Filamin A mediated Big2 dependent endocytosis: From apical abscission to periventricular heterotopia

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Accessibility
Periventricular heterotopia (PH) is one of the most common malformations of cortical development (MCD). Nodules along the lateral ventricles of the brain, disruption of the ventricular lining, and a reduced brain size are hallmarks of this disorder. PH results in a disruption of the neuroependyma, inhibition of neural proliferation and differentiation, and altered neuronal migration. Human mutations in the genes encoding the actin-binding Filamin A (FLNA) and the vesicle trafficking Brefeldin A-associated guanine exchange factor 2 (BIG2) are implicated in PH formation. Recent studies have shown that the transition from proliferating neural progenitors to postmitotic neurons relies on apical abscission along the neuroependyma. This mechanism involves an actin dependent contraction of the apical portion of a neural progenitor along the ventricular lining to complete abscission. Actin also maintains stability of various cell adhesion molecules along the neuroependyma. Loss of cadherin directs disassembly of the primary cilium, which transduces sonic-hedgehog (Shh) signaling. Shh signaling is required for continued proliferation. In this context, apical abscission regulates neuronal progenitor exit and migration from the ventricular zone by detachment from the neuroependyma, relies on adhesion molecules that maintain the integrity of the neuroependymal lining, and directs neural proliferation. Each of these processes is disrupted in PH, suggesting that genes causal for this MCD, may fundamentally mediate apical abscission in cortical development. Here we discuss several recent reports that demonstrate a coordinated role for actin and vesicle trafficking in modulating neural development along the neuroependymum, and potentially the neural stem cell to neuronal transition.

Introduction

Periventricular heterotopia (PH, Figure 1) is one of the most common malformations of cortical development (MCD) and causes seizures, dyslexia, and psychiatric disturbances.1 PH is characterized by bilateral ectopic neuronal nodules (arrows) found along the lateral ventricles.2 The nodules are caused by impaired migration from the ventricular zone (VZ) and disruption in the integrity of the neuroependymum.1 Mutations in the causative PH genes can also cause microcephaly (meaning small brain).3,4

The most common form of PH is inherited in an X-linked dominant fashion from mutations in the filamin A (FLNA) gene.5,7 Hemizygous males are generally embryonic lethal with PH manifesting in females. FLNA encodes the cytoplasmic actin binding protein FLNA, which serves as a scaffold for over 30 proteins.8 A second form of autosomal recessive PH with microcephaly (ARPHM) has been associated with mutations in the ARFGEF2 gene. ARFGEF2 encodes brefeldin A inhibited guanine exchange factor-2 (BIG2).3 BIG2 is a protein kinase A anchoring protein (AKAP) which regulates Golgi-vesicle trafficking through its...
Recent work has identified cadherin receptor ligands in causing PH due to mutations in \textit{FAT4} and \textit{DCHS1}.9
Some of the pathophysiological mechanisms giving rise to hallmarks of PH (diminished proliferation, impaired initial neural migration, and disruption of the neuroependymal lining causing heterotopia) have become apparent. Studies from this laboratory and others have demonstrated that a loss in cell-cell and cell-extracellular matrix (ECM) adhesion leading to loss of integrity along the ventricular lining causes heterotopia formation.10-12 The reduction in neuronal production seen with loss of FlnA is partially due to impaired degradation of cell cycle associated proteins, leading to a prolongation in the time to transit through the cell cycle and subsequent reduced proliferation.13 The migratory defects seen with loss of either FlnA or Big2 could be attributed to some disruption in actin dynamics.14 While these studies clarify individual cellular and molecular pathways for the specific PH phenotypes, they do not provide a unifying general mechanism for this disorder. Here we comment on recent experiments from this lab demonstrating both physical and functional interactions between FlnA and Big2. We suggest that coordinated interactions between actin and vesicle trafficking give rise to PH hallmarks and perhaps, serve primary roles in cell abscission along the neuroependyma.

**Apical Abscission in Cortical Development**

Normal development of the human cortex occurs through a series of well-described cellular events. During embryonic development, this process involves the proliferation, migration, and differentiation of neuronal progenitors located in the ventricular zone. Beginning along the lateral ventricles, neural ependymal progenitors undergo several rounds of cell division leading to the formation of the rapidly expanding ventricular zone. As this process continues, post-mitotic neurons attach and migrate along radial glial scaffolds out of the expanding ventricular zone, creating a new overlying layer of cells called the preplate. The laminar position of neurons is characteristic of their birthdate, such that younger neuroblasts migrate past their older counterparts to form the more superficial layers of the cortex. Finally, once neurons reach their cortical destinations, they differentiate ultimately forming the complex dendritic and axonal connections that are characteristic of fully mature cortical neurons.15

Recent studies have yielded new insights into how the transition from neural progenitor to post-mitotic neuron occurs along the neuroependyma.16 A form of cell subdivision takes place along the neuroependyma resulting in abscission of the apical membrane and detachment of the newly formed post-mitotic neuron from the ventricle (Fig. 2). Neuroependymal progenitors contain a subapical actin cable that contracts to mediate apical abscission at the ventricular surface. N-cadherin is connected intracellularly to the contractile actin cable and maintains apical junctions and cell-cell adhesion, thereby inhibiting abscission. Maintenance of the neuroependyma also preserves primary cilia function. Neuroependymal cells contain a primary cilium which transduces sonic hedgehog (Shh) signaling required for continued progenitor proliferation. With loss of N-cadherin function and activation of actin, abscission is initiated. Apical abscission leads to a dismantling of the cilium, loss of Shh signaling, and decline in proliferation with progenitors exiting the cell cycle.17,18 Abscission also regulates cell polarity as apical proteins such as the Par-complex protein, atypical protein kinase C, remain with the abscissed portion along the ventricular neuroependyma and is absent in the post mitotic, migratory neuron.

**Periventricular Heterotopia: A Genetic Disorder of Endocytosis**

FLNA is a 280 kD actin-binding phosphoprotein represented by an N-terminal actin-binding domain, followed by Immunoglobulin (IG) like repeat domains, that contain the receptor binding region at the C-terminus. FLNA also contains a PKA-dependent phosphorylation site at serine 2152 that has been shown to regulate its redistribution to the cell membrane.19,20 FLNA homodimers regulate the actin cytoskeleton through interactions derived from its multiple receptor binding regions, thereby directing cell stability, protrusion, and motility.21,22 Filamin also promotes actin branching, tethers large actin filaments and holds them in a perpendicular arrangement.23,24 The actin filaments represent a characteristic cortical actin structure at the leading edge of migrating cells. FLNA has also been shown to interact with numerous other proteins, suggesting many potential mechanistic roles that could influence cortical development. These interactors demonstrate great functional diversity including (1) regulation of cortical actin networks through molecules including \( \beta \)-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 SHIP-2 or SEK1.25-28 In a general sense, filamins serve to stabilize various receptors and cell signaling partners near the cell membrane.
and also promote co-localization of interacting proteins and molecules to facilitate potential activation of signaling cascades.

BIG2 is one of the three large Sec7 proteins isolated in mammalian cells (GBF1, BIG1/2) and distinguished by their sensitivity to the fungal metabolite Brefeldin A (BFA). The Sec7 domain of BIG2 gives it the ability to bind and activate ADP-Ribosylation Factor (ARF) proteins by accelerating the replacement of ARF-bound GDP to GTP. BIG2 proteins belong to the Ras-superfamily of GTPases and are primarily involved in intracellular vesicle trafficking through the trans-Golgi network, recycling endosome, and plasma membrane. BIG2 therefore regulates intracellular vesicle trafficking by activating ARF proteins (specifically ARFs 1 and 3). Additionally, the N-terminal domain of BIG2 binds Exo70, a member of the exocyst complex involved in vesicle exocytosis. Several AKAP (A-kinase anchoring protein) binding sites were identified within the Exo70 domain. These residues interact with PKA (Protein Kinase A) subunits to regulate BIG2 activation of the ARFs. Finally, at its C-terminal, BIG2 interacts with the β subunit of GABA receptors. Overall, these data suggest that BIG2 can coordinate vesicle trafficking (through ARF and Exo70 proteins) with cAMP-dependent signaling (through the A-kinase anchoring domain).

Recent studies from this laboratory have begun to reconcile how these two seemingly dissimilar proteins can give rise to PH. Either acute or chronic loss of either Big2 or FlnA leads to impairments in neural migration during development of the cortex. Migratory neural cells show defects in filopodia extension and attachment onto extracellular matrix coated surfaces. Both proteins physically bind and interact within neural progenitors, and loss of protein expression of either FlnA or Big2 leads to compensatory upregulation of the other. As with many proteins that FlnA binds, Big2 localization is dependent on phosphorylation of the actin binding protein which redirects Big2 from the Golgi to the cell membrane. Relocalization to the membrane allows Big2 to activate Arf1. Arfs have been shown to reside at the cell surface with ARF1 and ARF3 mediating endocytosis. This reciprocal regulation likely reflects a negative feedback, where loss of Big2 promotes phospho-FlnA expression to enhance redistribution of Big2 to the membrane. Conversely, loss of FlnA enhances Big2 expression to allow for Big2 delivery to the membrane. These studies begin to suggest an integral relationship between FlnA dependent actin dynamics and Big2 dependent regulation of vesicle formation and trafficking.

Changes in Arf-dependent endocytosis have the potential to disrupt several cell developmental processes. With respect to migration, Big2 and FlnA interactions regulate the stability and turnover of integrin/paxillin at the cell membrane, thereby directly impacting neural migration. Endocytosis also mediates the stability and turnover of cell-cell and cell-ECM adhesion molecules such as cadherins, and mediate neuroependymal integrity. Finally, changes in the expression and stability of receptors such as cadherins at the membrane would directly affect the processing, signaling and degradation of downstream effectors such as catenins, as these molecules are trafficked either back to the membrane or into lysosomal compartments. In this manner, a more generalized defect in vesicle trafficking may account for the multiple PH phenotypes.

**Apical Abscission and PH: Development and Disease through a Common Pathway?**

The gross anatomical features seen with PH suggest that it may reflect a disorder along the neuroependymal lining. Although a cell motility problem is in part implicated in heterotopia formation, the neuronal nodules along the lateral ventricles are primarily composed of later-born neurons. Impairments in cell autonomous motility would not be expected to favor different neuronal birth dates. We also observe a diminution of the neuroependymal lining adjacent to the heterotopia, suggesting that loss of the neuroependymal could lead to disruption of the radial glial fibers and heterotopias formation. Another hallmark of PH is a reduction in brain size, consistent with a proliferative defect. Neural progenitor proliferation occurs within the ventricular zone of the cortex and abnormalities in the integrity could also disrupt neural stem cell proliferation. Thus, a defect in neural stem cell development along the neuroependyma would collectively account for all the neurological phenotypes seen in this disorder.

Given that apical abscission takes place along the neuroependyma, genes implicated in PH might be expected to regulate this fundamental process. Both FlnA and Big2 are highly expressed along the apical portion of the neuroependyma during cortical development, suggesting a primary function along the neuroependyma. Loss of FlnA or Big2 also leads to diminished expression of various cell adhesion-associated proteins along the neuroependyma such as cadherins and...
Membrane recycling through vesicle trafficking is required for advancement of the cleavage furrow and separation of a single cell into daughter progeny. A similar process is likely invoked with apical abscission.

It remains to be seen what aspects of apical abscission are disrupted following FlnA or Big2 inhibition. Prior to abscission, Shh signaling is maintained through the primary cilium. Maintenance of N-cadherin expression and actin cables stabilize the neuroepithelium and inhibit cell abscission. Downregulation of cadherins through transcription factors such as FoxP2/4, promotes apical abscission, and presumptive loss in Shh signaling with subsequent dismantling of the primary cilium. Loss of Shh signaling leads to withdrawal from the cell cycle and a decline in proliferation. FlnA loss would be expected to impair Shh signaling and lead to diminished proliferation, if it truly affects primary cilium function. Loss of cadherin stability and expression through disruption of PH-associated proteins would also likely increase the number of cells undergoing abscission, inhibit Shh signaling, and promote withdrawal from the cell cycle. With respect to neuronal migration into the cortical plate, disruption of FlnA or Big2 dependent endocytosis could lead to failed abscission or a delay in the time required for separation of the main cell body from the abscissed portion at the apical surface. A delay in abscission would impede exit of post-mitotic neurons from the ventricular zone, leading to heterotopia formation.

Open Questions

The basic mechanisms underlying the transition from neural progenitor to neuron along the neuroepithelial lining may extend beyond normal corticogenesis and MCD. More broadly, apical abscission regulates an epithelial to mesenchymal type transformation (EMT) of various organ systems. As with the neuroependyma, epithelial cells in general maintain an apical to basal polarity, lack motility and form organized sheets. Conversely, mesenchymal cells lack this polarity and are loosely organized allowing such cells to dislodge and migrate to distant sites-a process similar to post-mitotic neurons which dislocate from the neuroepithelium and migrate to the cortical plate. Mesenchymal cells therefore possess greater malignant and metastatic potential. Genes associated with PH are implicated in these same processes. Beyond the brain, FlnA directs the development of various progenitors along the lining of various organ systems (i.e., gut, blood vessels, lung, etc). FlnA is also highly expressed and secreted in certain cancers. FlnA loss has been shown to inhibit the progression of cancers, consistent with the notion that it might inhibit EMT. Similarly, Big2 levels are increased in certain cancer cells and its effector ARF1 has been shown to regulate growth and migration of cancer cells. It remains to be seen whether genes associated with PH coordinate actin dynamics and vesicle formation in apical abscission and how these processes might mediate other disease pathogenesis, including cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Sheen VL. Periventricular Heterotopia: Shuttling of Proteins through Vesicles and Actin in Cortical Development and Disease. Scientifica (Cairo) 2012; 2012:480129; PMID:24278701; dx.doi.org/10.6064/2012/480129
2. Lu J, Sheen V. Periventricular heterotopia. Epilepsy Behav 2005; 7:143-9; PMID:15966530; http://dx.doi.org/10.1016/j.yebeh.2005.05.001
3. Sheen VL, Ganesh VS, Topcu M, Sebire G, Bodell A, Hill RS, Grant PE, Shugart YY, Imtota J, Khoury SJ, et al. Mutations in ARFGEF2 impair vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. Nat Genet 2004; 36:69-76; PMID:14647276; http://dx.doi.org/10.1038/ng1276
4. Sheen VL, Topcu M, Berkovic S, Valknizgul D, Blatt I, Bodell A, Hill RS, Ganesh VS, Cherry TJ, Shugart YY, et al. Autosomal recessive form of periventricular heterotopia. Neurology 2003; 60:1108-12; PMID:12682315; http://dx.doi.org/10.1212/01.WNL0000055898.00349.02
5. Fox JW, Lamperri ED, Ekgojlu YZ, Hong SE, Feng Y, Graham DA, Scheffler JE, Dobyns WB, Hirsch BA, Radke RA, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. Neuron 1998; 21:1315-25; PMID:9838725; http://dx.doi.org/10.1016/S0896-6273(98)00651-0
6. Sheen VL, Dixon PH, Fox JW, Hong SE, Kinton L, Sisoody SM, Duncan JS, Dubaux F, Scheffler JE, Schachter SC, et al. Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. Hum Mol Genet 2001; 10:1775-83; PMID:11532987; http://dx.doi.org/10.1093/hmg/10.17.1775
7. Sheen VL, Jansen A, Chen MH, Patrizi E, Morgan T, Ravenscroft C, Ganesh V, Underwood T, Wiley J, Leventer R, et al. Filamin A mutations cause periventricular heterotopia with Ehlers-Danlos syndrome. Neurology 2005; 64:254-62; PMID:16664422; http://dx.doi.org/10.1212/01.WNL.0000149512.79621.FD

8. Feng S, Reséndez JC, Lu X, Kroll MH. Filamin A binding to the cytoplasmic tail of glycoprotein Ibalpha regulates von Willebrand factor-induced platelet activation. Blood 2003; 102:2122-9; PMID:12796664; http://dx.doi.org/10.1182/blood-2002-12-3805

9. Carabalona A, Beguin S, Pallesi-Pocachard E, Buhler E, Pellegrino C, Arnaud K, Hubert F, Ouahila M, Siffert JP, Khantane S, et al. A glial origin for periventricular nodularity caused by impaired expression of Filamin-A. Hum Mol Genet 2012; 21:1004-17; PMID:22076441; http://dx.doi.org/10.1093/hmg/ddr591

10. Ronald JF, Bariz LF, Neal J, Lian G, Bundock E, Lu J, Huo YC, Diamond R, Mei D, Banham AH, et al. Disruption of neural progenitors along the ventricular and subventricular zones in periventricular heterotopia. Hum Mol Genet 2009; 18:497-516; PMID:18999016; http://dx.doi.org/10.1038/hmg/ddn.577

11. Gorlin JB, Yamin R, Egan S, Stewart M, Stossel TP, Li MG, Serr M, Yamamoto K, Nakayama K. Overexpression of an ADP-ribosylation factor-guanine nucleotide exchange factor, BIG2, uncouples brefeldin A-induced adaptor protein-1 coat dissociation and membrane tubulation. J Biol Chem 2003; 278:9668-73; PMID:13777925; http://dx.doi.org/10.1074/jbc.M112472200

12. Xu KF, Shen X, Li H, Pacheco-Rodríguez G, Moss J, Vaughan M. Interaction of BIG2, a brefeldin A-inhibited guanine nucleotide-exchange protein, with exocyst Exo70. Proc Natl Acad Sci U S A 2005; 102:7284-9; PMID:15705715; http://dx.doi.org/10.1073/pnas.0409871102

13. Lian G, Lu J, Zhang J, Hu J, Sheen V, Filamin A regulates neuronal migration through brefeldin A-inhibited guanine exchange factor 2-dependent Arf1 activation. J Neurosci 2013; 33:15735-46; PMID:24089482; http://dx.doi.org/10.1523/JNEUROSCI.2117-13.2013

14. Dong C, Zhang X, Zhou F, Dou H, Duverney MT, Zhang P, Wu G. ADP-ribosylation factors modulate the cell surface transport of G-protein coupled receptors. J Pharmacol Exp Ther 2010; 335:174-83; PMID:20933598; http://dx.doi.org/10.1124/jpet.109.161489

15. Kondo Y, Hanai A, Nakai K, Kato R, Nakayama K, Shin HW. ARF1 and ARF3 are required for the integrity of recycling endosomes and the recycling pathway. Cell Struct Funct 2012; 37:141-54; PMID:22971977; http://dx.doi.org/10.1007/s12972-0020-12105

16. Lu J, Tiao G, Folkers R, Hecht J, Walsh C, Sheen V. Overlapping expression of ARFGEF2 and Filamin A in the neurendoplasmic lamination of the lateral ventricles: implications into the cause of periventricular heterotopia. J Comp Neurol 2006; 496:474-84. PMID:16320251; http://dx.doi.org/10.1002/cne.20806

17. Feng Y, Chen MH, Moskowitz IP, Mendonça AM, Vidali L, Nakamura F, Kwikowski D, Walsh CA. Filamin A (FLNA) is expressed in the development of contacts in vascular development and cardiac morphogenesis. Proc Natl Acad Sci U S A 2006; 103:19836-41; PMID:17172441; http://dx.doi.org/10.1073/pnas.0609628104

18. Adams M, Robinson JR, Abdelmamed Z, Dawe HR, Szynmanska K, Logan CV, Wheway G, Pitt E, Gull K, Knowles MA, et al. A meckelin-Filamin A interaction mediates ciliogenesis. Hum Mol Genet 2012; 21:1272-86; PMID:22211117; http://dx.doi.org/10.1093/hmg/ddr575

19. Schiel JA, Childs C, Prekeris R. Endocytic transport and cytoskeleton: from regulation of the cytoskeleton to midbody inheritance. Trends Cell Biol 2013; 23:319-27; PMID:23522622; http://dx.doi.org/10.1016/j.tcb.2013.02.003

20. Sokol NS, Cooley L. Drosophila filamin encoded by the cheero locus is a component of ovarian ring canals. Curr Biol 1999; 9:1211-20; PMID:9506687; http://dx.doi.org/10.1016/S0960-9829(99)80502-8

21. Mi KG, Serr M, Edwards K, Ludmann S, Yamamoto D, Tilney LG, Field CM, Hays TY. Filamin is required for ring canal assembly and actin organization during Drosophila oogenesis. J Cell Biol 1999; 146:1061-74; PMID:10774759; http://dx.doi.org/10.1083/jcb.146.5.1061

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46. Feng B, Schwarz H, Jesuthasan S. Furrow-specific endocytosis during cytokinesis of zebrafish blastomeres. Exp Cell Res 2002; 279:14-20; PMID:12213209; http://dx.doi.org/10.1006/excr.2002.5579

47. Alper O, Stetler-Stevenson WG, Harris LN, Leitner WW, Ozdemirli M, Hartmann D, Raffeld M, Abu-Asab M, Byers S, Zhuang Z, et al. Novel anti-filamin-A antibody detects a secreted variant of filamin-A in plasma from patients with breast carcinoma and high-grade astrocytoma. Cancer Sci 2009; 100:1748-56; PMID:19594548; http://dx.doi.org/10.1111/j.1349-7006.2009.01244.x

48. Savoy RM, Ghosh PM. The dual role of filamin A in cancer: can’t live with (too much of) it, can’t live without it. Endocr Relat Cancer 2013; 20:R341-56; PMID:24108109; http://dx.doi.org/10.1530/ERC-13-0364

49. Boulay PL, Cotton M, Melançon P, Claing A. ADP-ribosylation factor 1 controls the activation of the phosphatidylinositol 3-kinase pathway to regulate epidermal growth factor-dependent growth and migration of breast cancer cells. J Biol Chem 2008; 283:36425-34; PMID:18990689; http://dx.doi.org/10.1074/jbc.M803603200