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Control of Outer Radial Glial Stem Cell Mitosis in the Human Brain

Bridget E.L. Ostrem,[1,2,3] Jan H. Lui,[1,2] Caitlyn C. Gertz,[1,2,3] and Arnold R. Kriegstein[1,2,*]
[1]Department of Neurology, University of California, San Francisco, 35 Medical Center Way, San Francisco, CA 94143, USA
[2]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, 35 Medical Center Way, San Francisco, CA 94143, USA
[3]Neuroscience Graduate Program, University of California, San Francisco, 1550 4th Street, San Francisco, CA 94158, USA
*Correspondence: kriegsteina@stemcell.ucsf.edu
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SUMMARY

Evolutionary expansion of the human neocortex is partially attributed to a relative abundance of neural stem cells in the fetal brain called outer radial glia (oRG). oRG cells display a characteristic division mode, mitotic somal translocation (MST), in which the soma rapidly translocates toward the cortical plate immediately prior to cytokinesis. MST may be essential for progenitor zone expansion, but the mechanism of MST is unknown, hindering exploration of its function in development and disease. Here, we show that MST requires activation of the Rho effector ROCK and nonmuscle myosin II, but not intact microtubules, centrosomal translocation into the leading process, or calcium influx. MST is independent of mitosis and distinct from interkinetic nuclear migration and saltatory migration. Our findings suggest that disrupted MST may underlie neurodevelopmental diseases affecting the Rho-ROCK-myosin pathway and provide a foundation for future exploration of the role of MST in neocortical development, evolution, and disease.

INTRODUCTION

The human neocortex is characterized by a marked increase in size and neuronal number as compared to other mammals. Neural stem cells called outer radial glia (oRG), present in large numbers during human, but not rodent, brain development, are thought to underlie this expansion (Hansen et al., 2010; Lui et al., 2011). oRG cells are derived from ventricular radial glia (vRG), the primary neural stem cells present in all mammals (LaMonica et al., 2013; Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001; Shitamukai et al., 2011; Wang et al., 2011). Both progenitor cell types display basal processes oriented toward the cortical plate, along which newborn neurons migrate (Hansen et al., 2010; Misson et al., 1991; Rakic, 1971, 1972). However, oRG cells reside primarily within the outer subventricular zone (oSVZ), closer to the cortical plate than vRG cells, and lack the apical ventricular contact characteristic of vRG cells (Chenn et al., 1998; Hansen et al., 2010). While vRG cell behavior, mitosis, and lineage have been extensively studied (Bentivoglio and Mazzarello, 1999; Hartfuss et al., 2001; Noctor et al., 2001, 2004, 2008; Qian et al., 1998; Taverna and Huttner, 2010), much less is known about regulation of oRG cell proliferation and the unique mitotic behavior of these cells (Betizeau et al., 2013; Gertz et al., 2014; Hansen et al., 2010; LaMonica et al., 2013; Pilz et al., 2013).

oRG cell cytokinesis is immediately preceded by a rapid translocation of the soma along the basal fiber toward the cortical plate, a process termed mitotic somal translocation (MST) (Hansen et al., 2010). Due to the relative abundance of oRG cells in humans, it has been hypothesized that genetic mutations causing significant brain malformations in humans, but minimal phenotypes in mouse models, may affect oRG cell-specific behaviors such as MST (LaMonica et al., 2012). However, the molecular motors driving MST have not been identified, hindering exploration of the function of MST in human brain development and its possible role in disease. MST is reminiscent of interkinetic nuclear migration (INM) of neuroepithelial and vRG cells, in which nuclei of cycling cells migrate back and forth along the basal fiber between the apical and basal boundaries of the ventricular zone in concert with the cell cycle. INM is controlled by the centrosome, the microtubule motors kinesin and dynein, and associated proteins, with actomyosin motors playing an accessory role (Taverna and Huttner, 2010). As oRG cells are derived from vRG cells and display analogous nuclear movements, it has been hypothesized that MST requires similar molecular motors as INM (LaMonica et al., 2012).

We find that MST requires activation of the Rho effector ROCK and nonmuscle myosin II (NMII), but not intact microtubules, centrosomal advancement into the leading process, or calcium influx. Conversely, oRG cell mitosis requires intact microtubules, but not NMII activation, demonstrating that MST and mitosis are mutually dissociable. We examine the expression profiles of genes implicated in the Rho-ROCK-myosin pathway that cause large developmental brain malformations when mutated in humans, but not in mice. Interestingly, several disease genes thought to primarily affect neuronal migration display expression profiles similar to known radial glial genes, consistent with expression in oRG cells. This observation suggests that defects in oRG behaviors such as MST may partially underlie cortical malformations currently attributed to defective neuronal

migration. Together, these results increase our understanding of the cellular and molecular basis for human cortical evolution and have important implications for studying disease mechanisms that cannot be effectively modeled in mice.

RESULTS

MST is thought to contribute to radial expansion of the oSVZ during human brain development (Lui et al., 2011). Supportive of this idea, we imaged oRG cell divisions in human fetal cortical slices at the border of the upper oSVZ and intermediate zone (IZ) during peak neurogenesis and oSVZ growth (gestational weeks 15–20). We observed many divisions in which oRG cells translocated out of the oSVZ and into the IZ, thereby increasing oSVZ size (Movie S1). We found that MST trajectory in the human oSVZ was overwhelmingly toward the cortical plate (Figure 1A). Furthermore, MST frequency and translocation distances were greater in humans as compared to ferrets and mice, species displaying proportionally smaller oSVZ sizes (Figures 1B–1D). These observations are suggestive of a role for MST in human oSVZ expansion. However, in-depth exploration of the function of MST in development and disease first requires an understanding of the underlying molecular mechanisms, which have remained elusive.

We initially hypothesized that MST depends on the same molecular machinery as INM. To determine the relative contributions of microtubule motors and actomyosin to MST, we applied inhibitors of microtubule polymerization and NMII (the most well-characterized myosin in brain development; Tullio et al., 2001; Vallee et al., 2009) to human fetal cortical slice cultures. We performed time-lapse imaging of oRG cell behaviors and quantified translocation (MST) and division frequency in each slice before and after addition of inhibitors or DMSO (control) (Figures 1E–1I and S1). Treatment of slices with a high concentration (100 μM) of blebbistatin, a selective NMII inhibitor, nearly abolished both translocations and divisions (data not shown). However, treatment with a low concentration (5 μM) of blebbistatin caused a significantly greater reduction in translocations than in divisions, suggesting that NMII plays a relatively larger role in MST than in mitosis. Conversely, treatment with the microtubule depolymerizing reagent nocodazole (1 μM) reduced divisions significantly more than translocations. Additionally, nocodazole, but not DMSO or blebbistatin, decreased the proportion of translocations that ended in division. We found that oRG cells express two isoforms of NMII, NMIIa and NMIIb (Figures 1J, 1K, and S1), which have both been shown to play essential roles in neuronal migration (Vicente-Manzanares et al., 2009). These results demonstrate that MST and mitosis are mutually dissociable in oRG cells. MST requires NMII activation, but not intact microtubules, and thus, not microtubule motors. Conversely, mitosis is relatively more dependent on intact microtubules than on NMII activation. We asked whether inhibition of MST directly affects daughter cell fate. Blebbistatin treatment of human fetal cortical slices did not alter the ratio of TBR2+ to SOX2+ cells in the oSVZ as compared to DMSO ($p = 0.38$, unpaired Student's t test), suggesting that MST does not control cell fate. Inhibition of MST may lead to cell crowding or have other indirect effects that could influence cell fate on a longer timescale than we could analyze using our slice culture system.

To control for non-cell-autonomous effects and to enable examination of subcellular mechanisms, we used dissociated neural progenitor cell cultures. Blocks of gestational week 15–20 (GW15–20) dorsal neocortical tissue spanning the ventricle to the cortical plate were dissociated by papain treatment and trituration. We previously observed that oRG-like cells undergo MST in dissociated fetal human cortical cultures (LaMonica et al., 2013). To confirm oRG identity of oRG-like cells, we performed fate staining on daughter cells after MST division (Figures 2A–2C and S2). Similar to oRG cells in slice culture (Hansen et al., 2010), daughters of MST divisions in dissociated culture expressed SOX2 (65 out of 65) and PAX6 (17 out of 17), usually expressed nestin (18 out of 24), rarely expressed TBR2 (2 out of 34), and never expressed βIII-tubulin (0 out of 20). As in slice culture, we observed expression of both NMIIa (14 out of 14 cells) and NMIIb (ten out of ten cells) in dissociated oRG cell daughters (Figures 2I and 2J). We concluded, based on morphology, behavior, and marker expression, that cells undergoing MST in dissociated culture are oRG cells, validating the use of dissociated cultures to study oRG cell behaviors.

We quantified translocation (MST) and division frequency in dissociated culture after motor protein inhibition. Similar to results in slice culture, blebbistatin treatment reduced translocations significantly more than divisions, while nocodazole treatment reduced divisions without significantly affecting translocations (Figures 2D–2H and Movie S2). Upon drug washout, blebbistatin-treated cultures showed an increase in translocations, while nocodazole-treated cells that had remained rounded up after MST underwent cytokinesis, suggesting that the effects of inhibitors were reversible and not due to cell death (Movie S3; Figure S2). Furthermore, inhibitor-treated cultures did not display increased staining for cleaved caspase-3, confirming that the effects of inhibitor treatment could not be attributed to apoptosis (Figure S2). Thus, results in dissociated culture confirm observations in slice culture that MST and mitosis are mutually dissociable in oRG cells. Intact microtubules are required for oRG cell mitosis, but not for MST, while NMII activation is required for oRG cell MST and is relatively less important for mitosis.

Though microtubule polymerization is not required for oRG cell MST in humans, nocodazole treatment significantly increased MST distance in both slice culture and dissociated cells (Figure 3A). Based on previous observations in rodent (Wang et al., 2011), we hypothesized that the centrosome migrates into the basal fiber prior to translocation, remains connected to the nucleus via a microtubule cage, and ultimately determines the location of translocation cessation and cytokinesis (Tsai et al., 2007). Nocodazole treatment would disrupt nucleus-centrosome coupling, eliminating the "stop" signal for translocation. To determine whether the centrosome precedes the nucleus into the basal process, we performed time-lapse imaging of centrosome behavior in dissociated human oRG cells after transfection with a construct encoding the centrosomal protein Centrin-2 (*Cetn2*) fused to the fluorescent reporter dsred (Figure S2) (Tanaka et al., 2004). While centrosome location was variable during interphase, centrosomes consistently returned to the soma prior to MST and remained adjacent to the nucleus throughout translocation (Figures 3B and 3D; Movie S4). In