ARTICLE ADDENDUM

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Brain size is controlled by the mammalian target of rapamycin (mTOR) in mice

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The number of neurons in the brain is mostly determined by neural progenitor proliferation and neurogenesis during embryonic development. Increase in postnatal brain size is largely dependent on cellular volume changes. The mammalian target of rapamycin (mTOR) signaling has been associated with cell proliferation and size determination in a variety of cell types. The role of mTOR signaling in neural development has been increasingly pursued due to its association with neurodevelopmental disorders and cancers. Surprisingly, however, there has been lack of *in vivo* genetic evidence that defines mTOR functions in neural progenitors during progenitor self-renewal and subsequent brain formation. Here, we discuss our recent evidence that mTOR signaling is required for the establishment of normal brain size during development. Mice lacking mTOR show smaller brain and reduced numbers of neural progenitors and neurons. Additionally, mTOR interacts with the Wnt signaling pathway in the control of neural progenitors. Our study establishes the mTOR signal as a key regulator of an evolutionarily conserved cascade that is responsible for vertebrate brain size.

Keywords: brain size, GSK-3, mTOR, neural progenitor, neurogenesis

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Control of Neural Progenitor Proliferation and Neuron Size

Cell cycle regulation plays an important role in the number of neurons produced in the developing brain.[1] Changes in cell cycle progression such as cell cycle length and re-entry/exit alter brain size.[2-4] Radial neural progenitors deficient in mTOR signaling fail to re-enter cell cycle and show abnormal cell cycle length (Ka et al., 2014). As a result, the number of radial progenitors and intermediate progenitors is decreased in mTOR-deficient brains. Consistent with this finding, neurogenesis is inhibited throughout the embryonic ages with cell counts and Western blot analysis showing that only around half of the normal number of neurons are generated in mTOR-deficient brains.[5] The decreased number of both post-mitotic neurons and intermediate progenitors in mTOR-deficient mice is expected because radial neural progenitors are the source of both cell types. Thus, neural differentiation is largely arrested at the radial progenitor stage in mTOR-deficient brain. Although deletion of mTOR inhibits neural differentiation beyond the radial progenitor phase, some progenitors are still capable of differentiation into intermediate progenitors and post-mitotic neurons. Whether some progenitors can truly progress independently of mTOR signaling or whether the differentiated cells represent a population of radial progenitors that have some persistent mTOR protein due to either late or incomplete deletion of mTOR remains to be determined. Kriegstein and colleagues have recently shown that there is another type of neural progenitor, outer subventricular zone radial glia-like (oRG) cells, in the developing brain.[6,7] It remains to be elucidated if mTOR plays a similar role in oRG cells as well as in radial neural progenitors and intermediate progenitors.

Neuronal cell size is also a critical determinant of overall brain size, especially the thickness of the cerebral cortex. mTOR and its downstream targets, S6K and 4EBP1, are thought to control mammalian cell size.[8-11] Intracellular

molecules that regulate mTOR activity such as AKT/PTEN are associated with neuronal cell size.[12] In mTOR-deficient brains, neurons in the cortical plate are smaller.[5] Thus, reduced cell size contributes to the smaller brain in mTOR-deficient mice. These findings demonstrate that mTOR is critical to determine the size of developing neurons.

The Size of the Brain and Cognitive Evolution

The evolution of cognitive function has been an intriguing topic in evolutionary and cognitive neuroscience. There is little information as to how cognition has evolved in vertebrates.[13-15] Brain size has been proposed as a factor in cognitive evolution.[16-18] There are remarkable variances in brain size across species. Evolutionary changes in brain size and cortical reorganization are thought to determine corresponding change in cognitive function.[17,19] A recent study has demonstrated that the species with the largest brain volume show superior cognitive powers in a series of self-control.[20] Larger brains have more neurons and tend to become more modularized, which may facilitate the evolution of new cognitive networks. These findings suggest that changes in brain size set up a foundation for evolutionary improvement in cognitive function. In this regard, the role of mTOR in brain size control may be a critical mechanism of cognitive evolution. Although mTOR is conserved throughout evolution, the amount and functional proportion of mTOR activity may vary across the species, critically contributing to the determination of brain size. It will be interesting to examine if mTOR activity is changed in different species.

Disease Implication

The abnormal regulation of neural progenitors and neurogenesis can lead to altered brain size and function, and is implicated in a number of neurodevelopmental disorders and brain malformations including mental retardation, schizophrenia, epilepsy, autism, lissencephaly, microcephaly, and heterotopias.[21,22] Genetic mutations and/or activity changes in mTOR and Tuberous Sclerosis Complex 2 (TSC2) are implicated in neurological diseases including autism spectrum disorders, schizophrenia, bipolar disorder, epilepsy, and brain tumors.[23,24] For example, the tuberous sclerosis complex, which is caused by a genetic mutation of TSC1 or 2, is associated with abnormal cell proliferation and differentiation in the brain. Patients with this disease show an activated mTOR signal. Thus, dysfunction of mTOR and TSC2 in neural progenitor regulation could be an important aspect of this pathophysiology.

The activity of Glycogen Synthase Kinase-3 (GSK-3) is altered in multiple diseases,[25,26] suggesting that manipulation of GSK-3 activity within neural progenitors and neurons is potentially a powerful tool for developing therapies against neurological disorders associated with brain size abnormality. However, the act of simply inhibiting or activating GSK-3 is expected to bring about undesirable side effects given that GSK-3 is associated with multiple cellular signals.[27] Thus, it is critical to identify novel downstream targets of GSK-3 signaling that offer more selectivity for the regulation of neural progenitors. We have previously identified GSK-3 downstream targets in neural progenitors such as β-catenin (Wnt), notch intracellular domain (Notch), and Gli (Shh) proteins.[28] Yet, difficulties arise in creating pharmacological interventions for these transcription factors. In particular, the Wnt/β-catenin pathway is notoriously difficult to create pharmacological interventions.[29] However, unlike Wnt, Notch, or Shh, the mTOR pathway can be easily controlled by pharmacological agents, including rapamycin, which is currently in clinical use such as cancer treatment. Our study demonstrates that the mTOR pathway interacts with GKS-3 signaling and that the interaction plays important roles in neural progenitor maintenance and neuron size determination.[5] Thus, the control of mTOR activity in neural progenitors and their derivatives may become a pharmacological strategy for treating GSK-3-associated diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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