Multiple roles of Ulk4 in neurogenesis and brain function

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ABSTRACT

Neurogenesis is essential for proper brain formation and function, and abnormal neural proliferation is an underlying neuropathology of many brain disorders. Recent advances on adult neurogenesis demonstrate that neural stem cells (NSCs) at the subventricular zone (SVZ) are largely derived during mid-embryonic neurogenesis from a subset of cells, which slow down in their pace of cell division, become quiescent cells and can be reactivated in need. The NSCs at birth constitute the stem cell pool for both postnatal oligodendrogenesis and adult neurogenesis. However, little is known about factors that control the size of NSC pool. The article published in Stem Cells on Jun 14, 2016 by Liu and colleagues described a member of the Unc-51-like serine/threonine kinase family, Ulk4, which plays a critical role in regulating the NSC pool size. Authors presented evidence of cell cycle-dependent Ulk4 expression in vitro and in vivo, and reduced NSC pool in targetedly disrupted Ulk4 newborn mice, with disturbed pathways of cell cycle regulation and WNT signaling (Fig. 1), suggesting that Ulk4 may be associated with neurodevelopmental, neuropsychiatric as well as neurodegenerative diseases.

Inspired by rare copy number variations of the ULK4 gene associated with schizophrenia, bipolar patients and autism, Shen and colleagues investigated the role of ULK4 in brain function. Further genetic evidence from the brain and body genetic resource exchange (BBGRE) cohort of patients showed that ULK4 was deleted in 1.2/1000 with clinical features including developmental delay, language delay and intellectual disability. The BBGRE cohort only constitutes patient samples with developmental disorders, with healthy controls. However, the Ulk4 mutation rate in this population is similar to that in the previous study, in which Xenopus Ulk4 gene is shown to be highly expressed in the ventricular zone (VZ) and SVZ of forebrain, and co-localized with Sox3 - a neural stem cell/progenitor marker in Xenopus, and a radial glial marker Blbp. In the article published in Stem Cells, Liu and colleagues demonstrate high Ulk4 expression in mouse neural proliferating zones during early and middle corticogenesis, which supports a role of Ulk4 in generating pyramidal neurons. Further examination reveals cell cycle-dependent Ulk4 expression, with the highest level in the G2/M phases in vitro and in vivo. Ulk4 expression is co-located with Ki67, a proliferation marker and phosphohistone 3, a marker for the M phase of mitotic cells in developing cortex. The G2/M phases of ULK4 expression is further supported by the

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in vitro study using human neuroblastoma cells, as nuclear morphology can be better visualized in cultured monolayer cells. These data also explain why no co-localization is observed between Ulk4 and BrdU in the earlier study, as Ulk4 expression is substantially weaker in G1 or S phase of cell cycle.

In keeping with cell-cycle specific Ulk4 expression, Liu et al observe that targeted Ulk4 disruption leads to a thinner cortex. The cerebral cortex is laminated with 6 layers of cells, and generated in a cell cycle-dependent and “inside out” manner during embryogenesis from the VZ of the dorsolateral telencephalon. The authors then perform immunochemistry with layer specific markers and demonstrate significant reduction of superficial layers (II-IV, Cdp/Cux1+) and deep layer (VI, Ctip2+) neurons in the mutants. The layers II-IV are produced during mid-neurogenesis, and the BrdU+ S phase cells are indeed decreased in E15.5 mutant embryos, and layer II-IV neurons are reduced at birth. The layer VI neurons are generated before E15.5, and interestingly, neither Tbr1+ (a layer VI marker) neurons at E15.5 nor Ctip2+ neurons at birth are significantly changed between wild type and mutants. Ulk4 homozygous mutants are shown to develop a hydrocephalus-related phenotype, which may contribute to the postnatal reduction of deep layer neurons.

Mammalian cortical neurogenesis is largely completed before birth. Limited NSCs are retained in the SVZ of lateral ventricles and subgranular zone (SGZ) of the hippocampus for adult neurogenesis. They remain as slowly dividing or quiescent cells which are activated for the need of postnatal oligodendrogenesis and adult neurogenesis. Indeed, the oligodendrocytogenesis is compromised in Ulk4 mutants (Liu et al., in preparation). In support of the role of Ulk4 in neural proliferation, a strong co-localization of anti-Ulk4 and Ki67 was observed in P0 SVZ region, whereas in neighboring areas of mature neurons Ulk4 expression was substantially reduced. The NSC pool at birth therefore links embryonic and adult neurogenesis. Remarkably, Ulk4 deficiency dramatically reduced Ki67+ cells in the SVZ of Ulk4 newborn brains. Together, Liu et al demonstrate that Ulk4 is not only required for embryonic neurogenesis, but also for the maintenance of the NSC pool (Fig. 1).

To address the molecular mechanism of this observation, the authors carry out whole genome RNA sequencing with cortical RNA. Quantitative expression analyses of 19,652 genes reveal 469 downregulated and 149 upregulated genes in the Ulk4 mutants. Pathway analyses show significant association of Ulk4 targets with “neural precursor cell proliferation” and “cell cycle,” with 16 genes involved in “cell cycle process,” 5 genes in “regulation of neural precursor cell proliferation,” 6 in cell-cycle phase transition, 8 as “positive regulators” and 6 as “negative regulators” of cell cycle. Evidence is also emerging from other studies that ULK family members can be involved in cell cycle progression and tumorigenesis. For example, knockdown of STK36 appears to increase the sensitivity to PARP inhibitor-induced cell death. Conversely, higher level of STK36 mRNA is found in patient cells with STK36 Ala687Thr mutation, which is associated with differential responsiveness to temozolomide chemotherapy in glioblastoma, the most aggressive form of brain tumors. Cells with STK36 Ala687 exhibit altered G2/M-checkpoint regulation and elevated sonic hedgehog pathway. In contrast, ULK1 and ULK2 are hypermethylated and downregulated in glioblastoma.

There is only limited literature published for ULK4, but one of the most prominent functions is its association with tumorigenesis. ULK4 polymorphism (rs1052501, Lys542Thr) was reported to be associated with multiple myeloma. In agreement with reduced proliferation and diminished Fzd6 expression in Ulk4 mutants, high FZD6 expression is linked to poor

Figure 1. Ulk4 is expressed in a cell cycle dependent manner and modulates cell cycle and Wnt signaling, thereby regulating embryonic corticogenesis, postnatal oligodendrogenesis and adult neurogenesis through the size of neural stem cell pool.
Recent review..12 Continuous production of new neurogenitor cells are comprehensively documented in a canonical pathways. Their roles in neural stem/pro-receptors (Fzd) and signal through canonical and non-glycolipoproteins, which bind to transmembrane neurogenesis. The Wnt family consists of 19 secreted signaling pathway is an essential regulator of adult mutants. This is particularly interesting as the Wnt behavior. Therefore, upregulation of Wnt signaling may counteract decline of age-related neurogenesis and cognition.18 Interestingly, key transducers of Wnt signaling also mediate knockout effects of CHD8, whose de novo mutations are strongly associated with autism spectrum disorder. Enhancing Wnt signaling is able to rescue the transcriptional and behavioral deficits caused by Chd8 knockdown,19 therefore Wnt pathway can be a drug target for both neurodevelopmental and neurodegenerative disorders.

Wnt signaling can be inhibited by adenosomatous polyposis coli (Apc), a multifunctional protein promoting β-catenin degradation by forming a destructive complex with β-catenin, axin and Gsk3β. Importantly, Apc is directly involved in the maintenance of NSCs in the adult neurogenic zones. Loss of Apc markedly reduces neurons in the SVZ, leading to a decreased olfactory granule cell layer.20 Binding of Wnts to Fzd1–10 can prevent β-catenin destruction, induce nuclear translocation and activate target gene transcription in association with the Tcf/Lef family.

Significantly, Fzd2, Fzd6 and Fzd10 are also reduced in Ulk4 mutants. Mutations of human FZD6 are 5-fold enriched for neural tube defects, and Fzd3−/−/Fzd6−/− mice are associated with neural tube defects.21 Wnt target genes are also dys-regulated in Ulk4 mutants. For example, Nr5a2 is involved in maintenance of the stemness of embryonic stem cells but reduced in Ulk4 mutants. On the other hand, expression of Gfap and Vim, negative regulators of neurogenesis is markedly increased in Ulk4 mutants.22 Together, Liu et al suggest that the Wnt pathway may play a critical role in mediating the effects of Ulk4 on NSCs.

Interestingly, new neurons migrate in parallel to the flow of cerebrospinal fluid (CSF) in the adult brain, and synchronized beating of ependymal cilia is required for CSF flow and directional migration of neuroblasts.23 Normal production and circulation of growth, and dendritic spine formation in adult hippocampus.16 Similarly, Lrp6, a co-receptor to Frizzled in the Wnt pathway, is identified as a negative regulator of NSC proliferation through system genetics, overexpression and silencing approaches.17 In addition, it is shown that expression of the Wnt antagonist Dkk1 increases with age and memory decline. Inducible loss of the Dkk1 can elevate Wnt activity, enhance self-renewal and increase generation of immature neurons. Mice deficient in Dkk1 exhibit enhanced spatial working memory and memory consolidation with improved affective behavior. Therefore, overexpression of Lrp6 signaling may counteract decline of age-related neurogenesis and cognition.18 Interestingly, key transducers of Wnt signaling also mediate knockout effects of CHD8, whose de novo mutations are strongly associated with autism spectrum disorder. Enhancing Wnt signaling is able to rescue the transcriptional and behavioral deficits caused by Chd8 knockdown,19 therefore Wnt pathway can be a drug target for both neurodevelopmental and neurodegenerative disorders.

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the CSF are known to be crucial for proper brain development.\textsuperscript{24,25} For example, Wnt4 and Wnt modulator Tgm2 are regulated by Otx2, a master regulator of choroid plexus development which produces CSF.\textsuperscript{24} We showed recently that Ulk4 is essential for ciliogenesis and CSF flow.\textsuperscript{26} Ependymal layer constitutes polarized cells with motile cilia projecting to ventricles, and the planar cell polarity cadherins Celsr2 and Celsr3 are shown to regulate basal body orientation at the foot of cilia. Ciliogenesis is markedly impaired in Celsr2/ Celsr3 double mutants, resulting in lethal hydrocephalus, with disturbed membrane distribution of Fzd3 and Vangl2.\textsuperscript{25} Remarkably, mutations of Celsr2, Fzd3, Ulk4 and Stk36 are all found as risk factors of congenital hydrocephalus.\textsuperscript{27} We show that Ulk4 deficiency impairs cilia formation and function, leading to hydrocephalus. This may be mediated by dysregulated Foxj1 and Wnt signaling. Expression of Apc, Fzd2, Fzd6 and Fzd10 are decreased in the Ulk4 mutant brain. Therefore dysregulation of the Wnt pathway may mediate Ulk4 effects on both ciliogenesis and neurogenesis.

Both increased and decreased neurogenesis has been associated with neurodevelopmental diseases. A good example of this is that reciprocal deletions and duplications of \textasciitilde 600kb region at 16p11.2 are strongly associated with behavioral disorders, cognitive deficits and brain volume. 16p11.2 deletions are associated with speech/language and motor deficits, intellectual impairment, restrictive and repetitive behavior and macrocephaly,\textsuperscript{28,29} whereas duplications are associated with ADHD, schizophrenia and microcephaly.\textsuperscript{28,30} Decreased neurogenesis in the Ulk4 mutants has provided a cellular mechanism for its association with neurodevelopmental disorders.

A disturbed balance of excitation and inhibition is proposed as a fundamental mechanism of a range of neurodevelopmental and neuropsychiatric disorders. Interesting, ULK4 is shown to be highly expressed in the inhibitory GABAergic neurons in adult brain,\textsuperscript{5} and at P0 Ulk4 expression appears to be higher in the ventral forebrain where inhibitory neurons originate, than in the cortex where the excitatory neurons reside.\textsuperscript{4} The fact that ULK4 regulates NSC pool at an early stage\textsuperscript{4} and is highly expressed in the inhibitory neurons in adult stage\textsuperscript{5} suggests that Ulk4 may be involved in a range of neurodevelopmental, neuropsychiatric and neurodegenerative disorders.

Defective neuritogenesis is one of the most consistent neuroanatomical findings in schizophrenia. Human post-mortem studies reveal shorter/less branched dendrites,\textsuperscript{31,32} and lower density of dendritic spines\textsuperscript{33} in schizophrenic brains. Using induced pluripotent stem cells it is shown that neurons derived from schizophrenic fibroblasts have decreased neurite numbers and reduced neuronal connectivity.\textsuperscript{34,35} ULK4 lesions also compromise neurotogenesis in the mouse brain and in human neuroblastoma cells.\textsuperscript{5} However, it remains to be seen if ULK4 deletion/mutation affects neuronal differentiation in human pluripotent stem cells.

While Liu \textit{et al.} have made the discovery that Ulk4 regulates cell cycle and NSC proliferation, many questions remain to be answered. For example, what is the nature of Ulk4 splicing variants in embryonic and adult brains? How do they function differently? What are the relationships among the ULK4 family members? Does ULK4 have kinase activity? What are the substrates or binding partners then? How does the binding of Ulk4 change the property of its partners? What is the nature of mutations/polymorphisms associated with multiple myeloma? Will ULK4 lesions in human pluripotent stem cells reproduce the phenotypes observed in the mouse model? What is the role of ULK4 in neurodegenerative diseases? Are there compounds available to rescue the defects of Ulk4 mutants? The results in the current manuscript suggest that it may be the time to address some of these questions, including the roles that ULK4 plays in both the central nervous and other systems.

\textbf{Abbreviations}

\begin{tabular}{ll}
Apc & adenomatous polyposis coli \\
BBGRE & brain and body genetic resource exchange \\
NSCs & neural stem cells \\
SGZ & subgranular zone \\
SVZ & subventricular zone \\
Ulk4 & unc-51-like serine/threonine kinase 4 \\
VZ & ventricular zone \\
\end{tabular}

\textbf{Disclosure of potential conflicts of interest}

No potential conflicts of interest were disclosed.

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