of these proteins is remarkably conserved.⁶⁷ Profilins catalyze the nucleotide exchange of monomeric actin (G-actin) and funnel ATP-actin to the barbed ends of actin filaments (F-actin).⁸⁹ Thus, profilins can promote actin polymerization by recharging G-actin and by adding it to the filaments’ growing ends. Apart from binding to actin, profilins can interact with phosphoinositides and proteins that contain poly-L-proline-rich domains.¹⁰¹¹ In fact, phospholipid-binding regulates profilin’s interaction with actin and poly-L-proline proteins.¹⁰¹² A plethora of proteins interacting with profilin have been identified (for a review see ref. 2). Among them are Rac and Rho effectors, regulators of membrane trafficking, synaptic scaffolding proteins, and focal adhesion proteins, such as VASP (vasodilator-stimulated phosphoprotein), Mena (mouse homolog of Drosophila enabled), EVL (Ena/VASP-like) and palladin. The large number and heterogeneity of interaction partners suggests a role for profilins in various cellular processes. Yet, the physiological relevance of profilin-ligand binding has been shown for only a few interactions. Moreover, whether these

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interactions provide a mechanism to link cellular processes to actin dynamics, or whether they function independently of actin polymerization is still unclear.

Role of Profilin in Cell Migration and Adhesion of Non-Neuronal Cells

A role for profilin activity in cell migration was discovered almost two decades ago in the soil-living amoeba Dictyostelium discoideum.13 Interestingly, the migration phenotype was present only in double mutant cells, but not in mutants lacking either profilin1 or profilin2, suggesting functional redundancy of both profilin isoforms. The importance of profilins for cell migration of multicellular organisms was first observed upon mutating the Drosophila melanogaster profilin-gene chickadee.14 Moreover, silencing profilin1 expression in human vascular endothelial cells slows down migration.15,16 These data led to the general idea that profilin1 fulfills a pro-migratory function in various species and cell types. However, this idea was challenged by studies demonstrating lowered expression levels of profilin1 in various adenocarcinoma cell lines, suggesting that, in these cells, a reduction of profilin1 activity is required for cell migration and cancer cell invasion.17-20 Loss or profilin1 activity also enhances motility of breast cancer cells as well as motility of normal mammary epithelial cells; conversely, a moderate overexpression of profilin1 inhibits cell motility.21,22 Altogether, these studies show that profilin1 functions in a more complex and cell-type specific manner than initially anticipated. Interestingly, defective cell migration upon profilin1 manipulation is associated with impaired formation of focal adhesions and adherence junctions suggesting that primary defects in cell-matrix and cell-cell adhesions contribute to the observed migration phenotypes.15,21,23 Both the actin and the poly-L-proline-binding domains of profilin1 appear to be important for cell migration.16 Poly-L-proline ligands of profilin1 that are of particular relevance for cell adhesion and migration are those directly involved in cytoskeletal dynamics such as members of the Ena/VASP protein family or the Rac/Rho effectors WASP (Wiskott-Aldrich syndrome protein), WAVE (WASP family verprolin-homologous protein) and mDia (mammalian diaphanos).24-27 Since all these profilin1 interaction partners were implicated in actin polymerization at or in the proximity of adherence junctions,28-32 it was hypothesized that they act as scaffolds to spatially recruit profilin1-actin complexes to the zone of active actin remodeling.21 Thus, in cell adhesion, profilin1 may be required to organize actin cytoskeletal structures that are needed to reinforce adhesion complexes. Notably, a recent study also linked profilin1’s phospholipid-binding to cell migration via a mechanism independent of actin or poly-L-proline ligands, adding phospholipid-binding to the complexity of profilin1 function in cell migration.33 The studies summarized above highlight the relevance of profilin activity for the crawling-like movement of cultured cells, which depends on lamellipodial actin-rich protrusions that advance a leading edge in the direction of migration. Although cell culture experiments are informative, the remaining open questions are (1) whether or not profilin activity is relevant for the migration of mammalian cells in a tissue context and (2) whether it plays different roles in specific modes of cell migration, e.g. in neuronal migration. To tackle these two important questions, mouse mutants for profilin1 and profilin2 were generated. Inactivation of profilin2 did not affect brain development or neuronal migration in mice.34 Although profilin2 mutant mice expressed normal levels of profilin1 in the brain, they displayed defects in vesicle exocytosis, neurotransmitter release and novelty-seeking behavior. Based on these data, it was suggested that profilin1 is responsible for the diverse functions required for neuronal migration and early brain development.35 A fundamental role of profilin1 for development was also suggested by the early embryonic lethal phenotype of systemic mutants.35 Using conditional mutant mice, profilin1’s relevance for chondrocyte migration and lamellipodia formation as well as proper focal adhesions was shown.36

Profilin1 is Important for Glial Cell Binding and Radial Migration of Neurons

By combining a conditional profilin1 mouse mutant with a nestin-cre transgenic line, we deleted the profilin1 gene specifically in the brain.37 Nestin-cre mediated deletion occurs in all brain cells and starts around embryonic day 10.5, at the onset of brain development. Mutant mice are viable and show a cerebellar hypoplasia, aberrant organization of cerebellar cortex layers, and ectopic cerebellar granule neurons (CGN). An aberrant organization of cerebellar cortex layers as well as ectopic CGN in the molecular layer result from impaired CGN radial migration, as shown by BrdU (5-bromo-2’-deoxyuridine) tracing experiments. Conversely, proliferation of CGN progenitors, CGN survival rates, and axonal outgrowth is normal in the absence of profilin1, suggesting a specific role for profilin1 in CGN migration. Interestingly, the ectopic CGN in profilin1 mutants represent mature neurons which appear to be well-integrated into the neuronal network. This is confirmed by positive staining for CaMKIV (Ca2+/calmodulin-dependent protein kinase IV), a marker for mature and postmigratory CGN,38 and the presence of rosette-shaped VGluT2 (vesicular glutamate transporter 2) signals, typical for presynaptic terminals of CGN innervating mossy fibers.39 The morphological defects are restricted to the neuronal compartment, as we found Bergmann glia organization and morphology to be unchanged in profilin1 mutants. Furthermore, this finding excluded structural defects in glial cells as the primary cause for impaired radial migration of CGN.

The alterations, which ultimately translate into the observed radial migration defect, were revealed by co-culture experiments of glial cells and neurons. As CGN can be separated from glial cells by centrifugation, we were able to plate profilin1-deficient CGN on top of control glial cells and, vice versa, control CGN on top of profilin1-deficient glial cells. In these experiments, we noted a significantly impaired glial cell-CGN interaction. Interestingly, we found glial cell-binding
and radial migration of CGN equally impaired when profilin1 was absent from either CGN or glial cells, demonstrating that—at least under cell culture conditions—profilin1 activity in CGN as well as in glial cells is required for CGN-glial cell adhesion and radial migration. Hence, we suggest that impaired glial cell adhesion is largely responsible for the radial migration defect of CGN. Future analysis of mouse mutants, in which profilin1 is either deleted from CGN or from glial cells, will allow us to dissect whether profilin1 activity in CGN and/or in glial cells is relevant for radial migration in vivo.

Surprisingly, we detected no migration defect of cultured CGN, when they were allowed to migrate in a glial cell-independent fashion. Before CGN migrate radially along glial fibers, they first perform a glial cell-independent tangential migration, which we found to be unaffected in profilin1 mutants. Thus, one can conclude that profilin1 is crucially important for radial migration, yet dispensable for tangential migration. One aspect that should be kept in mind, however, is the presence of profilin2 in the profilin1 mutant cells. It is conceivable that profilin2 has limited functional overlap with profilin1, which might result in a specific rescue in tangentially migrating neurons. Indeed, compensatory mechanisms for profilin1 and profilin2 in neurons were recently suggested.

How Does Profilin1 Control CGN-Glial Cell Adhesion and Radial Migration?

Similar to other radially migrating neurons, CGN express specific cytoskeletal features, including a perinuclear cage of microtubule, a thick band of cortical actin that lines the cell soma and glial cell contact sites beneath the cell body (interstitial junction), and short filopodia (punctae adherentia) that extend from the leading process as it wraps the glial process. In a current model of radial CGN migration, these structures operate synergistically to translocate the nucleus and to move the soma toward the direction of migration. Actin dynamics within the proximal region of the leading process depends on myosin II motor activity, and is controlled by the conserved polarity protein PAR6ε during radial migration. It was suggested that contractility of the actomyosin complex is relevant for pulling the nucleus and soma forward during glial-guided migration. Hence, it is tempting to speculate that regulators of actin dynamics, such as profilin1, are required for the functionality of the acto-myosin complex in the leading process and thereby control radial migration of neurons. Notably, other actin-binding proteins were shown to be relevant for radial migration, such as the F-actin depolymerizing factor n-cofilin. It will therefore be interesting to assess whether actin dynamics and actomyosin contractility in migrating CGN are profilin1-dependent.

A number of studies have confirmed the importance of CGN-glial cell adhesion for radial migration, and different molecules, including astrotactin1 and 2, N-cadherin, neuregulin/erbB4 or notch/jagged1, were implicated in this process. Our analysis adds profilin1 as another novel regulator of CGN-glial cell interaction. In fact, we found reduced levels of vinculin and Mena in junctures of profilin1-deficient CGN and control glial cells. Vinculin is expressed in cell contacts of a variety of cell types, including neurons, where it strengthens the mechanical link between cell-cell adhesion receptors and the actin cytoskeleton. The functional link of profilin1 to cell-cell contact formation is further confirmed by the reduced Mena localization to CGN-glial cell junctions in response to profilin1 inactivation. Mena is a member of the Ena/VASP protein family that is critical for the formation of cell-cell contacts in various cell types. Interestingly, a close interaction of Ena/VASP proteins with profilins was suggested by data showing that these molecules act synergistically in promoting local actin filament elongation and second, a genetic interaction of profilin1 and Mena had been shown in the mouse. Our study directly suggests that in CGN profilin1 acts in common with vinculin and Mena as one possible pathway to control actin dynamics at cell-cell contacts to coordinate glial-guided cell migration. Until now, a role for vinculin or Mena for glial cell-binding of radially migrating neurons has not been demonstrated. However, neutralization of Mena activity in neurons was shown to interfere with radial migration.

Does Profilin1 Contribute to Neuronal Migration Defects in Humans?

Our mouse model demonstrated that genetic mutation of profilin1 has a major impact on radial migration and the development of layered structures in the brain. As yet, profilin1 mutations have not been described in humans. Remarkably, the human profilin1 gene PFN1 is located on a chromosomal region that has been linked to the autosomal dominant disorder Miller-Dieker Lissencephaly Syndrome (MDLS). Additionally, a hereditary cerebellar hypoplasia was recently mapped to this region, but the responsible mutation of this severe neuropathy remains elusive. Lissencephaly is characterized by disturbed neuronal migration, microcephaly and delayed growth. Genetically, haploinsufficiency of PAFAH1B1 is the major cause for the lissencephalic phenotype in MDLS. However, other genes must contribute to MDLS in order to explain the broad spectrum of phenotypes described for patients with MDLS and those with isolated PAFAH1B1 mutations. For example, deletion of the genes encoding 14-3-3ε and CRK enhance the phenotype in MDLS, but it is tempting to speculate on a similar modifier activity of PFN1.

Acknowledgments

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