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Overlapping Expression of ARFGEF2 and Filamin A in the Neuroependymal Lining of the Lateral Ventricles: Insights into the Cause of Periventricular Heterotopia

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ABSTRACT

Periventricular heterotopia (PH) is a malformation of cortical development characterized by nodules of neurons, ectopically located along the lateral ventricles of the brain. Mutations in the vesicle transport ADP-ribosylation factor guanine exchange factor 2 gene (*ARFGEF2*) or the actin-binding Filamin A (*FLNA*) gene cause PH. Previous studies have shown that FLNA expression is developmentally regulated, with strongest expression observed along the ventricular zone (VZ) and to a lesser degree in postmitotic neurons in the cortex. Here we characterize the expression patterns for ARFGEF2 within the central nervous systems of human and mouse in order to better understand their potential roles in causing PH. *ARFGEF2* mRNA was widely expressed in all cortical layers, especially in the neural precursors of the ventricular and subventricular zones (SVZ) during development, with persistent but diminished expression in adulthood. *ARFGEF2* encodes for the protein brefeldin-inhibited guanine exchange factor 2 (BIG2). BIG2 protein immunoreactivity was most strongly localized to the neural progenitors along the neuroependymal lining of the VZ during development, with decreased expression in adulthood. Furthermore, overlapping BIG2 and FLNA expression was greatest in these same neuroependymal cells of human embryonic brain and was co-expressed in progenitors by Western blot. Finally, transfection of a dominant-negative construct of *ARFGEF2* in SHSY5Y neuroblastoma cells partially blocked FLNA transport from the Golgi apparatus to the cell membrane. These results suggest that mutations in *ARFGEF2* may impair targeted transport of FLNA to the cell surface within neural progenitors along the neuroependyma and that disruption of these cells could contribute to PH formation. J. Comp. Neurol. 494:476–484, 2006.

Indexing terms: ARFGEF2; Filamin A; periventricular heterotopia

Periventricular heterotopia (PH) is a malformation of cortical development characterized by the failure of a subset of neurons to migrate into the cerebral cortex. This migrational arrest results in the formation of neuronal nodules, ectopically positioned along the lateral ventricles of the brain and beneath an otherwise normal appearing cerebral cortex (Sheen and Walsh, 2003). Prior studies have shown that mutations in the actin-binding Filamin A (*FLNA*) gene cause the more common X-linked dominant form of PH (Fox et al., 1998). More recently, an autosomal recessive form of PH has been attributed to mutations in the vesicle transport ADP-ribosylation factor guanine exchange factor 2 (*ARFGEF2*) gene (Sheen et al., 2004).

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FLNA encodes a large (280-kDa) cytoplasmic actin-binding phosphoprotein that links the actin cytoskeleton to reportedly more than 30 cellular proteins (Stossel et al., 2001). These direct FLNA interactors demonstrate great functional diversity including 1) regulation of cortical actin networks through molecules including β-integrin and the Rho family of small GTPases; 2) interaction with transmembrane receptors and signaling molecules such as the dopamine and G-protein-coupled calcium sensing receptors; and 3) serving as signaling scaffolds with diverse intracellular cell signaling kinases, phosphatases, and adaptor molecules such as SH2 containing inositol polyphosphate 5-phosphatase (SHIP-2) or SAPK/ERK kinase (SEK1). Most commonly, FLNA interactions with integrins and extracellular matrix (ECM) adhesion proteins have been implicated in migration (Meyer et al., 1997; Loo et al., 1998; Dulabon et al., 2000; Calderwood et al., 2001), in which loss of adhesion may disrupt the initial attachment by neuroblasts onto radial glial fibers (Dulabon et al., 2000). Alternatively, FLNA directly binds the actin cytoskeleton, and FLNA-deficient melanocytes fail to undergo locomotion in response to factors that elicit migration (Cunningham et al., 1992); both observations are suggestive of a motility problem. Thus, although PH due to *FLNA* mutations has been broadly viewed as a disorder of neuronal migration, exactly where this disruption occurs en route as neuroblasts migrate from the ventricular zone to the cortical plate is not clear.

The ADP ribosylation factor (Arf) GTP-binding proteins are major regulators of vesicle trafficking, and the Arf guanine nucleotide exchange factors (GEFs) regulate the cycling of Arf proteins between the inactive GDP-bound and active GTP-bound forms. There are various GEFs that interact with the ARFs (ARFGEF), but all uniformly share a Sec7 domain, thought to be necessary for guanine-nucleotide exchange activity (Togawa et al., 1999; Pacheco-Rodriguez et al., 2002). *ARFGEF2* is one of three large molecular weight GEFs in the brain (Brefeldin-inhibited GEF1 [BIG1] and Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 [GBF1] being the other two). *ARFGEF2* encodes for the protein brefeldin-inhibited GEF2 (BIG2) (Chavrier and Goud, 1999; Cox et al., 2004). BIG2 regulates the vesicle trafficking of some key proteins, possibly adhesion molecules such as β-catenin and E-cadherin (Sheen et al., 2004), and BIG2 inhibition also causes retraction of axonal and dendritic growth cones in vitro (Jareb and Banker, 1997; Ruthel and Banker, 1999). Impairments in neurite extension and/or intercellular adhesion could potentially lead to PH in an analogous fashion by causing retraction of the leading process of migratory neurons or weakening of the neuroependymal lining.

The virtually identical characteristic of bilateral nodular heterotopia in PH from *ARFGEF2* and *FLNA* mutations suggests that these causative genes may share a final common mechanistic pathway. In this paper, we further characterize the *ARFGEF2* mRNA and BIG2 protein expression patterns during cortical development in both mice and human central nervous system (CNS) and investigate the possible functional relationship between *Filamin A* and *ARFGEF2*. The developmentally regulated CNS expression of *ARFGEF2*/BIG2, the overlapping co-expression between FLNA and BIG2 in neuroependymal cells along the lateral ventricles, and the disruption of FLNA transport by *ARFGEF2* inhibition all support the hypothesis that dysregulation of FLNA and BIG2 in the neural progenitors along the neuroependymal lining could lead to PH.

MATERIALS AND METHODS

Animals and human tissues

Our study using discarded human tissues was approved by the Institutional Review Boards at the Beth Israel Deaconess Medical Center and Brigham and Women's Hospital. De-identified human discarded tissue between 19 and 22 gestational weeks was obtained during autopsy from individuals with no known neurological disorders. The study includes Swiss-Webster (Taconic Farms, Germantown, NY) and C57bl/6J mice (Jackson Laboratories, Bar Harbor, ME) from our institutional colony. The mice were housed and treated in accordance with protocols approved by the IACUC of Harvard Medical School.

Cellular dissociation, isolation, and culture

Methods for ventricular zone dissection and dissociation of human and mice subventricular zone tissue follow general guidelines used previously in murine cortical cell dissociations (Sheen and Macklis, 1995; Bahn et al., 2002). In brief, samples are obtained along the periventricular zone within the frontal cortex, minced, washed in cold Hanks' buffered saline solution (HBSS), and placed in trypsin solution at 37°C for 30 minutes. The sample is then passed through a cell strainer to isolate single cells and washed in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum to inactivate the trypsin. The dissociated cells are spun down, the media is aspirated, and cells are placed in neurosphere medium (NM, Cambrex bullet kit, Walkersville, MD) containing epidermal growth factor (EGF), fibroblast growth factor (FGF), neural cell survival factor, gentamycin, and amphotericin B for expansion. The cultures are maintained in a 37°C/5% CO_2 incubator and media are aspirated and renewed on a weekly basis. Individual neurospheres can be dissociated and reexpanded to ensure a clonal population. HEK293 and SHSY5Y cell lines were grown in DMEM (Gibco, Carlsbad, CA), 10% v/v fetal calf serum, and 1% penicillin-streptomycin in a 37°C/5% CO_2 incubator.

Mouse in situ hybridization

The in situ hybridization was carried out by a previously published method (Sheen et al., 2004). The probes were obtained from polymerase chain reaction (PCR) products using *ARFGEF2* expressed sequence tag (EST) templates from mouse EST clones BF181048 and AI413956. The *ARFGEF2* primers contained flanking T7 and T3 promoters. Sense control probes examined gave no specific hybridization.

Microarray assay

The methods follow modifications from previously published work on gene expression in human neural stem cells (Wright et al., 2003). Microarray analysis is performed by Expression Analysis (Affymetrix® Genomic Processing Facility in Durham, NC) using the Affymetrix HU 133 plus 2.0 chip. Total RNA (10 μg) is extracted from the neural precursors and labeled. The target of labeled

cRNA is hybridized to the GeneChip, expression values are calculated based on the difference of the perfect match (PM) oligos and mismatch (MM) in the probe set, the signal values from each array are normalized, and a data file is generated. Data sets are analyzed by a pairwise comparison and Wilcoxon's signed-rank test to derive biologically meaningful results.

Cell transfection

The BIG2 (E738K) mutant construct subcloned into pcDNA4-HAN with an N-terminal HA tag was previously described (Shinotsuka et al., 2002). HEK293 cells or SHSY5Y cells grown in eight-well Biocoat chamber slides (Becton Dickinson, San Jose, CA) were transfected with expression vectors encoding HA-tagged BIG2 (E738K) by using the FuGene6 regent. Wild-type BIG2 contains the Sec7 domain, which catalyzes the conversion of GDP to GTP in the GEFs, thereby activating the ARFs. Thus, transfection of wild-type BIG2 could potentially have unintended effects. A GFP construct was felt to be a more reasonable control. After 24 hours, the cells were harvested for Western blot or fixed for immunocytochemistry.

Western blot

Proteins were extracted from both transfected and untransfected HEK293 cells by previously described methods (Wang et al., 1998). Briefly, cells were solubilized in lysis buffer, separated on a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and transferred onto PVDF membrane. The membrane was probed with the appropriate antibody and detected by enhanced chemiluminescence.

Immunohistochemistry

The appropriately aged mice were perfused with saline and fixative (2% paraformaldehyde in phosphate-buffered saline [PBS]). Coronal sections (50 μm) were cut on a Vibratome, permeabilized with Tween-20, and washed. Cell cultures were washed and fixed with 4% paraformaldehyde. Samples were treated with protease (type XXIV, Sigma, St. Louis, MO), placed in blocking solution with PBS containing 10% fetal calf serum, 5% horse serum, and 5% goat serum, incubated overnight in the appropriate antibody (BIG2: generated by Sigma, cat. no. GN-9790); the immunizing antigen is RLKHSQAQSK, raised in rabbit. No cross-reactivity with BIG1 is found in cytosol and microsomal fractions of in HepG2 and HeLa cells by Western blot. Antibody staining co-localizes with GM-130, 58K, and AP-1 in the perinuclear region and also distributes throughout the cells in a punctate pattern by immunohistochemistry consistent with a function in the Golgi apparatus. The localization of BIG2 antibody staining is also similar to that seen with transfected myc-tagged BIG2 (following staining with an antibody against myc; Yamaji et al., 2000; Filamin A: Novocastra, Newcastle, UK, cat. no. NCL-FIL); the immunizing antigen is platelet filamin membrane, epitope unknown, raised in mouse, clone number PM6/317.

Positive controls include skin by immunohistochemistry and skeletal muscle by Western blot analyses, processed through standard avidin/biotin amplification (Vectastain, Vector, Burlingame, CA) or fluorescent secondaries (CY3, Jackson Immunoresearch, West Grove, PA and FITC, Sigma). Specimens were examined by using through-light or fluorescence microscopy after mounting in appropriate media. Cells staining positive for Filamin A or expressed markers were counted with respect to the total number of cells in five randomly chosen microscopic fields (0.072 mm^2; magnification 400×) across the long axis of each object; an average of 200 cells was sampled on each well, and the results shown represent values from three wells per treatment.

Statistical analysis

All data are depicted as means ± SD and represent the observations from three to five independent experiments, with three to four replicates for each data point. Data were analyzed for statistical significance by using t-tests in which $P \leq 0.05$ was set as the minimum level of significance.

RESULTS

Expression of *ARFGEF2* messenger RNA throughout developing cerebral cortex and cerebral wall

To examine whether the temporal and spatial patterns of *ARFGEF2* expression were consistent with a role in affecting neuronal migration and proliferation, messenger RNA levels of *ARFGEF2* were evaluated in the brains of embryonic, early postnatal, and adult mice.

Throughout the developing embryo (embryonic day 17 [E17]), low levels of *ARFGEF2* mRNA were widely distributed both inside and outside the CNS (Fig. 1). High levels of *ARFGEF2* mRNA staining could be appreciated in the ventricular (VZ) and subventricular zones (SVZ), the cerebral cortex, the striatum, and the spinal cord. Within the cerebral wall and cortex, RNA message was seen more clearly in the VZ/SVZ and cortical plate, compared with the intermediate zone, although this observation could partially reflect the cell densities in the respective layers. Levels of ARFGEF2 mRNA in the CNS diminished with increasing age and in adult mice were detectable primarily in the hippocampus and cerebellum. Extra-CNS expression was seen in the somites, thymus, intestines, and brown fat of embryonic mice.

ARFGEF2 mRNA expression within the central nervous system complements the pattern previously seen with *FLNA* mRNA (Fig. 2). At E12.5, *ARFGEF2* mRNA was seen in the developing VZ and SVZ. At E16.5, expression of *ARFGEF2* showed predominant staining of the periventricular region, medial ganglionic eminence, and cortical plate. A similar pattern was present but was diminished in early postnatal mice. Finally, lower levels of *ARFGEF2* mRNA generally persisted throughout the adult cortex, although labeling was still seen in the dentate gyrus and CA area of the hippocampus.

Expression of ARFGEF2 protein (BIG2) throughout the developing cerebral cortex and cerebral wall

Given that the overlapping patterns of *ARFGEF2* and *FLNA* mRNA expression in cortex and cerebral wall implicate these genes in neural progenitor and migratory neuron development, we performed immunohistochemical studies by using antibodies generated against BIG2 to localize protein expression. Western blot analysis with the BIG2 antibody demonstrates an approximate 180-kDa band corresponding to the BIG2 protein derived from pro-

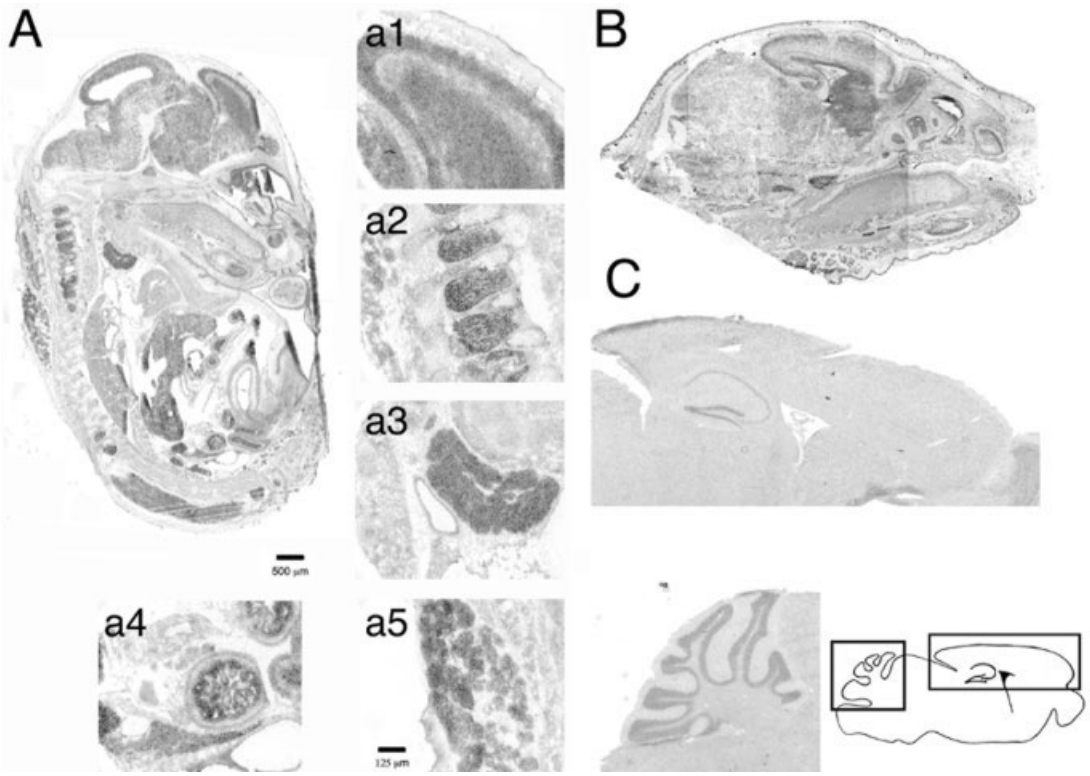

Fig. 1. The mRNA distribution of *ARFGEF2* in various mouse organ systems during development. Sagittal brightfield photomicrographs of an embryonic day 17 (E17; **A**), postnatal day 0 (P0; **B**), and adult (**C**) mouse brain following *ARFGEF2* in situ hybridization reveal significant expression of *ARFGEF2* mRNA throughout mouse brain. *ARFGEF2* shows highest levels of expression in the cerebral cortex (**a1**), somites (**a2**), thymus (**a3**), duodenum (**a4**), and brown fat (**a5**) during development. More specifically, in the CNS, *ARFGEF2* appears to be most abundant in the developing forebrain. *ARFGEF2* mRNA localizes preferentially to the embryonic subventricular and ventricular zones (VZ) and embryonic cortical plate (CP) and, to a lesser degree, to the intermediate zone (IZ). *ARFGEF2* signal was also appreciated in the underlying basal ganglia and spinal cord of the E17 mouse. *ARFGEF2* expression is developmentally regulated, with weaker expression noted in the CNS of early postnatal and adult mice, although some level of *ARFGEF2* expression persisted in the hippocampus and cerebellum by adulthood (boxed areas of camera lucida in C correspond to the photomicrographs in C). Scale bar = 500 μm in A (applies to A–C); 125 μm in a5 (applies to a1–a5).

tein extracts of HEK293 cells transfected with *ARFGEF2* (Fig. 3A).

On mouse embryonic day 14, BIG2 expression was highly restricted to the neural progenitor population along the neuroependymal lining of the VZ, with much weaker staining in the cortical plate. BIG2 staining along the ventricular lining persisted into adulthood, with progressively decreasing intensity (Fig. 3B). A similar pattern of staining was found in the human cortex and cerebral wall from 19 weeks of gestational age, with persistent expression seen at postnatal 2 months of age (Fig. 3C).

BIG2 and FLNA co-localization in neuroependymal progenitors

As the expression studies would indicate that *ARFGEF2* plays an important role in neural progenitor proliferation and migration during cortical development, we examined whether both FLNA and BIG2 localized to the same neuroependymal precursors along the ventricular lining. We performed immunohistochemistry with double FLNA/BIG2 labeling, isolated neuroependymal progenitors from both mouse and human, and analyzed the mRNA and protein expressions for both proteins by microarray gene profiling and Western blotting.

In the human gestational age 19-week brain, FLNA is expressed most strongly by the neuroependymal progenitor cells along the ventricular lining but is also present in progenitors and migratory neuroblasts within the VZ and SVZ (Fig. 4A). Intracellular localization of FLNA also appeared to be more restricted to the cell surface boundaries. BIG2 similarly localized to the same neuroependymal progenitor population, with intracellular expression more confined to the apical surface (CSF side) of the cells. Furthermore, both *FLNA* and *ARFGEF2* mRNA were expressed within isolated mouse and human neuroependymal progenitors by microarray gene profiling (Fig. 4B). All three large molecular weight GEFs (*GBF1*, *ARFGEF2*, and *BIG1*) were present in the neural progenitors. Among the filamin genes (*Filamin A*, *B*, and *C*), *FLNA* mRNA appeared to be more strongly expressed than the other homologous forms. Finally, abundant levels of BIG2 and FLNA protein were found in the neural progenitors by Western blot studies (Fig. 4C).

Inhibition of BIG2(E738K) inhibits transport of FLNA to the cell periphery

ARFGEF2/BIG2 and FLNA are expressed within the same neuroependymal precursors, and the GEFs regulate

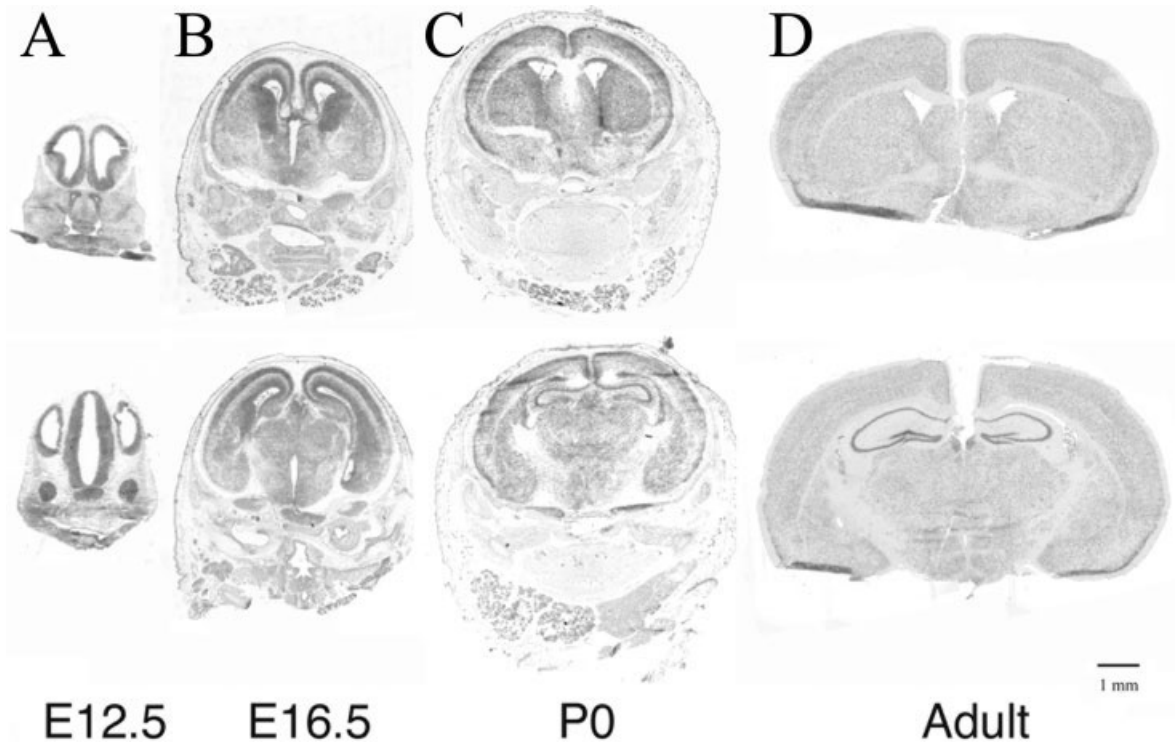

Fig. 2. **A–D:** Expression of *ARFGEF2* messenger RNA in mouse cerebral cortex at various ages of development. Coronal brightfield photomicrographs following in situ hybridization with *ARFGEF2* mRNA show labeling within the ventricular zone at E12.5, with increasing expression at E16.5 and relatively diminished expression at P0 and adult ages. *ARFGEF2* appears to be widely distributed across the cortical mantle and striatum during early development. Expression is limited to the hippocampal structures in adulthood. Scale bar = 1 mm in D (applies to A–D).

the transport of proteins to various cellular compartments. To examine whether loss of BIG2 function could disrupt *FLNA* transport and distribution, we inhibited BIG2 function in a human SHSY5Y neuroblastoma cell line and observed consequent changes in FLNA expression. FLNA is characteristically localized to the cell periphery, and no consequent changes were noted following transfection with a control GFP construct (Fig. 5). By 24 hours following transfection using the dominant negative BIG2(E738K) construct, FLNA appeared to be redistributed from the cell surface to the cell cytoplasm. Overlapping FLNA expression with BIG2 suggested that transport may be arrested or disrupted in the Golgi apparatus.

DISCUSSION

PH is often viewed as a disorder of neuronal migration due primarily to mutations in the X-linked *FLNA* gene (Fox et al., 1998). The recent identification of a second genetic cause of PH due to mutations in *ARFGEF2* now allows for a direct evaluation of potential interactions between these two proteins in regard to both their expression patterns and function (Sheen et al., 2004). Here, we performed extensive characterization of *ARFGEF2* mRNA and BIG2 protein expression during cortical development in both mice and humans. Both *ARFGEF2* mRNA and BIG2 protein are most strongly expressed in neural progenitors along the ependyma of the VZ. Moreover, both FLNA and BIG2 expression primarily overlap in these same neural progenitors, and BIG2 inhibition impairs FLNA transport and distribution to the cell surface. These observations raise the possibility that disruption of the neural progenitor population along the neuroependyma may contribute to PH formation and that PH is not necessarily a disorder of neuronal motility but rather reflects a problem with initial migration or cell adhesion.

Several observations support the idea that a disruption along the VZ gives rise to PH. The current studies demonstrate restricted expression of BIG2 within neural precursors along the ependyma and furthermore, the colocalization of both BIG2 and FLNA in these same cells. Overexpression of an FLNA-interacting protein (FILIP) in ventricular zone progenitors causes FLNA degradation and also prevents neuroblast migration from the VZ (Nagano et al., 2002). Finally, males with PH due to an *FLNA* mutation have a grossly normal-appearing cerebral cortex, suggesting that neurons expressing the mutated form of the FLNA protein are largely able to migrate into the cortical plate (Guerrini et al., 2004).

If PH truly results from some abnormality in the development of neural progenitors along the ventricular lining, several processes may contribute to PH formation: overproliferation of progenitors, disruption in radial glia and migratory neuroblast interactions, and weakening of the neuroependyma lining. Prior studies would suggest that *ARFGEF2* impairs proliferation, and the clinical human phenotype for this disorder is one of PH with microcephaly, thereby arguing against overproliferation (Sheen et

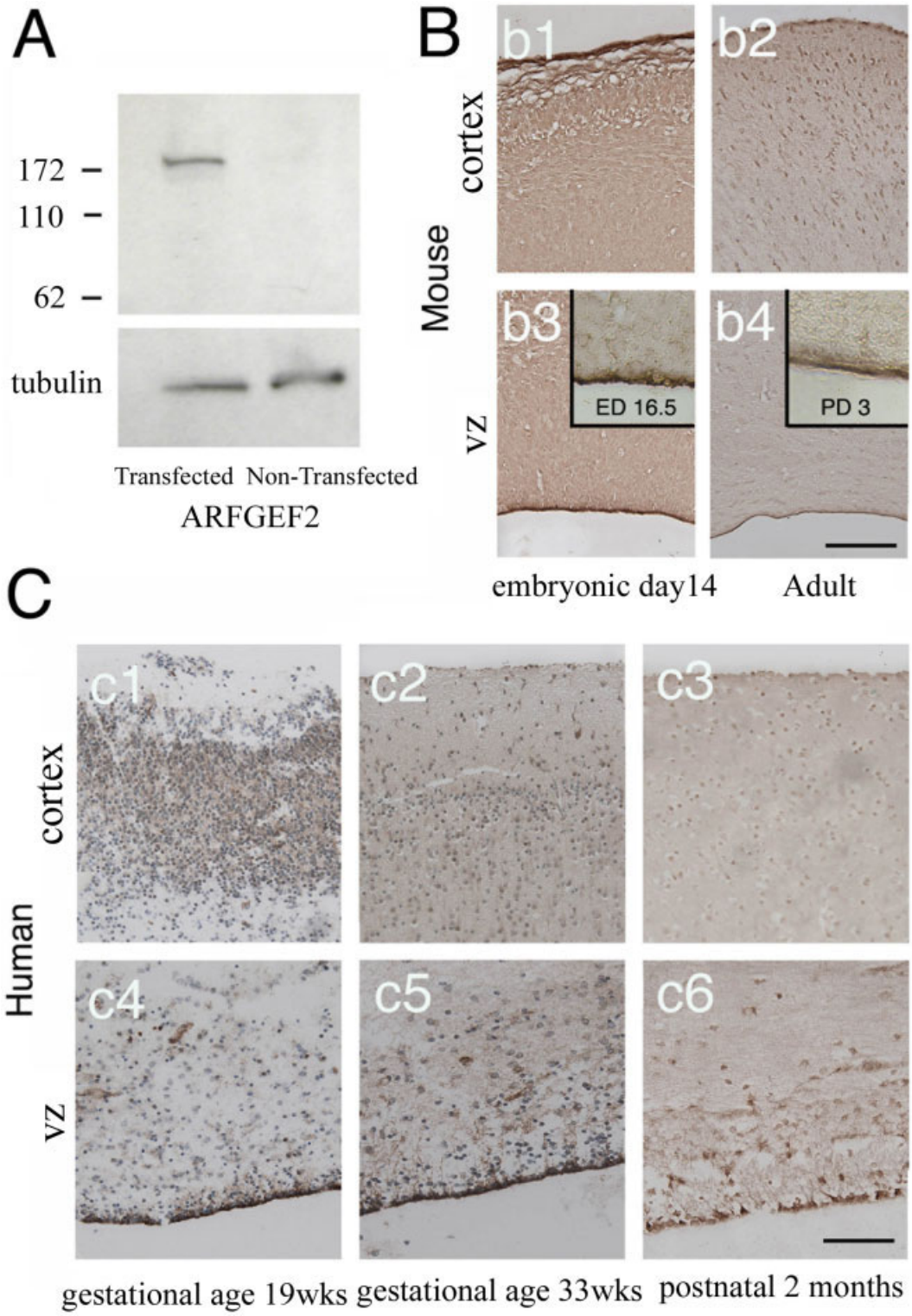

Fig. 3. BIG2 protein is strongly expressed in neuroependymal cells along the ventricular zone. **A:** Specificity of BIG2 (encoded by *ARFGEF2*) antibody is apparent following transfection into HEK293T cells and Western blot analyses. BIG2 protein corresponds to an approximately 180-kD band. **B:** BIG2 immunostaining at embryonic day 14 (ED14), E16.5, postnatal day 3 (PD3), and adulthood within murine cerebral cortex (**b1,b2**) and wall shows strong signal in the neuroependymal cells along the ventricular zone (VZ, **b3, b4**), which decreases with age. **C:** This same temporal progression is seen in human brain from embryonic weeks 19 and 33 to postnatal 2 months, with BIG2 more strongly expressed in the VZ and subventricular zone (**c4–c6**) than other areas of the cerebral cortex (**c1–c3**). Hematoxylin and eosin counterstaining in blue is shown in the gestational aged brains with DAB immunostaining of BIG2 in brown. Scale bars = 200 μm in b4 (applies to b1–b4); 100 μm in b4 (applies to insets in b3,4); 100 μm in c6 (applies to c1–c6).

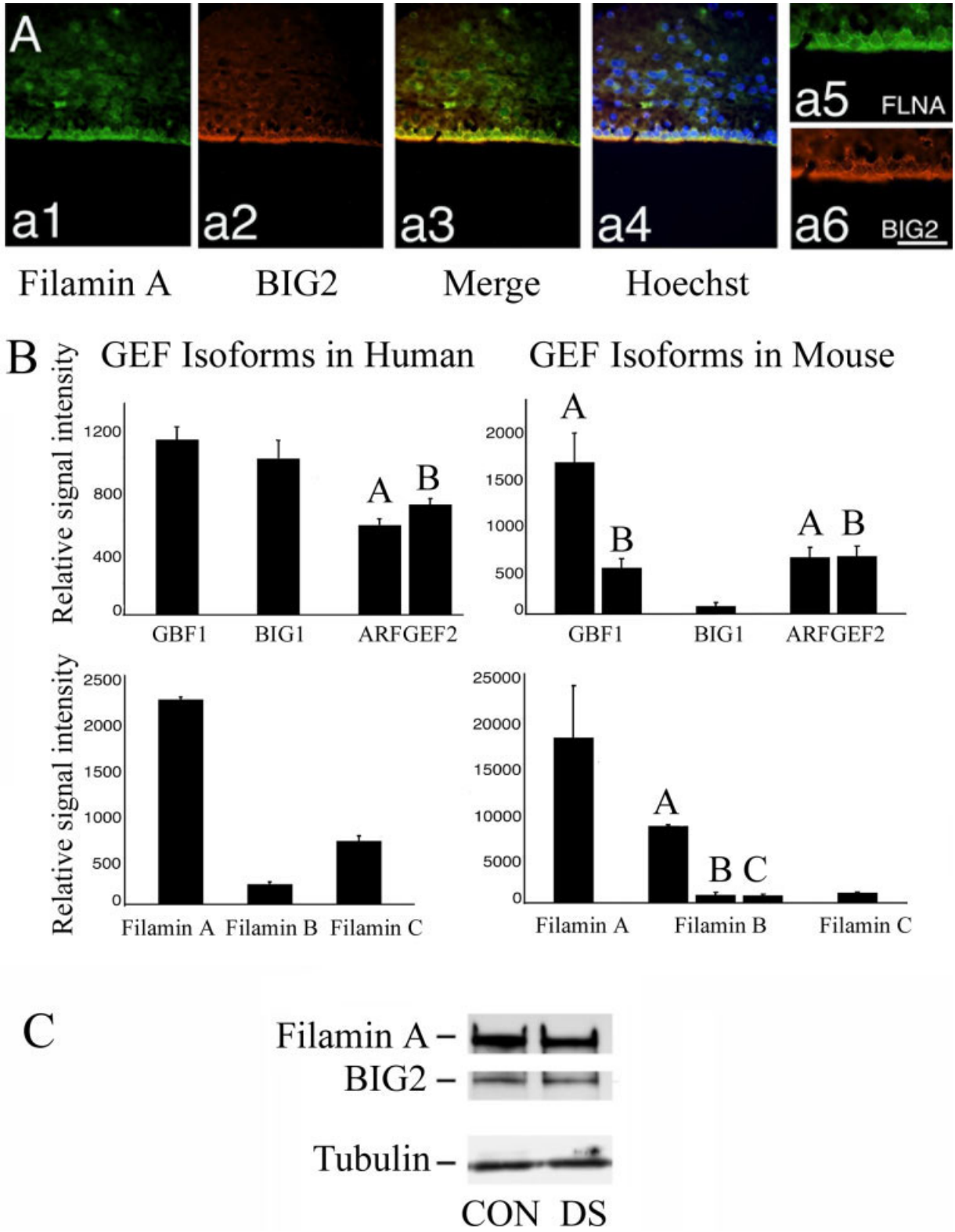

Fig. 4. ARFGEF2/BIG2 and FLNA co-localize along the neuroependyma and are expressed in human and mouse neural stem cells. **A:** Fluorescence photomicrograph of 19-week human brain shows co-localization of Filamin A (**a1**, fluoroscein) and BIG2 (**a2**, rhodamine) along the ventricular zone. Higher magnification photomicrograph of the neuroependyma (**a5,a6**) shows co-localization of FLNA and BIG2 within these cells. **B:** Expression of messenger RNA levels for the large molecular weight guanine exchange factor (GEF) and actin-binding filamin genes. **C:** Both 19-week human and Down syndrome neural progenitors express both Filamin A and ARFGEF2 by Western blot analyses. Neural stem cells from gestational 19–22 weeks aborted fetus and embryonic day 17 mice neural stem cells were expanded in culture, mRNA was isolated, and expression levels were determined with gene profiling by using Affymetrix microarrays. All the large molecular weight GEFs (GBF1, BIG1, and ARFGEF2) are expressed in both human and mice neural progenitors, although the BIG1 signal appears much weaker in the mouse. Filamin A is most strongly expressed in the CNS progenitor lines, consistent with a role in periventricular heterotopia formation. To a lesser degree, *Filamin B* and *C* are also present in the precursors. Different isoforms for the respective genes are noted above the quantified relative signal intensity (A, B, or C). Scale bars = 50 μm in a6 (applies to a1–4); 25 μm in a6 (applies to a5,a6).

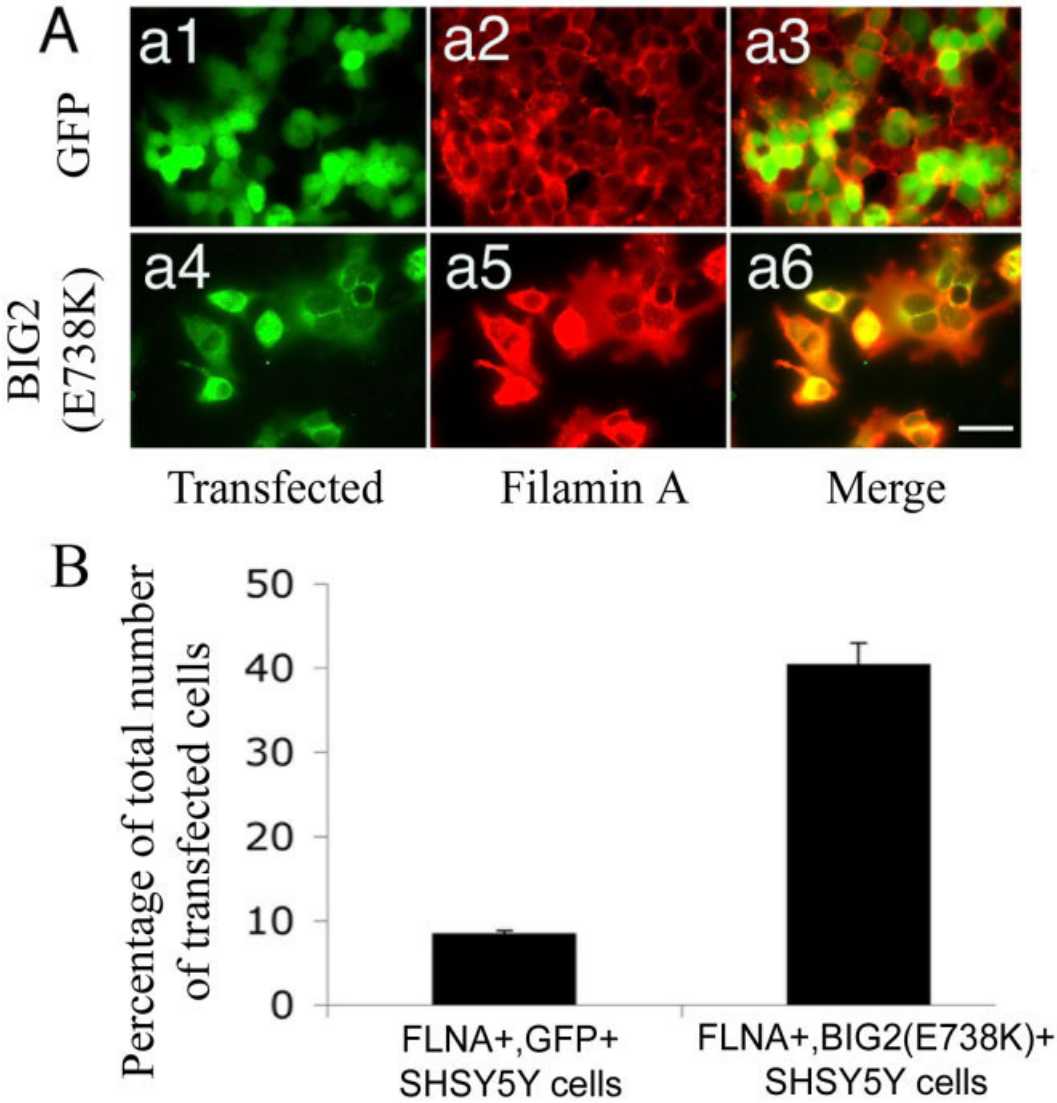

Fig. 5. Inhibition of BIG2 blocks Filamin A transport within the neuroependyma. **A:** SHSY5Y cells were transfected with either dominant negative HA-tagged BIG2 (E738K; **a4–a6**) or GFP control (**a1–a3**) constructs and then stained with antibody to HA (fluoroscein) and Filamin A. The perinuclear accumulation of Filamin A (rhodamine) in the cells transfected with the dominant negative BIG2 (E738K) and the co-localization with BIG2 (fluoroscein) suggest that filamin transport is arrested in the Golgi apparatus. Similar co-localization is not appreciated following transfection of control GFP. **B:** The percentage of transfected cells with overlapping FLNA and GFP or BIG2 expression is graphically quantified to the right. Scale bar = 25 μm in a6 (applies to a1–a6).

al., 2004). *ARFGEF2* restricts neurite extension by promoting collapse of axon growth cone and, by analogy, may inhibit the extension of leading process in migratory neurons and attachment to radial glia. FLNA similarly affects membrane protrusion and cell stability and could impair initial neuronal attachment onto radial glia (Dulabon et al., 2000). Finally, FLNA localizes to the cell surface and interacts with cell matrix adhesions and perhaps other focal adhesion contacts (Tu et al., 2003).

Recent studies have also implicated FLNA in the cause of the disorder of the extracellular matrix, Ehlers-Danlos syndrome (EDS; Sheen et al., 2005). Thus, much in the same manner by which loss in the tensile strength of the vessel wall, skin, or joints leads to EDS due to *FLNA* mutations, a similar process may arise from weakening of the neuroependymal lining and cause PH. The current findings suggest that BIG2 regulates not only trafficking of lipid membranes and cell adhesion molecules but also FLNA. To what extent and how selective this transport is and how it contributes to the neuronal/radial glial interactions or the integrity of the neuroependyma remains to be determined.

Several observations can be made from the profiling of GEF and FLN expression in neural precursors. As expected, FLNA appears to be the predominant form of filamin in the CNS and is consistent with the protein's role in PH formation (Sheen et al., 2002). To date, FLNB and FLNC have not been reported to have any demonstrable CNS phenotype. All the high molecular weight GEFs (BIG1, BIG2, and GBF1) are more or less equally ex-

pressed in the neural progenitors, suggesting that these proteins have both distinct and shared roles in vesicle transport. Interestingly, the function of BIG2 is distinct enough to cause primarily a neurological disorder. On the other hand, clear complementary roles exist for these proteins in the brain, because other vesicle and lipid-related disorders (α-SNAP in the HYH mouse and Zellweger syndrome in humans) have been associated with PH (Goldfischer et al., 1986; Chae et al., 2004).

PH is clearly a heterogeneous disorder, but the relatively rare frequency of presentation and the strikingly similar radiographic features between the various causes of PH would suggest a shared final common cellular and molecular mechanism. The current studies demonstrate a common neural progenitor population that highly expresses both of these genes, suggesting that FLNA localization within a cell may in part be dependent on ARFGEF2. Further characterization of the pathological features seen in the PH brain due to mutations in *FLNA* and the consequent changes that loss of FLNA function has on neural progenitors along the neuroependyma will provide insight into the underlying process leading to this malformation of cortical development.

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