Interaction of Syntenin-1 and the NG2 Proteoglycan in Migratory Oligodendrocyte Precursor Cells*

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Migration of oligodendrocyte precursors along axons is a necessary prerequisite for myelination, but little is known about underlying mechanisms. NG2 is a large membrane proteoglycan implicated in oligodendrocyte migration. Here we show that a PDZ domain protein termed syntenin-1 interacts with NG2 and that syntenin-1 is necessary for normal rates of migration. The association of syntenin-1 with NG2, identified in a yeast two-hybrid screen, was confirmed by colocalization of both proteins within processes of oligodendroglial precursor cells and by coimmunoprecipitation from cell extracts. Syntenin-1 also colocalizes with NG2 in “co-capping” assays, demonstrating a lateral association of both proteins in live oligodendrocytes. RNA interference-mediated down-regulation of syntenin-1 in glial cells results in a significant reduction of migration in vitro, as does the presence of polyclonal antibody against NG2. Thus syntenin plays a role in the migration of oligodendroglial precursors, and we suggest that NG2-syntenin-1 interactions contribute to this.

The NG2 proteoglycan is a type I membrane protein that is expressed by a variety of immature cells of several embryonic tissue origins including glia, muscle progenitor cells, and pericytes (1). In the central nervous system, expression of NG2 was originally thought to specify oligodendroglial progenitor cells, but more recent data suggest that NG2-expressing cells encompass a wider range of immature glial cells in white and gray matter. These include glia that make synaptic-like contacts with neurons in the hippocampus and cerebellum (2) and glial cells specifically associated with the nodes of Ranvier (3). Interestingly, many NG2-positive cells are both proliferative and motile or exhibit local process motility (4, 5). Antibodies to the NG2 extracellular domain inhibit migration of oligodendroglial progenitor cells and immature Schwann cells in in vitro migration assays (5, 6), and NG2 also plays a role in cell spreading in melanoma tumors, which express melanoma chondroitin sulfate proteoglycan (MCSP), the human ortholog of NG2 (7). Identifying the intracellular NG2-interacting proteins should aid in elucidating the function of this multidomain protein in migratory cells of the oligodendrocyte lineage.

The intracellular domain of NG2 consists of the C-terminal 76 amino acids and has the PDZ (postsynaptic density-95/discs large/gona occludens-1) binding motif QYWV, which can interact with PDZ domain-containing proteins (8). To define relevant intracellular partners of the glycoprotein, we carried out a yeast two-hybrid screen using the complete intracellular domain of NG2 as bait. One of the proteins that we identified is syntenin-1 (also termed MDA-9). Syntenin-1 is a widely expressed PDZ protein that is often overexpressed in highly migratory metastatic tumors including melanoma (9).

Here we demonstrate that syntenin is expressed by primary oligodendrocytes and show functional studies using the oligodendroglial precursor cell line Oli-neu. We provide biochemical, morphological, and functional data demonstrating that NG2 and syntenin-1 form a complex, which we suggest is one component that regulates oligodendroglial precursor migration.

EXPERIMENTAL PROCEDURES

Animals—NMRI mice were obtained from the Central Animal Facility of the University of Mainz.

Antibodies—The following primary antibodies were used: polyclonal (pc)4 antibodies against rat syntenin-1 (10), against syntenin-1 (generated using a synthetic peptide corresponding to amino acids 286–298 or 250–C terminus in mouse; Synaptic Systems, Göttingen, Germany, and Abcam, Cambridge, UK), against EGFP (BD Biosciences), and against AN2 (which recognizes mouse NG2) (5); and monoclonal antibodies against AN2 (which recognize mouse NG2) (5) and monoclonal antibodies against AN2 (5), against glial fibrillary acidic protein (GFAP; Boehringer, Mannheim, Germany), against neurofilament (SNI-31; Sternberger Monoclonals Inc., Lutherville, MD), against PLP (AA3; M. B. Lees, Waltham, MA), against myelin oligodendrocyte glycoprotein (8-18-C5; C. Linington, Aberdeen, Scotland), against

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4 The abbreviations used are: pc, polyclonal; EGFP, enhanced green fluorescent protein; GFAP, glial fibrillary acidic protein; siRNA, small interfering RNA; PBS, phosphate-buffered saline; X-gal, 5-bromo-4-chloro-3-indolyl-β-d-galactoside; div, days in vitro; PLP, proteolipid protein; GRIP, glutamate receptor interacting protein; CNP, 2',3'-cyclic nucleotide 3'-phosphodiesterase.
β-tubulin isotype III (SDL3D10; Sigma-Aldrich, Munich, Germany), and against CNP (11-5B; Sigma-Aldrich).

Cell Culture and Transfection—HEK293T cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum and 2 mM glutamine. Primary oligodendrocyte cultures were prepared from embryonic day 14–16 mice as described previously (11). Cells were grown on poly-L-lysine-coated coverslips in modified Sato medium (12) supplemented with B27, 10 ng/ml platelet-derived growth factor, 5 ng/ml basic fibroblast growth factor, and 1% horse serum.

Proliferation of astrocytes was prevented by treatment with mitomycin C (Sigma-Aldrich) at 10 µg/ml for 2.5 h when the primary cells were used for Western blot analysis. The Oli-neu cell line was cultured on poly-L-lysine-coated coverslips in Sato medium containing 1% horse serum according to Trotter and co-workers (13).

Expression vectors were transfected by conventional electroporation (20 µg/300 µl, 4 × 10⁶ cells/ml). 2 mM sodium butyrate was added to enhance expression of constructs with a cytomegalovirus promoter. Syntenin-1-directed synthetic siRNA (target sequences TAGTGCAGCATAGCATTTA and CAGATTGGAGATTGATA, 80 pmol each) and non-silencing control siRNA (target sequence AATTCCTCGAGGCTTGCAGT, 160 pmol) were purchased from Qiagen and nucleofocted into 10⁶ Oli-neu cells using the AMAXA basic nucleofection protocol for primary mammalian neural cells.

Coimmunoprecipitation—HEK293T cells were transfected with EGFP-syntenin-1 (10) and the NG2del construct, which contains one-fourth of the extracellular domain, the complete transmembrane domain, and the cytoplasmic tail of NG2 (8). 24 h after transfection, cells were washed with phosphate-buffered saline (PBS), incubated for 1 h in methionine/cysteine-free medium, and metabolically labeled with 100 µCi/ml [³⁵S]Met/Cys for 4 h. Cells were washed twice with Hank’s balanced salt solution fortified with cold Met/Cys and then lysed on ice in 1% Triton X-100, 50 mM Tris, pH 7.4, 150 mM NaCl, and a protease inhibitor mixture of iodoacetamide (18 mg/ml in H2O), phenylmethylsulfonyl fluoride (100 mM in isopropyl alcohol), pepstatin (5 mg/ml in Me₂SO), antipain (1 mg/ml in Me₂SO), aprotinin (1 mg/ml in H₂O), benzamidine HCl (26 mg/ml in H₂O), and leupeptin (5 mg/ml in Me₂SO). The lysates were chilled for 30 min and centrifuged at 300 × g for 5 min to remove nuclei. For immunoprecipitation the following antibodies were used: rabbit pc AN2, rabbit pc EGFP, and rabbit pc syntenin-1 (Synaptic Systems).

Isolation of Syntenin-1 with Yeast Two-hybrid Screen—The entire 76-amino acid C-terminal region of mouse NG2 (NH₂-RKRKNKT...NGQYWW-COOH, GenBank™ accession number AF352400) was fused to the Gal4 binding domain by cloning it into the pGBT9 vector (Clontech) with XbaI/HindIII. The resulting bait construct was designated pGBT9cyto. Using the lithium acetate method, the yeast strain CG1945 was transformed sequentially with pGBT9cyto and a 9–12-week-old postnatal mouse brain MATCHMAKER cDNA library in pACT2 (Clontech). 33 × 10⁶ transformants were screened. Transformants were grown on synthetic defined media/Leu⁻/Trp⁻/His⁻ plates; 5 mM 3-amino-1,2,4-triazole was added to the medium to suppress leaky HIS3 reporter gene expression. Positive clones were tested for β-galactosidase gene activity.

Yeast colonies were grown on synthetic defined media/Leu⁻/Trp⁻/His⁻ plates, transferred onto reinforced nitrocellulose membrane, submerged in liquid nitrogen, and placed on a Z-buffer/X-gal solution-soaked Whatman paper (Z-buffer: 16.1 g/liter Na2HPO4·7H2O, 5.5 g/liter NaH2PO4·H2O, 0.75 g/liter KCl, 0.246 g/liter MgSO4·7H2O, pH 7; Z-buffer/X-gal solution: 100 ml of Z-buffer, 0.27 ml of β-mercaptoethanol, 1.67 ml of 20 mg/ml X-gal stock solution). Blue color was allowed to develop for 30 min to 3 h. The specificity of the NG2-syntenin-1 interaction was confirmed by β-galactosidase activity.
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The growth selection of cotransformed yeast cells was confirmed with pGBT9cyto and isolated library plasmids.

To map the PDZ binding motif at the C terminus of NG2, individual mutations of the 0-, 1-, 2-, and 3-positions of the C-terminal peptide QYWV* were introduced by PCR; cloned into pGBT9; and designated NG2 0G (Val mutated to Gly), NG2 1G (Trp to Gly), NG2 2G (Tyr to Gly), NG2 2F (Tyr to Phe), and NG2 3G (Gln to Gly). Mutant NG2 constructs were cotransformed with full-length syntenin-1 and syntenin-1-PDZ1/2. Yeast cells were grown on double dropout medium, assayed for β-galactosidase gene activity, and additionally selected for growth on triple dropout medium.

Scratch Migration Assay—Transfected cells were plated on poly-l-lysine-coated gridded coverslips (15 mm; Bellco Glass). Cells were cultured until they reached 80–90% confluence but were kept at least 16 h prior to experimental manipulation. Cell-free areas were generated by gently scratching the cell monolayer with a sterile blue Gilson pipette tip. Coverslips were subsequently washed with PBS and placed in 3-cm dishes containing preconditioned Sato medium. Phase images (×40 magnification) were captured approximately every 6 h over a period of 1.5 days. The "magnetic pen tool" of Photoshop CS2 was used to manually define the area covered by cells. The marked (covered) areas were exported and quantified using Image-Pro Plus software (MediaCybernetics). The software calculated the marked area as a percentage of the whole picture. The results at each time point were normalized by subtracting the value of the starting area covered by the cells. In this study we have equated the percent increase of marked areas with the migration of the cells.

RESULTS

Identification of Syntenin-1 as an Intracellular Partner of NG2 via Yeast Two-hybrid Screening—To elucidate the mechanism of NG2 function in migration, we sought to identify novel NG2-interacting proteins using a yeast two-hybrid analysis. The complete mouse NG2 cytoplasmic region consisting of 76 amino acids (RKRN...QYWV*, where * indicates the translation stop codon) was used as a bait to screen a postnatal day 76 amino acid library (Clontech). Screening of 33 × 10^6 transformants identified several potential binding partners including GRIP (8) and four independent different library plasmids encoding the PDZ protein syntenin-1 (14). Each plasmid contained the entire syntenin-1 cDNA, which corresponds to the published mouse sequence (14). The interaction was verified by cotransforming yeast cells with NG2 and syntenin-1. The transformed yeast cells were viable on double and triple dropout media and in addition tested positive in the β-galactosidase assay (Fig. 1A, row 1). This finding suggests that syntenin-1 is a novel interacting partner of NG2.

The C terminus of NG2 displays a canonical PDZ binding motif (QYWV). To validate the requirement of the PDZ motif for the interaction of NG2 with syntenin-1, we carried out point mutation analyses and found that the terminal three amino acid residues in the PDZ recognition site of NG2 are important for binding to the PDZ domains of syntenin-1 (Fig. 1A, rows 2–7). These data demonstrate that the PDZ binding motif QYWV of NG2 is critical for the binding to syntenin-1.

Syntenin-1 harbors a tandem repeat of two PDZ domains. To determine which PDZ domain is required for the interaction with NG2, we carried out deletion analyses. Syntenin-1 deletion mutants were generated by PCR using full-length mouse syntenin-1 as a template, were cloned into EcoRI/BamHI sites of the pACT2 vector, and were verified by sequencing. The following deletion mutants were tested for interaction with NG2: syntenin-1 N terminus-PDZ1 (MSLYP-HKDS), syntenin-1-PDZ1 (RAEI-HKDS), syntenin-1-PDZ2 (FERTV-TPEV*), and syntenin-1-PDZ1/2 (RAEI-TIPEV*). Both PDZ domains were required for binding to NG2 (Fig. 1B).

| Construct | Syntenin full length | Syntenin PDZ1-2 |
|-----------|---------------------|-----------------|
| wt (QYWV*) | +                  | + |
| NG2 -0G (QYWV*) | +                  | + |
| NG2 -1G (QYWV*) | +                  | + |
| NG2 -2G (QYWV*) | +                  | + |
| NG2 -3G (QYWV*) | +                  | + |
| NG2 -2F0G (QYWV*) | +                  | + |

FIGURE 1. Syntenin-1 binds to NG2 via both PDZ domains. A, the amino acids tryptophan, tyrosine, and valine in the PDZ binding motif of NG2 are essential for binding to syntenin-1. Yeast cells were transformed with full-length syntenin-1 or a syntenin-1 deletion mutant containing PDZ1/2 together with different PDZ binding motif mutants of NG2. These were then tested for β-galactosidase (beta-Gal) activity in triple amino acid-deficient medium. nd, not determined. B, both PDZ domains of syntenin-1 are required for binding to NG2. Yeast was transformed with the cytoplasmic tail of NG2 together with different syntenin-1 deletion mutants and tested for β-galactosidase activity and growth in triple amino acid-deficient medium. C, a schematic diagram of syntenin and NG2 is shown. PDZ indicates two postsynaptic density-95/discs large/zona occludens-1 domains; LNS indicates two laminin G/neurexin/sex hormone-binding globulin domains; TM indicates the transmembrane domain; and the arrow indicates the position of the deletion (amino acids aa 478-2164) in NG2del. The amino acids QYWV represent the PDZ binding motif. wt, wild type; Mm, Mus musculus.
Syntenin-1 Is Expressed by Immature NG2-positive and by More Mature Oligodendrocytes in Vitro—We determined the expression of syntenin-1 in primary oligodendrocytes and the cell line Oli-neu (Fig. 2). Lysates of primary oligodendrocytes (2 div), Oli-neu cells (Fig. 2A, lanes 2 and 3), and total brain were subjected to Western blot analysis using the syntenin-1 antibody. We found that syntenin-1 is expressed in both primary oligodendrocytes and Oli-neu cells (Fig. 2A, lanes 2 and 3). Total brain lysate served as a positive control to validate the 36-kDa syntenin-1 band (lane 1). Interestingly, the highly migratory Oli-neu cells reveal a high expression of syntenin-1. These results show that syntenin-1 is expressed in oligodendroglial cells.

Next, we characterized the developmental profile of syntenin-1 in primary oligodendrocytes and the cell line Oli-neu (Fig. 2B). The expression of syntenin-1 increases with time in culture. The decrease of NG2, which is expressed by immature oligodendrocytes, and the increase of PLP, a marker for more mature oligodendrocytes, suggest that the increase in syntenin-1 expression parallels oligodendrocyte maturation. The possibility that the syntenin-1 signals arise from neuronal and/or astrocytic contamination was excluded by controlling for expression of the neuronal protein neurofilament and the astrocyte-specific protein GFAP. We did not observe detectable amounts of neurofilament, and the low amount of GFAP expression did not correlate with the increase of syntenin-1 over time. These results suggest an increase in syntenin-1 expression in cultures of maturing oligodendrocytes.

To corroborate this finding, we subjected cultured oligodendrocytes to immunocytochemistry using stage-specific markers for oligodendrocytes. We found syntenin-1 expression at different stages of oligodendrocyte differentiation (Fig. 3). All NG2-positive cells at 2 div expressed syntenin-1. In addition, we found that many more mature oligodendrocytes in the culture, identified by CNP, myelin oligodendrocyte glycoprotein, and PLP expression, also express syntenin-1. These data demonstrate that syntenin-1 is expressed by immature as well as by differentiated oligodendrocytes. The extensive syntenin-positi

Coimmunoprecipitation of NG2 and Syntenin-1—To validate the interaction of syntenin-1 and NG2 biochemically,
HEK293T cells were cotransfected with a plasmid encoding EGFP-syntenin-1 (10) and a plasmid encoding NG2del (encompassing one-fourth of the extracellular domain, the complete transmembrane domain, and the cytoplasmic tail of NG2) (Fig. 1C) (8). A complex of EGFP-syntenin-1 and NG2 was isolated from the transfected cells after metabolic radiolabeling by precipitation with polyclonal AN2 antibodies against NG2del and polyclonal EGFP antibodies. Lane 1, preclear; lane 2, non-transfected cells, immunoprecipitation with pc AN2; lane 3, transfected cells, immunoprecipitation with pc AN2; lane 4, transfected cells, immunoprecipitation with pc EGFP. B, Oli-neu cells were transfected with the EGFP-syntenin-1 plasmid, and lysates were subjected to immunoprecipitation. Lane 1, non-transfected cells, immunoprecipitation with pc AN2; lane 2, transfected cells, immunoprecipitation with pc AN2; lane 3, transfected cells, immunoprecipitation with pc EGFP; lane 4, transfected cells, immunoprecipitation with pc syntenin-1.

NG2 Colocalizes with Syntenin-1 in NG2-expressing Primary Glial Cells in Vitro—Primary oligodendrocytes were double-labeled with monoclonal AN2 antibody, which recognizes NG2, and with polyclonal antibody against syntenin-1 (Fig. 5, A–C). Although NG2 was predominantly localized at the cell surface, on the cell body, and in cell processes, syntenin-1 is expressed throughout the whole cell in a punctate manner. Strikingly, we found that NG2 and syntenin-1 colocalize at the surface of the cell as indicated by the yellow punctate fluorescence in the overlay (Fig. 5, A–C). The colocalization is particularly intense at distinct varicosities in the processes. These regions may indicate points where branching has just started.

Capping of NG2 on Glial Cells Causes a Parallel Redistribution of Syntenin-1—To corroborate that NG2 and syntenin-1 form a complex in primary oligodendrocytes, we performed a co-capping assay (Fig. 5, D–I). Clustering and parallel movement of NG2 and syntenin-1 were explored by incubating live oligodendrocytes (2 div) with monoclonal AN2 antibody for 10 min at 4 °C. This was followed by the addition of a fluorescent secondary antibody and incubation at 37 °C. Patching and capping of NG2 were induced. A similar redistribution of a population of endogenous syntenin-1 resulted in clustering of NG2 and syntenin-1 at or near the cell surface, bolstering our finding that NG2 and syntenin-1 associate in oligodendrocytes.
Syntenin-1 Promotes Migration of Oli-neu Cells—NG2 has been implicated in spreading and migration (4–6, 16, 17). If syntenin-1-NG2 binding were functionally important, loss of syntenin-1 should reduce the migratory behavior of Oli-neu cells and primary NG2-positive oligodendrocytes. We took an siRNA approach to acutely knock down syntenin-1 in Oli-neu cells. Oli-neu cells were transfected with either control siRNA or syntenin-1 siRNA. By Western blot analysis (Fig. 6, inset), we found an efficient knockdown of syntenin-1 in Oli-neu cells 16 h after transfection as compared with control siRNA-transfected cells (Fig. 6A, inset). Western blot analysis at different times after transfection demonstrated that the syntenin knockdown was stable at least 54 h after transfection (data not shown) and thus maintained during the whole time course of the migration assays. Control and syntenin-1 siRNA-transfected Oli-neu cells were then analyzed in a scratch assay. Knockdown of syntenin-1 significantly reduced the migration of Oli-neu cells as compared with control cells (Fig. 6C). Inclusion of polyclonal AN2 antibody directed against the whole NG2 molecule, which recognizes primarily the protein core (5), reduced migration to a greater extent than syntenin-1 siRNA alone (Fig. 6D). When both these approaches were combined in the assay, a stronger inhibition of migration was found than with either syntenin-1 siRNA or AN2 antibodies alone, suggesting that the signaling pathways in the migration-promoting effects of NG2 and syntenin are not completely overlapping. These data demonstrate that like NG2, syntenin-1 promotes migration of Oli-neu cells.

DISCUSSION

PDZ Proteins as Partners of NG2—The NG2 proteoglycan binds to several PDZ domain proteins with its C-terminal QYVV motif. This has been shown for MUPP1 and GRIP (8, 18). PDZ domain proteins act as intracellular scaffolds coordinating and compartmentalizing molecules involved in signal transduction. The majority of the PDZ domain-containing proteins are associated with plasma membrane proteins, and they are generally restricted to specific subcellular domains such as synapses or cell-cell contact points (19). Here, we characterize syntenin-1 as an additional intracellular ligand of NG2.

Expression of Syntenin-1—Syntenin-1 was first identified as an adhesion molecule neurofascin (10). In vivo subpopulations of neurons have been reported to express syntenin-1; cell bodies and dendrites of pyramidal cells show diffuse expression of syntenin-1 (23).

Cloning of mouse and rat syntenin-1 (also known as melanoma differentiation-associated gene-9, or MDA-9) revealed that the molecules are highly homologous (9): rodent and human MDA-9/syntenin-1 are nearly identical at the level of PDZ-1 and the C-terminal domains. The N-terminal portion is more divergent: in humans this domain is 81 and 77% identical to the mouse and rat domains, respectively. A second family member, syntenin-2, also exists (10).

Cell lysates from cultured primary oligodendrocytes, the cell line Oli-neu, and whole mouse brain homogenate all show expression of syntenin-1 by Western blotting and immunofluorescence. Both a membrane-associated and a cytosolic distribution of the protein were observed, similar to observations in lymphocytes (24). In both Oli-neu and primary cells, the intracellular staining at high resolution often appeared punctuated and may represent vesicular association.
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Colocalization of NG2 and Syntenin-1—In primary oligodendrocyte cultures, an intense colocalization of NG2 and syntenin-1 at varicosities in oligodendroglial progenitor cell processes was seen. Recent work reported that in neurons overexpression of syntenin-1 increased the number of dendritic protrusions (25).

Subpopulations of syntenin-1 and NG2 co-cap. Syntenin-1 is a component of early secretory pathways (26) and has been found to be expressed by early apical recycling endosomes (27) and to bind to the GTPase Rab5 (28).

Only a subpopulation of syntenin-1 colocalizes with NG2, further reiterating that syntenin-1 (like NG2) has several binding partners and that only a subpopulation of syntenin-1 interacts with NG2 at any given time point. Namely, it has been reported that NG2 can interact with MUPP1 (18) or GRIP1 (8). At present, no experimental evidence exists that MUPP1 or GRIP1 is involved in the migration or process motility of NG2-positive glia or oligodendrocyte progenitors. Our results suggest that a subset of NG2 molecules cooperates with syntenin-1 in a molecular complex required for cellular motility, whereas the interaction with MUPP1 or GRIP1 is important for other cellular functions (discussed in Refs. 8 and 18). Our results are similar to observations in T and B lymphocytes where a subcellular functions (discussed in Refs. 8 and 18). Our results are similar to observations in T and B lymphocytes where a subcellular functions (discussed in Refs. 8 and 18). Our results are similar to observations in T and B lymphocytes where a subcellular functions (discussed in Refs. 8 and 18). Our results are similar to observations in T and B lymphocytes where a subcellular functions (discussed in Refs. 8 and 18). Our results are similar to observations in T and B lymphocytes where a subcellular functions (discussed in Refs. 8 and 18).

Molecular Characteristics of NG2-Syntenin-1 Binding—The interaction of syntenin-1 with NG2 requires both PDZ domains; it has not been ascertained whether the binding is cooperative. Syntenin-1 has the capacity to multimerize (10); this may explain the observation that an endogenous syntenin-1 band was detected in immunoprecipitates of Oli-neu cells expressing EGFP-syntenin-1. PDZ domains have the propensity to multimerize as homomultimers or heteromultimers (30) and can multimerize via PDZ-independent mechanisms (31). Moreover, not all interactions with syntenin-1 require both domains (32). Syntenin-1-NG2 interaction does not thus exclude simultaneous binding of other ligands. The ability to form multimers further increases the possibility of simultaneous interaction with several ligands.

Functional Implications of the NG2-Syntenin-1 Interaction—Actin-binding domains have not been identified in the cytoplasmic tail of NG2; however, NG2 plays a role in migration, metastasis, and adhesion complexes. The association of NG2 with downstream cytoskeletal machinery must therefore be mediated by linker proteins. The high expression levels of both NG2 and syntenin-1 in immature glia correlate with the motility of these cells as well as the localized motility of their processes. Knockdown of syntenin-1 reduces migration of Oli-neu cells. Syntenin-1 may be an adaptor molecule that connects NG2 to downstream components and may play a role in mediating cytoskeletal changes during the movement of NG2-expressing cells and/or their processes. However, in many cell types NG2 interacts in cis with integrins, which may promote migration independent of syntenin (33). Similarly, syntenin-1 may additionally promote migration by mechanisms independ-
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