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oRGs and mitotic somal translocation — a role in development and disease

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Abstract

The evolution of the human brain has been characterized by an increase in the size of the neocortex. Underlying this expansion is a significant increase in the number of neurons produced by neural stem cells during early stages of cortical development. Here we highlight recent advances in our understating of these cell populations, consisting of ventricular radial glia and outer radial glia. We highlight how gene expression studies have identified molecular signatures for radial glial cell populations and outline what has been learned about the mechanisms underlying the characteristic mode of division observed in outer radial glia cells, mitotic somal translocation. Understanding the significance of this behavior may help us explain human cortical expansion and further elucidate neurodevelopmental diseases.

Introduction

The considerable increase in the size and complexity of the human neocortex as compared to other mammalian species can be attributed to a greater number of neural progenitor cells during development, and to an expanded proliferative potential of each progenitor cell. These changes have recently been linked to an evolutionary increase in the number of outer radial glia cells (oRG), highly proliferative cells which are capable of producing neural precursors [1]. oRG cells display a characteristic migratory behavior, mitotic somal translocation (MST), in which the soma rapidly translocates towards the cortical plate immediately prior to cytokinesis [1]. It has been postulated that MST is important for germinal zone expansion during development. Until recently, the molecular motor driving this behavior had not been identified, but recent evidence indicates that actin-myosin motors are primarily involved [2••]. Here, we review what is known about oRG cell origin, function, and motility (MST), and speculate on how MST may be altered in neurodevelopmental diseases.

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Conflict of interest statement

Nothing declared.

Two types of radial glia

oRG cells reside primarily within the outer subventricular zone (oSVZ) of the developing cortex. The inner fiber layer and the inner subventricular zone separate the oSVZ from the ventricular radial glial (vRG) cells lining the developing cerebral ventricles. The oSVZ is present in the developing primate brain, but generally not in the developing rodent brain, although there are exceptions such as the agouti that displays a distinct oSVZ and a substantial population of oRG cells [3]. The oSVZ contains a large number of cycling cells throughout the period of neurogenesis [4,5] and the progenitor cells within the oSVZ are thought to be directly responsible for the increased number and complexity of upper layer neurons in primates, as the oSVZ is the principal proliferative region in the dorsal cortex during upper layer neurogenesis [6]. oRG cells were identified within the oSVZ after time-lapse imaging experiments demonstrated the direct generation of neuronal lineage-committed cells from oSVZ cells expressing radial glial markers [1]. These cells were named oRG based on their similarity to vRG cells in marker expression, morphology, and progeny. They have also been called basal radial glia (bRG) in reference to their position closer to the basal lamina and removed from the ventricular (apical) surface [7].

As oRG cells share many molecular features with ventricular radial glial (vRG) cells, an examination of the defining features of vRG cells is essential for gaining a comprehensive understanding of oRG cell function. vRG cells reside within the ventricular zone (VZ) lining the cerebral ventricles, and generate the vast majority of cortical excitatory neurons in rodents. Both vRG and oRG cells express an identifying set of radial glial markers, including nestin (NES), vimentin (VIM), and PAX6 [8], while oRG cells also express several newly discovered markers, including HOPX, TNC, and ITGB5, giving them a unique transcriptional profile [9••]. Early studies demonstrated that vRG cell divisions result in vRG self-renewal, direct neurogenesis, or production of intermediate progenitor (IP) cells that divide once to produce two neurons [10,11]. In contrast, only a small proportion of human oRG cell divisions produce IP cells, while the vast majority of divisions appear to lead to oRG cell self-renewal [1]. This observation has been interpreted to mean that oRG cells likely self-renew many times before producing IP cells, which similarly self-renew many times before producing neurons [8]. This process, termed ‘transit amplification,’ appears to be a defining feature of human as compared to rodent neural development, and has likely contributed to evolutionary expansion of the primate neocortex.

Morphology of oRG cells

Both types of radial glia share important morphological characteristics. vRG cells are bipolar, contacting the ventricular surface via an apical process, and the pial surface through a basal fiber. Newborn neurons migrate along the basal fiber towards the cortical plate [12,13]. oRG cells possess a basal fiber analogous to that of vRG cells, which similarly functions as a guide for neuronal migration [1,14]. Approximately one quarter of oRG cells in the fetal human cortex display a short apical process [15], but oRG cells lack ventricular contact and do not express markers of apical polarity seen in vRGs, such as PROM1, PARD3, or ZO-1 [16]. oRG cells with one prominent basal fiber have also been described in the ferret oSVZ, where they were initially termed intermediate radial glia cells (IRGCs)

[17]. Although rodents lack an oSVZ, a very small population of unipolar oRG cells was identified within the dorsal telecenphalic mouse SVZ and intermediate zone [18,19].

Currently, the defining morphology of oRG cells is controversial [7,20,21]. Studies in the macaque showed that oSVZ progenitor cells may display an apical process, a basal process, both types of processes, or neither, and may alternate between morphologies during a given cell cycle or divide to produce daughter cells that are morphologically distinct from the parent cell [21]. oSVZ progenitors within two other gyrencephalic species, the ferret and the sheep, also display a mix of bipolar, unipolar, and multipolar morphologies [22]. It is likely that many of these cells are more similar to IP cells than to radial glia in function, as 30% of macaque oSVZ cells with classical oRG morphology (unipolar with a basal process) co-express the IP cell marker TBR2, while only 60% have an expression pattern reminiscent of human oRG cells (PAX6+/TBR2−) [1,21]. Data from the macaque also show TBR2 co-expression in at least 30% of oSVZ cells with apical fibers [21]. Supportive of the presence of an IP cell population with oRG-like morphology, analysis using a novel cytoplasmic IP cell marker, PPP1R17, which colocalizes with TBR2, demonstrated that IP cells within the human oSVZ may be multipolar, unipolar, or bipolar, with radially or tangentially oriented processes [9••]. Taken together, these data suggest that there may be a greater proportion of IP-like oSVZ progenitors compared to radial glia-like progenitors in the macaque compared to the human, as TBR2 is expressed to a greater extent in macaque oSVZ cells than human oSVZ cells regardless of morphology [21]. Recent advances in high-throughput RNA-sequencing technology, applied at the population and single-cell level, have started to provide a more comprehensive understanding of oSVZ precursor diversity, which will ultimately lead to a consensus definition of oRG cell morphology and molecular signature [9••,23•,24,25•].

oRG cell origins

At least two distinct mechanisms lead to the generation of oRG cells. Delamination of vRG cells results in a direct transformation of vRG to oRG cell in both human and ferret [26]. vRG divisions can also generate oRG cells in a cleavage angle-dependent manner. In contrast, vertical vRG cell divisions produce a self-renewed vRG cell and either an additional vRG cell or an IP cell daughter [15]. The division of vRG cells with a horizontal cleavage angle, where the cleavage furrow is parallel to the ventricular surface, generates a self-renewed vRG cell (the daughter that inherits the apical endfoot) and an oRG cell (the daughter that inherits the basal fiber) [15]. Interestingly, mouse vRG cells divide with predominantly vertical cleavage angles throughout cortical development, while human vRG cells shift to approximately equal proportions of vertical and horizontal divisions at the start of oSVZ growth and oRG cell generation [15,27–29]. Furthermore, perturbations of the mitotic spindle that decrease vertical divisions in mouse lead to generation of oRG-like cells [19]. Sustained expression of PAX6 in mouse vRG cells, a transcription factor highly expressed in human but not mouse oRG cells, similarly leads to decreased vertical divisions and generation of oRG-like cells [30]. These data suggest that an evolutionary shift from a vertical cleavage plane angle to a mix of vertical and horizontal divisions may have contributed to an increased oRG cell population and the development of an oSVZ in humans. Studies in the ferret suggest there may be a restricted period for the formation of

the oSVZ during which vRG cells generate large numbers of oRG cells. This window is delineated by down regulation of *Trnp1* and *Cdh1* [31]. The DNA-associated protein Trnp1 had previously been identified as a regulator of mammalian brain development, and shRNA knockdown in the mouse leads to increased production of oRG-like cells [32••]. The blockade of Chdh1 incidentally favors horizontal cleavage planes and an excess of daughter cells delaminating from the ventricular surface [33,34]. A number of recent studies have identified other genes that may be important for oRG cell genesis and neocortical expansion. The human specific gene ARHGAP11B, highly enriched in human RG cells, was found to increase oRG generation and oRG amplification when transiently expressed in the mouse neocortex, resulting in thickening of the SVZ [23•]. Hedgehog signaling has also been proposed as a central signaling mechanism underlying the evolutionary expansion of oRG cells and neocortical growth [35].

Molecular mechanism of MST

oRG cells display a unique behavior called mitotic somal translocation (MST), in which the soma rapidly ascends towards the cortical plate in the hour before cytokinesis [1]. Recent studies have begun to explore the possible roles of MST in normal development, human brain evolution, and disease [2••,26]. MST appears to expand the basal border of the oSVZ outwards towards the cortical plate, which may reduce cell crowding, thereby allowing further transit amplification within the oSVZ by oRG and IP cells [8]. Until recently, the lack of understanding of MST at a molecular level prevented a detailed exploration of its role in development and disease. Based on the lineage relationship between vRG and oRG cells, and on the assumption that MST involved nuclear movement towards the centrosome, it had been speculated that the mechanism could be similar to vRG cell interkinetic nuclear migration (INM) [36]. During INM, the nucleus translocates along the basal fiber in synchrony with the cell cycle, first basally and then apically towards the centrosome prior to cytokinesis. The apical, centrosome-directed component of INM is dependent on the microtubule motor dynein and associated proteins such as LIS1 [36]. Therefore, it was speculated that dynein was involved in MST as well.

A recent study from our group used live imaging of cortical organotypic slices and fluorescent labeling of oRG cells to ask what molecular motor was responsible for driving MST. Surprisingly, microtubule depolymerization using nocodazole did not inhibit MST, implying that microtubule motors such as dynein do not control MST. Instead, MST required non-muscle myosin II (NMII) and the Rho effector ROCK, implicating the RhoA-ROCK-NMII pathway in MST [2••]. In contrast to INM, the centrosomes maintained a perinuclear localization throughout MST [2••]. Thus, oRG cell MST is dependent on the RhoA-ROCK-NMII pathway, but not microtubule motors or centrosomal guidance, and is mechanistically distinct from both INM and neuronal migration (Figure 1).

Cortical malformations and MST

MST may be targeted in neurodevelopmental disorders caused by mutations in genes interacting with the RhoA-ROCK-NMII pathway. Genes shown to interact with this pathway have been linked to several types of cortical malformations, including forms of primary

microcephaly (*ASPM*), periventricular nodular heterotopia (*ARFGEF2, FLNA, FMR1*), cobblestone malformations (*GPR56*), and lissencephaly (*RELN*) [2••,37]. Neuronal migration was first hypothesized to be the primary target of many of these mutations because NMII plays an important role in neuronal migration [38], and the motility of neural stem cells such as oRG cells was not previously appreciated. However, attempts to model these disorders in mouse have only sometimes resulted in altered phenotypes, and often produce a grossly normal brain [2••]. This phenotype discrepancy would be explained if oRG cell MST was the primary target of these mutations, as mice lack an oSVZ and display very few oRG cells [18].

A role for MST in brain evolution

Interestingly, MST length, frequency and direction correlate with oSVZ size. Despite a strong correlation between oSVZ size and degree of brain folding (gyrencephaly), currently there are no functional studies showing the role of MST in brain development. MST distance in the fetal human brain (highly folded) is, on average, greater than in the ferret brain (intermediate level of folding), while mice, with smooth (lissencephalic) brains, display a shorter MST distance than both ferrets and humans [2••]. In the human fetal cortex, almost half of all divisions within the oSVZ are preceded by MST, compared to approximately 15% in ferret [2••]. In the macaque, 24% of oRG cells undergo MST [21]. While MST is overwhelmingly directed towards the cortical plate in the fetal human brain [2••], oSVZ progenitor cells in the fetal macaque monkey display approximately equal proportions of translocations directed towards the cortical plate and towards the ventricle [21], and up to 46% of MST trajectories in the ferret are oblique or even parallel to the ventricular surface [26]. These data suggest that an increase in MST length and frequency may partially underlie evolutionary expansion in primate oSVZ, and that a consistent pial-directed translocation trajectory may be particularly prevalent in human. As discussed above, an increased number of IP cells with oRG-like morphology in non-human primates may explain discrepancies in molecular marker expression, and MST behavior of oSVZ progenitors in different species [1,21].

2-Dimensional and 3-dimensional cell culture to model oRG cells and MST

While organotypic slice culture using human tissue is ideal for studying unique aspects of human brain development that cannot be recapitulated in model organisms, important caveats limit the utility of this method. Tissue availability is often scarce, particularly of disease specimens, while viability, and therefore experiment length, does not extend past one week. Variability in experimental results may exist due to potentially inaccurate estimates of gestational age, and difficulty in determining brain region of partial specimens. A number of experimental systems using human primary cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells, are addressing these issues and greatly expanding the types of questions we can ask about MST and human brain development [15,39•,40–42]. Dissociated human fetal progenitor cells can be cultured for up to six weeks or longer, allowing for more extensive lineage analysis and examination of cell-intrinsic behaviors [9••,15]. oRG cells have been identified in dissociated dorsal cortical tissue from human fetal brain specimens, based on morphology, marker expression and MST prior to cytokinesis [15]. MST in

dissociated primary culture, as in slice culture, is ROCK-dependent and NMII-dependent, suggesting that the molecular mechanisms regulating MST may be reliably studied using this method [2••].

ES and iPS cell cultures are particularly attractive for studying human oRG cells given the widespread availability of cell lines, the ease of performing genetic modifications using techniques such as CRISPR [43], and the ability to use patient-derived iPS cells. Neural progenitor cell cultures derived from human ES and iPS cells have been shown to display apicobasal polarity, with vRG-like cells that undergo interkinetic nuclear migration, and oRG-like cells that undergo MST [40,44]. Recently, a 3-dimensional stem cell culture system has been developed to grow cerebral organoids using both ES and iPS cells. The organoids recapitulate many aspects of human brain development, including formation of ventricle-like structures and the organization of progenitor zones. Initial studies of cerebral organoids reported the presence of radial glial-like cells sharing morphological characteristics with oRG cells, and like oRG cells in fetal tissue, displaying a horizontal cleavage angle, with the cleavage furrow perpendicular to the ventricular surface [15,39•,45]. However, a lack of molecular markers for oRG cells at the time of these studies, and an absence of evidence for MST behavior, limited the validation of this cell population in 3D cerebral organoids. Following the discovery of molecular markers of oRG cells [9••,25•] and with ever improving *in vitro* protocols, a recent study demonstrated the generation of an OSVZ-like layer with cells expressing the oRG markers HOPX, FAM107A and PTPRZ1 [46••]. Studies from our laboratory using live time-lapse imaging of cerebral organoids show that the MST behavior is also recapitulated by oRG cells in cerebral organoids (Figure 2). Given these latest advances, this culture system would be ideally suited for studying how genetic mutations implicated in cortical malformations affect MST.

Conclusion

Since its initial description as a neural stem cell of the developing human neocortex our understanding of the oRG cell has greatly advanced. This is mainly due to the molecular profiling of this cell type with single cell transcriptomics [9••,25•] and the elucidation of the molecular motors underlying MST, the unique oRG cell mitotic behavior [2••,9••,25•]. Future studies building on this knowledge should determine the regulators that drive MST behavior, especially regarding the directionality of the movement which varies between different species. It will also be important to investigate the significance of MST, a phenomenon largely unexplored, in cortical development. 3D modeling of developmental diseases with cerebral organoids offers a promising platform to undertake such studies. However, molecular profiling of these *in vitro* derived cells and their comparison to their fetal counterparts will be an important step in validating oRG cells in such models.

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Asian ZIKV strains. ZIKV infection leads to increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer volume resembling microcephaly. [PubMed: 27118425]

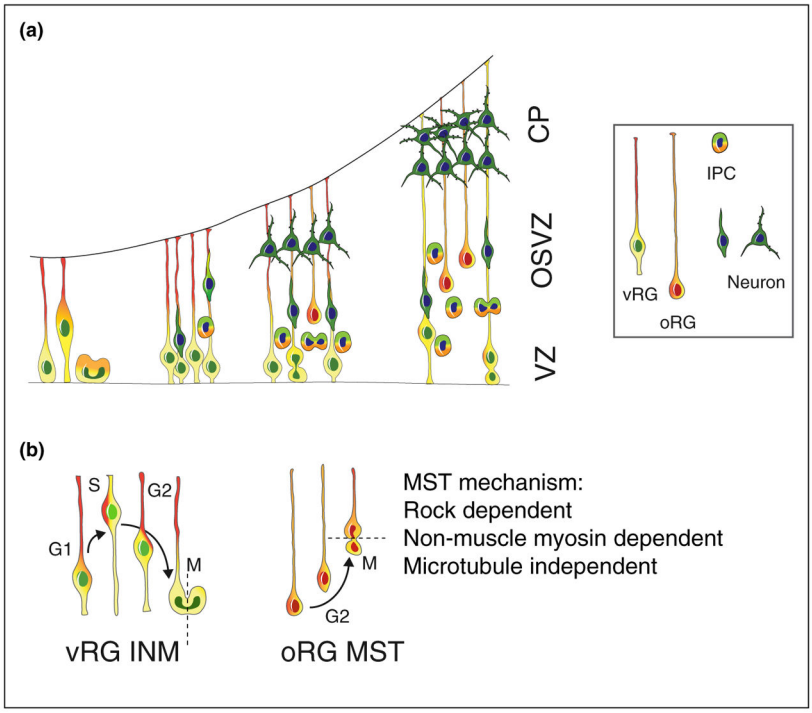

Figure 1.

Increased cellular complexity during neurogenesis. **(a)** Schematic representation of neural stem cell populations and their progeny as neurogenesis proceeds (left to right). Early vRG cells undergo symmetric divisions to give rise to a pool of progenitor vRG cells. vRG cells can divide asymmetrically to generate intermediate progenitors or neurons. Newly born neurons migrate along the RG radial fiber scaffold. As neurogenesis proceeds vRG cells also undergo horizontal divisions to give rise to oRG cells, creating a second neurogenic niche of neural stem cells. Neurons migrate through the oSVZ to populate the cortical plate. Ventricular zone (VZ), outer subventricular zone (oSVZ), cortical plate (CP). **(b)** vRG cells undergo interkinetic nuclear migration (INM). oRG cells undergo MST. Horizontal and vertical cleavage planes shown.

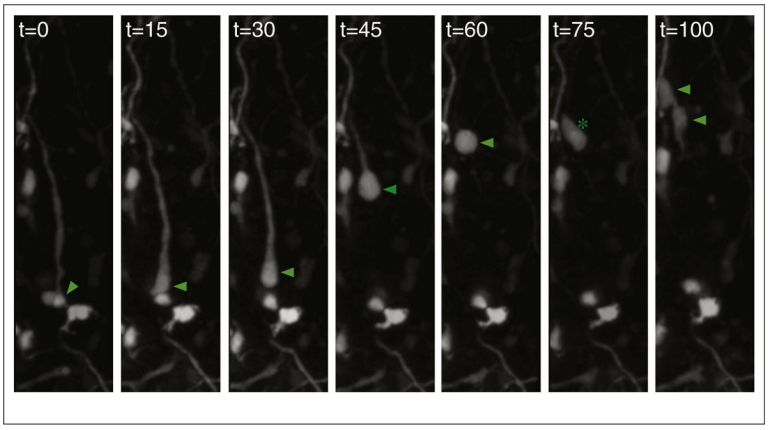

Figure 2.

MST behavior in iPS cell derived organoids. Time-lapse stills of oRG-like cells labeled with CMV-GFP adenovirus in iPS cell derived organoid slices following 90 days culture *in vitro*. Time elapsed from start of imaging indicated at the top of image (minutes). An oRG-like cell (closed arrowhead) maintains the basal fiber during mitosis and exhibits an MST before dividing (star) to give 2 daughter cells (2 closed arrowheads).