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Neocortical Expansion and Neurodevelopmental Disease*

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Abstract Changes of cerebral cortex size and brain volume have experienced evolutionary expansion across mammals, which is the cellular base of neuronal network complexity. The existence of cortex folding, allowing huge area of cortex to fit into a relatively small cranial space, is one of important evolutionary features in primate. The adult human CNS contains approximately 86.1 billion neurons and equal number of glial cells. Among which, human cerebral cortex consists of roughly 16.34 billion neurons. Based on algorithm analysis, approximately 3.86 million projection (excitatory/pyramidal) neurons are generated per hour during prenatal cortical neurogenesis in human. Such an incredible fast speed of neurogenesis suggests that human neocortex development requires strictly organized molecular regulations and cellular processes. Here we discuss the molecular and cellular characteristics of mammalian cerebral cortex expansion and the related disease.

Key words neocortex, evolution, development, neurogenesis, OSVZ
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1 OSVZ and neural progenitor cells

After closure of neural tube, a layer of neuroepithelial cells serve as neural stem cells, dividing symmetrically to expand founder progenitor pool in a short period of time, called ventricular zone (VZ). Then progenitor cells in neocortical VZ begin to generate earliest neurons or progenitor cells *via* asymmetrical divisions[1–7]. At the same time, another proliferative area appears above the VZ, called the subventricular zone (SVZ). Smart *et al*. first identified the remarkable size of the SVZ in the developing cerebral cortex of macaque monkeys, and in primate, SVZ was defined into two compartments, the ISVZ (inner SVZ) and OSVZ (outer SVZ), separated by a thin layer, the inner fiber layer (IFL)[8–10]. The proliferative OSVZ exhibits a number of distinctive characteristics in primates compared to rodents with only relatively small SVZ[10–12]. Other than primates, OSVZ-like structure is also discovered in carnivores, such as ferrets, which also exhibit gyrencephalic brains, suggests that OSVZ presence may be a key evolutionary process for cortical expansion[8, 13–15]. However, the findings that the lissencephalic primate marmoset also exhibits large OSVZ indicate that OSVZ is not the only requirement for gyrus formation

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in mammals[16–17].

Neurogenic speed is incredible fast in primates, which is a result from the diversity and proliferative behavior of neural precursors. VZ and SVZ of dorsal pallium are two regions enriched with progenitor cells and give rise to excitatory glutamatergic neurons in neocortex [8, 12, 18]. In early embryonic development, neural progenitor cells localized in VZ are called ventral radial glia(vRG), with one side anchoring to the VZ surface and the other side to the pia surface with long processes. In mammals, vRG cells initially divide symmetrically to enlarge the progenitor pool and then divide asymmetrically to maintain one daughter cell as vRG and the other as a progenitor cell, such as an outer radial glia (oRG) or an intermediate progenitor cell (IPC), or a newborn neuron (Figure 1) [11, 13, 19–21]. oRGs only exhibit long processes attached to pia surface, reside mainly in SVZ. Compared to only a few oRG in mouse SVZ, oRGs are abundant in ferrets, macaque, and human brains, indicating that the expansion of this type of precursor population correlates with brain size[8, 12, 15, 18]. In human and ferret brains, oRGs undergo multiple rounds of self-renewing divisions and then asymmetric divisions to generate IPCs or neurons [8, 12, 15, 18, 22–23]. IPCs, serving as highly proliferative progenitors, play an important role in neuron transient amplification in cortical development. IPCs are generated from vRGs or oRGs, capable of self-renew and divided as neurons. Experimental increase of the oRG abundance by genetic manipulations in lissencephalic animal models (such as, mice) results in promoted neurogenesis and some cortical folding with enlarged surface size[24–27], further suggesting that oRGs are essential for neuron burst in a short period of cortical development in primates.

Comparative studies indicate that not only oRG abundance is different, but proliferative properties are also distinguishable between lissencephalic mouse and gyrocephalic ferret or human. While majority of rodent oRGs undergo asymmetric division to give rise to nascent neurons, primate oRGs are able to self-renew for several successive cell cycles and then divide asymmetrically for IPCs or neuron production (Figure 1) [8, 12, 15, 18, 22–23]. In addition, compared to mouse, the oRG morphology is more diverse in ferret with different types of visible basal processes [15, 23], indicating parallel increases in cell number, morphological diversity and proliferative capacity in gyrocephalic mammals serving as cellular basis of neurogenesis.

Classically, progenitor cells in VZ and SVZ give rise to excitatory neurons and precursors located at ganglionic eminences of the ventral telencephalon primarily generate inhibitory interneurons[28–29]. Recent studies in primate OSVZ suggest that some interneuron progenitor cells also reside in this zone in developing human and macaque brains, which are considered as newly identified resource for calretinin-positive interneuron generation [29–35]. This observation is not clear in rodent, suggesting that the complicity of primate neocortex comes from the diversity of neural progenitor cells at the beginning of brain development. Other than neural precursors, cells with oligodendrocyte progenitor cells with Olig2 expression are also present in OSVZ[36–37], indicating the existence of localized origin of non-neural cells in a highly proliferative neurogenesis zone in primate, which may account for facilitating connectivity build-up in late prenatal development.

2 Cell cycle and cell migration of precursors

Cell cycle regulation is always one of critical determinants for cell proliferative ability. Interestingly, *ex vivo* cortex culture and imaging results in primate indicate that cell cycle duration of oRGs is shorter than vRGs, primarily due to a reduction of G1 time[10, 18, 38]. Shortened G1 period promotes progenitor cells to continuously enter cell cycles without exit, contributing to a rapid progenitor pool enrichment, and subsequently neuron generation and eventually fast cortical expansion in developing brain. Compared to primate, cell cycle duration of progenitors keeps increasing in rodent corticogenesis process, due to extended G1 phase[39], indicating that the fast cortical expansion in developing primate brain is a combined consequence along a long evolutionary road.

Experimental regulation of cell cycle plays roles in controlling the cortical thickness. For example, ectopically overexpressing CyclinD1/Cdk4 results in more proliferating progenitor cells and increased cortical surface in model animals[25, 40]. Moreover, loss of cyclin-dependent kinase inhibitors p57 (KIP2) or p27 (KIP1) increased primarily layer 5～6 or layer 2～5 neurons respectively *via* regulating different stages of precursor proliferation [41–42]. Deletion of histone acetyltransferase(HAT) cofactor transformation/ transcription domain-associated protein (Trrap) specifically disrupts E2F cell-cycle target gene

transcription, leading to cell-cycle lengthening in neural progenitor cells and microcephalic features in mice [43]. Disruption of centrosomal protein CENPJ in neural progenitors also causes cell cycle extension and microcephaly in mouse [44]. Therefore, cell-cycle regulation plays an important role in cortical expansion in mammals.

Neocortex exhibits a six-layered organization, a key feature general to all mammals. Progenitor cell division behavior and neuron migration regulation are the cellular and molecular basis of this elaborative lamination structure. Neurogenesis follows inside-first outside-last pattern where late-born neurons migrate and pass the early-born neurons in mammals. Radial position of excitatory projection neurons largely depend on their birthplace because the nascent neurons migrates along the basal processes of progenitor cells, such as vRGs and oRGs [4, 11, 28, 45-48]. In primate, fibers from vRGs in VZ and oRGs in OSVZ extend part of the way to the pia surface, which provide the possibility to neurons to switch fibers and move tangentially to the final destination [11, 15, 19, 49]. Additionally, recent studies in ferrets exhibit some oRG fibers reach to other area than pia surface, suggesting oRG possibly provide scaffolds to guide cells migrate primarily radially and also horizontally to some extent [14-15]. This could be an important mechanism in the expansion of neocortical surface observed in gyrencephalic species (Figure 1).

One important feature of vRGs is interkinetic nuclear migration (INM), a classic migration that soma move down towards apical VZ surface where vRGs find centrosomes and undergo M-phase. INM requires centrosome, microtubule and actomyosin system [22, 50]. The minus-end microtubule associated motor protein dynein, and the dynein-interacting protein Lis1 regulate nuclear movements in INM [51-53]. Moreover, inhibition of the small GTPase Rac in actomyosin motor system specifically affect INM in developing mouse brain [54]. Different from vRGs, oRGs undergo a distinct movement prior to mitosis, called mitotic somal translocation (MST). During MST, oRG cell body moves rapidly up towards the pia surface side before entering mitosis in human and mouse [12, 18, 22]. Along with the discovery of different cellular morphology of oRGs, the movement of MST is observed to be basally or apically, and even no movement (stationary) in primate and ferret before mitosis [15, 38].

3 Molecular identity of neural progenitor cells

Transcription factor expression patterns are used to define neural progenitor diversity. In mouse, vRGs and oRGs express Pax6 but no Tbr2 in VZ and SVZ respectively and IPCs express Tbr2 but no Pax6 in SVZ [1, 12, 18]. In ferret and primate, other than these markers, cortical OSVZ precursors are more diverse with expression of Pax6, Tbr2, Sox2, Ascl1, and Olig2 in a non-mutually exclusive manner [8, 18, 36, 55], suggesting many different kinds of cells, such as excitatory neurons, inhibitory interneurons, oligodendrocytes could be produced in this area, resulting in a more complicated cortical structure in gyrencephalic species.

Similar to vRGs, oRGs express radial glia markers and progenitor markers but lack an apical attachment to the ventricular surface. With a fast development of single-cell transcriptomic technologies, identification of cell-type-specific markers would open a window to explore the correlation between morphology, proliferative behavior, and offspring cell fate determinants. Pollen and colleagues have revealed that oRGs preferentially express TNC, PTPRZ1, FAM107A, HOPX, and LIFR genes, that related to extracellular matrix compartment, migration regulation, and stemness maintenance in human cortical development [56], suggesting that oRGs are maintained in subventricular niche, where LIFR/STAT3 self-renewal pathway is selectively activated in oRGs [56]. Additional work demonstrate ANXA1 and CRYAB as vRG specific expression markers in human cortex by Fixed and Recovered Intact Single-cell RNA (FRISCR), a new method for single-cell transcriptomics study in fixed cells [57]. Molecular differences between species are also important for understanding the cortical expansion from an evolutionary aspect. The growth factor PDGFD and its receptor PDGFRbeta are specifically expressed by RG in human, but not in mouse in corticogenesis. Inhibition of PDGFD-PDGFRbeta signaling in human cortical culture blocks cell cycle of RG [58]. Expression of human-specific gene ARHGAP11B in mouse neocortex promotes RG self-renewal and induces gyrification in late developmental stage [24]. These discoveries indicate that some molecules and signaling pathways may contribute to evolutionary expansion of human neocortex.

4 Cortical development diseases

Accumulated experiments reveal more and more understanding of the anatomy structure, cellular dynamics and molecular regulation of the cortical expansion in development. Our knowledge of the cellular and molecular basis of spatial-temporal organization of the developing neocortex in human drive us to look into neurodevelopmental disease[9, 12, 59-60]. Here we discuss some neurodevelopmental disease, focusing on excitatory-neurogenesis-related disorders in the cortex. Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder that is characterized by reduction of circumference of the head, brain volume and cortex thickness with normal brain architecture. Patients experience neurological and psychiatric symptoms, including cognition defects, mental retardation, motor deficit, balance and coordination difficulties, *etc*. [61]. Twelve MCPH loci (MCPH1-MCPH12) have been identified all around the world, including *Microcephalin*, *CDK5RAP2*, *CENPJ*, *ASPM*, *WDR62*, *CASC5*, *STIL*, *CEP135*, *CEP152*, *ZNF335*, *PHC1* and *CDK6*. These gene mutations cause disturbed mitotic spindle orientation, premature chromosomal condensation, DNA damage signaling deficiency, progenitor cell apoptosis, cell migration defects, leading to impaired neurogenesis and reduced neocortex size[61-79].

Neuronal migration plays an essential role in mammal cortical development as discussed. Hence, defects in neural progenitor cell or newborn neuron migration directly cause brain developmental diseases. Doublecortin (DCX) is a microtubule-associated protein expressed in some neuronal precursor cells and immature neurons. DCX mutations lead to X-linked lissencephaly and the double cortex syndrome, showing a subcortical band of misplaced neurons, due to disrupted neuronal migration [80-83]. Another lissencephaly-related gene is LIS1, encoding dynein-interacting protein, regulating centrosomal and nuclear translocation in neuronal migration and hence impairing brain cortical expansion and folding[22, 51, 84]. Reelin is an extracellular molecule secreted by Cajal-Retzius cells, regulating microtubule dynamics and triggering neurons to migrate into their proper destination in the cortex and promote neuron to detach from RG fibers [85-87]. Disruptions in Reelin signaling pathway lead to layer disorganization in mice and lissencephaly and cerebellar hypoplasia in human due to neuron migration defects. Other than developmental disorders, mutations of Reelin have also been identified to associate with neuropsychiatric disorders and neurodegenerative disease, such as, schizophrenia, autism and Alzheimer disease (AD)[85-86, 88-91].

5 Involved in contribution to cortical expansion

Dr. Xiao-Qun Wang has been working on researching the cellular and molecular regulation of cortical expansion using different animal models. It has been know that vRGs experience symmetrical division at early development stage for processor cell pool enrichment, and then asymmetrical mitosis for self-renewal and neuron production in mammals. However, the cellular characteristics of asymmetrical division of vRGs are still not clear. Wang and colleagues [7] have demonstrate that the centrosome maintaining old mother centriole preferentially stays in the new progenitor cell while the centrosome with new mother centriole mostly leaves the VZ and is enclosed in post mitotic differentiating cell. In addition, this asymmetrical division behavior is regulated by ninein, a mature centriole-specific protein in mouse. More than findings in vRG, in 2011, Wang *et al*.[12] observe a new subtype of neural progenitor cells, oRGs, which arise from asymmetric divisions of vRGs and undergo asymmetric divisions to generate one oRG and one neuron in mice (Figure 1). Although the amount of oRGs in mice are much fewer than in primates, and the OSVZ does not exist in mice; however, the exploration of oRGs in mice not only broaden the biological basis of neurogenesis in rodents, but also open an avenue to the evolutionary comparison among different mammal species to understand the correlations among oRGs, OSVZ and cortical expansion. Other than cellular behaviors, Dr. Wang also works on how neurogenesis is regulated by signaling pathways. Epigenetic regulatory complexes play key roles in the modulation of transcriptional regulation underlying neural progenitor proliferation and progeny specification. How specific cofactors guide histone demethylase LSD1/KDM1A complex to regulate distinct gene activation and repression in neural processors, and consequently to play roles in cortical neurogenesis remains unclear. Wang and colleagues demonstrate that RCOR2, a co-repressor of LSD1, is mainly expressed in central nervous system (CNS), and plays

a key role in epigenetic regulation of cortical development. RCOR2 have been identified to directly bind to promoters of *Dlx2* and *Shh* and represses their transcriptions, suggesting that co-repressor RCOR2 is critical for cortical development by regulating SHH signaling pathway in dorsal telencephalon [92]. This work illustrates how developmental-essential SHH signals are regulated epigenetically. Moreover, ferrets have been used in development studies, serving as a non-primate gyrencephalic model for decades, and ferrets are considered as a great example for cortical expansion research. Wang leads a team to create several new disease models in ferrets for the first time, including *Dcx* or *Aspm* gene disruptions, which result in lissencephaly and microcephaly in ferrets [82]. The neurogenesis of ferret is closer to primate, compared to rodent; hence, this work offers neurobiologists novel and important tools for neurodevelopmental disease research, which are hardly studied in mouse, a widely used but lissencephalic animal model.

6 Conclusions and perspectives

Cortical expansion and neurogenesis has been one of the most important and hottest research fields over hundreds of years, because consciousness, motion, intelligence *etc*. are all highly related to cortex surface size and neural network complicity, which are all based on abundance of cell number and diversity of cell types in neural system. Other than studies of cellular mechanism and molecular regulators of neurogenesis in different species, the comparisons across species also shed lights on the understanding of the evolution of cortical folding and how that results in improving and creating new abilities of organism, such as vision, movement, learning memory, *etc*. Neurogenesis is an elaborative temporal-spatial process that is influenced by neural progenitor diversity, proliferative capacity and migration behavior. Tremendous molecules have been identified to be involved in neurogenesis regulation, including cell-cycle activators/inhibitors, centrosomal proteins, microtubule-associated kinesin or proteins, epigenetic factors, *etc*. Loss function of most of these molecules leads to neurodevelopmental diseases or neuropsychiatric disorders as discussed in previous context [9, 13, 22, 65, 84, 93]. Recent rapid development on

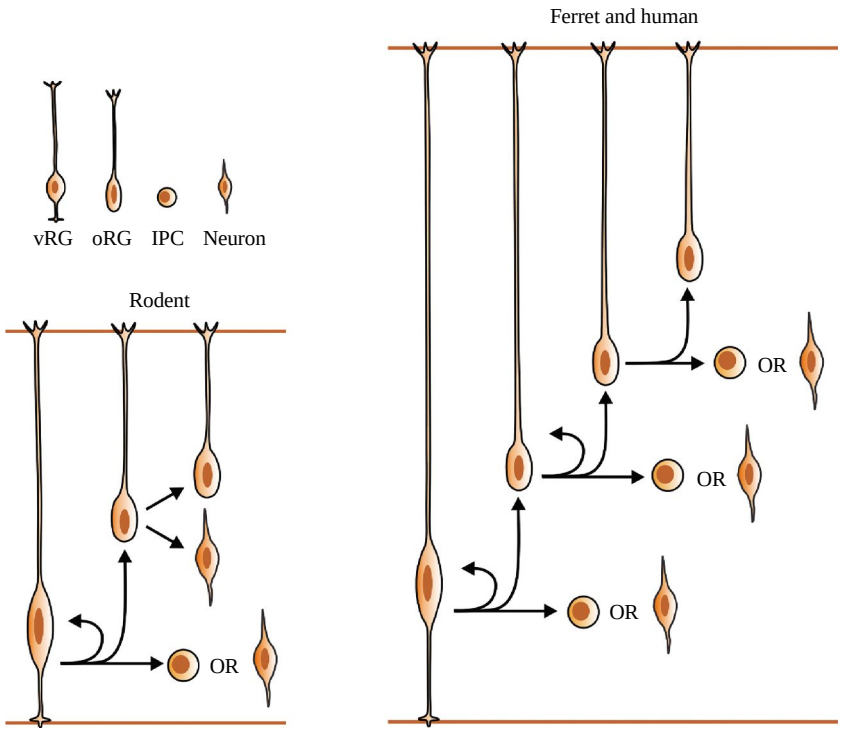

Fig. 1 Contrasting rodent and ferret/human cortical progenitor cell division behavior

Current views of the lineage relationship among vRGs, oRGs, IPCs and neurons. In rodent, ferret and human, vRGs all divide for self-renewal and generation of oRGs, IPCs or neurons. However, in rodent, oRGs typically divide to maintain one daughter cell as an oRG and the other as a neuron. In ferret or human, oRGs divide for several rounds to amplify oRG pool first, and then oRGs divide for accumulation of IPCs and neurons. IPCs, serving as transit-amplifying cells, are important sources for neuron generation. The differences of oRG cell division behavior lay the ground for fast neurogenesis and cortical expansion in gyrencephalic mammals.

analysis of single-cell genomics, transcriptomics or proteomics has enabled the detection of genomic variations in not only specific type of cells but also healthy or diseased human brains. These technologies have opened a big window to understand basic regulations of cortical expansion and disease mechanisms [56-58, 94-95]. With the fundamental understanding of molecular basis of different types of cells involved in cortical expansion, we would have an accurate and detailed map to direct us possibly not only to prevent neurodevelopmental diseases in babies and strengthen brain function of health people, but also open new avenues to find prospective candidate drugs and therapeutic strategies for neural system diseases and injury.

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大脑新皮层和神经发育疾病 *

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摘要 哺乳动物进化过程中，大脑皮层逐渐增大增厚和脑容量增大，从而构成了脑神经环路复杂性的细胞生物学基础．皮层出现皱褶是非人类灵长类演化的重要特征．成体人脑大约由近 860 多亿个神经细胞组成，其中，在人脑神经发生高峰，每小时有近 400 多万个兴奋性神经细胞产生．如此高速的神经生成过程需要精确的细胞与分子调控机制．本文主要讨论调控大脑皮层增大增厚的细胞与分子机制和相关的脑发育疾病．

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