

# Enhancing Lysosomal Activation Restores Neural Stem Cell Function During Aging

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**ABSTRACT:** Adult neurogenesis supports cognitive and sensory functions in mammals and is significantly reduced with age. Quiescent neural stem cells are the source of new neurons in the adult brain and emerging evidence suggests that the failure of these cells to activate and re-enter the cell cycle is largely responsible for reduced neurogenesis in old animals. However, the molecular mechanisms supporting quiescence and activation in the adult and aged brain remain undefined. Recent work published by Leeman et al. in *Science* uncovers a novel role for lysosomes in supporting neural stem cell activation, and reveals that loss of lysosome function during aging contributes to reduced neural stem cell activity. Using a combination of transcriptomics and functional analysis, the authors show that quiescent and activated neural stem cells employ different branches of proteostasis networks, with quiescent stem cells particularly dependent on the lysosome-autophagy system. Excitingly, stimulation of lysosomal activity in the aged quiescent population significantly enhanced their ability to activate and increased the frequency of activated neural stem and progenitor cells within the neural stem cell niche. This work for the first time identifies lysosomal dysfunction as a cause of reduced neurogenesis during aging, and shows that enhancing lysosomal function is sufficient to restore healthy stem cell activity in the aged brain.

**KEYWORDS:** Neural stem cell, aging, lysosome, proteostasis

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## Introduction

Protein homeostasis, or proteostasis, is critical to maintain cellular integrity and function. Dysregulation of the proteome, including accumulation of damaged and aggregated proteins, is a major hallmark of aging.<sup>1</sup> Accumulation of protein aggregates is also associated with pathological conditions, including neurodegenerative diseases. Though not much is known about the etiology of aggregates in many cases, their clearance can extend lifespan and alleviate the symptoms of neurodegeneration in some model systems.<sup>2,3</sup> Thus, understanding the mechanisms responsible for aggregate accumulation and clearance will have important implications for preventing and treating age-related diseases.

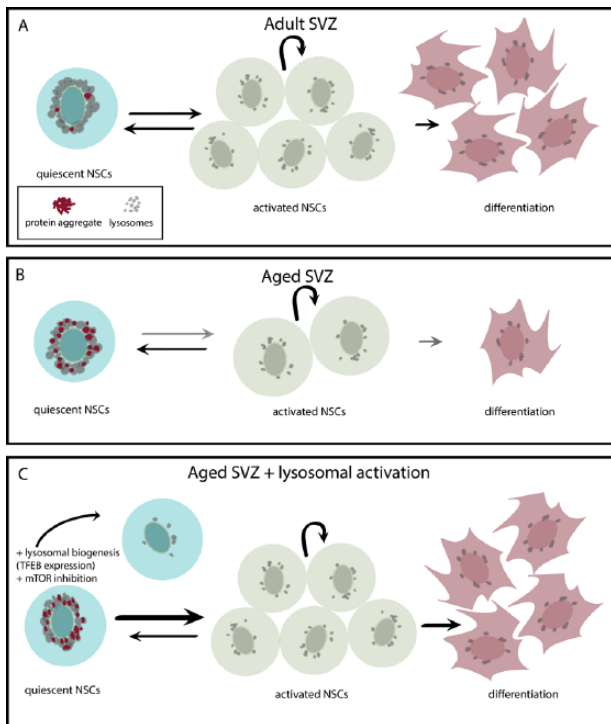
There are three main mechanisms or branches of the protein homeostasis and clearance network: the lysosome-autophagy proteolytic system, molecular chaperones, and the proteasome. Macroautophagy, generally referred to as autophagy, is a tightly regulated process by which cellular organelles, proteins, and cytoplasm are engulfed into autophagosomes for degradation and recycling.<sup>4</sup> The lysosomal-autophagy pathway is also important for the degradation of potentially toxic protein aggregates. Cellular quality control through this system may be particularly important in tissue-specific stem cells, which are used for lifelong tissue regeneration and repair. However, the extent to which these processes are dysregulated in particular stem cell niches within aged animals remains

unclear. A study recently published in *Science* provides the first evidence that regulation of protein homeostasis is critical to support a healthy neurogenic lineage in the adult and aging mammalian brain.<sup>5</sup>

## Protein Homeostasis in the Mammalian Brain

Dysregulated protein homeostasis is associated with many neurodegenerative diseases, including Alzheimer's, Huntington's, and Parkinson's disease. Though protein homeostasis has been studied extensively in post-mitotic neurons, little is known about its role in the neurogenic regions of the adult brain. Neural stem cells (NSCs) reside in two neurogenic niches in the mammalian brain: the subventricular zone (SVZ) lining the walls of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus. The majority of stem cells present within these two niches are in a quiescent, rarely dividing state. In response to external or internal cues, quiescent NSCs (qNSCs) enter a transient activated state and can undergo several rounds of cell division. Activated NSCs (aNSCs) have the ability to self-renew and give rise to mature neurons, astrocytes, and oligodendrocytes.<sup>6</sup> Accumulating evidence indicates that the ability of quiescent NSCs to become activated and undergo neurogenesis declines with age, although the underlying molecular mechanisms have yet to be fully elucidated.<sup>7–9</sup>





**Figure 1.** Clearance of protein aggregates in aged quiescent NSCs through lysosomal activation increases NSC activation. (A) In the adult subventricular zone (SVZ), quiescent NSCs accumulate large lysosomes containing protein aggregates. (B) In the aged SVZ, quiescent NSCs have increased lysosomal content and aggregate buildup when compared to the adult. Overall, there is a reduction of NSC activation and neurogenesis. (C) Induction of aggregate clearance through activation of lysosomal biogenesis (TFEB overexpression) or mTOR inhibition in the aged quiescent NSCs increases the frequency of activated NSCs in the SVZ.

Evidence suggests that the maintenance of protein homeostasis through autophagy is integral to the function of NSCs beginning early in development. NSC-specific ablation of the autophagy-related genes *Atg5* or *Atg7* during embryogenesis is associated with progressive neuronal loss and motor dysfunction starting shortly after birth.<sup>10,11</sup> Moreover, *Atg5* knockdown reduces the differentiation and neurogenic potential of the NSC pool.<sup>12</sup> These data suggest that cellular quality control through autophagy supports both the lineage progression and differentiation of NSCs beginning early on in development.

More recent studies indicate that autophagy also regulates neurogenesis in the adult mammalian brain. NSC-specific genetic ablation of *FIP200*, a gene necessary for the induction of autophagy, significantly reduces the size of the NSC pool within both neurogenic niches. Moreover, there was an overall reduction in the number of new neurons that formed in the olfactory bulb and dentate gyrus in the *FIP200* knockout.<sup>13</sup> A similar phenotype was also observed in mice heterozygous for *Beclin1*, a gene required for early autophagosome formation.<sup>14</sup> Together, these data suggest that the flux through the autophagy-lysosomal system is necessary for the maintenance and lineage progression of the adult NSC pool. These findings also raised a

number of interesting questions regarding the precise role of autophagy in the NSC lineage in the adult and aging brain. For example, is autophagy critical for all stages of neurogenesis, or are specific transitions during lineage progression particularly dependent on this process? As the current studies in the field only examine the early stages of autophagosome formation, could later stages involving the lysosome similarly regulate neurogenesis? Moreover, does the age-related decline neurogenesis arise from progressive autophagic or lysosomal dysfunction?

### Discovery of a New Mechanism of Aggregate Control in NSCs

In order to systematically evaluate whether protein homeostasis is differentially regulated throughout the NSC lineage with age, Leeman et al. first performed transcriptional profiling of the neurogenic lineage using freshly isolated cells from the mouse SVZ. Comparison of the activated and quiescent NSCs revealed striking differences in the expression of genes involved in protein homeostasis between the two cell types. Further analysis revealed that genes specifically associated with lysosomal function were selectively upregulated in the quiescent population. This is in contrast to aNSCs, which had higher expression of various molecular chaperones and displayed a signature associated with the proteasome and ubiquitin-mediated proteolysis. Functional assessment of the quiescent and activated NSCs confirmed the transcriptional profiling results, revealing greater quantities of lysosomes in the quiescent pool of NSCs (Figure 1), and higher proteasome activity in the activated cells. Interestingly, lysosomal size was also significantly greater in the qNSCs. These data could suggest either increased autophagic flux or more gradual lysosome degradation in the quiescent state. Further characterization of the qNSC lysosomes showed normal acidification, indicative of proper function. The use of a reporter system with manipulation of autophagic flux revealed that qNSCs degrade their lysosomal contents at a much slower rate than aNSCs. Together, these data show that the two cellular states utilize independent branches of the protein homeostasis network, indicating that the activated and quiescent populations rely on different mechanisms to maintain proteome integrity and function.

What are the consequences of employing distinct quality control mechanisms in quiescent and activated NSCs? Interestingly, qNSCs showed significantly more insoluble aggregates than their activated counterparts, even in young adult animals (Figure 1). Further assessment of the nature of the aggregates present in the qNSCs revealed multiple protein species, including a large fraction of amyloid-beta-type aggregates. This finding is surprising given that decreased protein synthesis is a hallmark of quiescence.<sup>15,16</sup> Moreover, these experiments suggest that rapid clearance of protein aggregates, including those potentially associated with Alzheimer's disease, occurs during the first stage of neurogenesis, possibly allowing for the generation of new neurons devoid of aggregated or damaged proteins.

### Is Lysosomal Activation Sufficient for NSC Activation?

The correlation between lysosome activation and NSC activation raises the question of whether activation of lysosomes is sufficient to drive NSCs out of the quiescent state. NSC activation involves cell cycle re-entry in response to intrinsic or extrinsic cues from the neurogenic microenvironment, although the molecular mechanisms are not fully understood. Could lysosome activation be a novel intrinsic stimulus to break quiescence? The work by Leeman et al. provides compelling evidence that this may be the case. The authors observed that blocking lysosomal acidification induced aggregate accumulation in qNSCs and significantly reduced their ability to become activated. In contrast, induction of autophagic flux reduced the quantity of aggregates and enhanced the response of qNSCs to activation cues. This evidence suggests that clearance of protein aggregates is sufficient to induce activation of qNSCs in response to growth factor stimulation, although it cannot be ruled out that other unidentified cargo are critical for activation. Nevertheless, pathological lysosome dysfunction and aggregate buildup may have a causative role in the age-associated decrease in NSC activation and neurogenesis.

### Aggregate Formation Increases in Aged Quiescent NSCs

A significant reduction in the ability of quiescent NSCs to activate in the aged brain is in part responsible for decreased neurogenesis with age, but the underlying mechanisms are not well understood. The study by Leeman et al. reveals a novel mechanism for this decline. Transcriptome analysis of the neurogenic lineage during aging revealed that qNSCs exhibit more significant transcriptome changes with age compared to aNSCs and neural progenitor cells. Genes associated with lysosomal function were particularly dysregulated in aged qNSCs. Quantification of LAMP-1 positive lysosomes showed that indeed aged qNSCs have a significant reduction in their lysosome levels. Moreover, a subpopulation of qNSCs exhibited lysosomal dysfunction and accumulation of protein aggregates, which could be reversed by nutrient deprivation or overexpression of a master regulator of lysosome biogenesis (Transcription Factor EB; TFEB; Figure 1). Importantly, induction of the lysosome-autophagy pathway using TFEB overexpression or mTOR inhibition (rapamycin treatment) in aged qNSCs also rescued the age-associated decline in NSC activation. These findings are important because they indicate that although the lysosome-autophagy system becomes defective in NSCs with age, this deficiency is reversible given the right stimulus.

### Insights into the Mechanism of NSC Activation and Its Age-Associated Decline

Altogether, the report from Leeman et al. for the first time implicates lysosomal dysfunction and aggregate accumulation in the well-known age-associated decline in neurogenesis.

Excitingly, this study also reveals a novel mechanism, the restoration of lysosomal activity, that is sufficient to clear aggregates and enhance NSC reactivation. Thus, this work suggests new therapeutic targets to ameliorate the age-associated decline in neurogenesis. This work also raises several interesting questions that will be important to address in future studies. For example, it is unknown whether the aggregates reappear later in the lineage as the activated NSCs exit the cell cycle and commit to terminal differentiation. Thus, to expand on these findings, it will be important to follow the activated NSCs until they complete terminal differentiation and integrate into the functional circuitry of the brain. In addition, although the data show that proteins present in the aggregates are from multiple species, it is unclear if the aggregates consist of certain families of proteins or are similar to pathological aggregates. Moreover, how aggregate clearance is mechanistically linked to NSC activation is unclear. For example, this catabolic activity may provide the metabolic fuel and nucleotides necessary for qNSC activation and proliferation. Alternatively, the presence of aggregates could function as a negative feedback mechanism to ensure reduced protein synthesis and maintenance of the quiescent state.

It will also be important to identify the direct upstream regulators of proteostasis in the quiescent and activated NSCs. The authors show that overexpression of the known master regulator of lysosomal biogenesis, TFEB, was sufficient to induce activation of aged quiescent NSCs. This finding raises the question of whether changes in TFEB activity may underlie the age-associated decrease in NSC activation. The observation that rapamycin treatment can rescue qNSC activation in aged animals supports this possibility, as mTOR is known to inhibit TFEB.<sup>17,18</sup> This resembles other neurodegenerative conditions where decreased TFEB activity has been linked to disease pathology. For example, in human post-mortem Alzheimer's disease and amyotrophic lateral sclerosis (ALS) brain specimens, a reduction in nuclear TFEB has been observed in the hippocampus.<sup>19</sup> Similarly, TFEB activity is reduced in the midbrain of Parkinson's disease patients, likely through sequestration in the cytoplasm by  $\alpha$ -synuclein.<sup>20</sup> Whether the aggregates in the aged qNSCs can similarly sequester TFEB outside the nucleus has not been explored. In future work, it will be interesting to investigate if inactivation of TFEB may contribute to the decreased ability of qNSCs to activate with age.

The extent to which other key regulators of NSC quiescence and activation regulate proteostasis in these cells also remains unknown. For example, the pro-longevity FOXO transcription factors support stem cell function, and the quiescent state in particular.<sup>21–24</sup> FOXOs are also known to regulate autophagy and the proteasome in embryonic and tissue-specific stem cells.<sup>25,26</sup> Moreover, several studies suggest that FOXOs may contribute to longevity through direct regulation of autophagy and proteostasis in neural progenitors and other

mammalian cells.<sup>27–29</sup> FOXOs can also act in a reciprocal manner with TOR signaling.<sup>30</sup> It will be important to determine whether dysregulation of FOXOs may contribute to the observed buildup of protein aggregates and lysosomes in the NSC lineage and whether modulation of these factors may induce clearance programs to alleviate the age-associated decrease in NSC activation.

In sum, this work provides new insight into the mechanisms supporting quiescence and activation of mammalian NSCs. For the first time, this study shows that lysosomal dysfunction and loss of protein homeostasis in the quiescent NSC pool may contribute to the decline in neurogenesis with age. Excitingly, these findings suggest a novel intervention to enhance NSC activation and neurogenesis in the context of aging and neurodegenerative disease.

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### Author Contributions

AJA and AEW prepared the manuscript.

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### REFERENCES

- Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153:1194–1217.
- David DC. Aging and the aggregating proteome. *Front Genet*. 2012;3:247.
- Menzies FM, Fleming A, Caricasole A, et al. Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. *Neuron*. 2017;93:1015–1034.
- Reggiori F, Klionsky DJ. Autophagosomes: biogenesis from scratch? *Curr Opin Cell Biol*. 2005;17:415–422.
- Leeman DS, Hebestreit K, Ruetz T, et al. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science (New York, NY)*. 2018;359:1277–1283.
- Lim DA, Alvarez-Buylla A. The adult ventricular-subventricular zone (V-SVZ) and olfactory bulb (OB) neurogenesis. *Cold Spring Harb Perspect Biol*. 2016;8:a018820.
- Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J Neurosci*. 2004;24:8354–8365.
- Capilla-Gonzalez V, Cebrian-Silla A, Guerrero-Cazares H, Garcia-Verdugo JM, Quinones-Hinojosa A. Age-related changes in astrocytic and ependymal cells of the subventricular zone. *Glia*. 2014;62:790–803.
- Giachino C, Basak O, Lugert S, et al. Molecular diversity subdivides the adult forebrain neural stem cell population. *Stem Cells (Dayton, Ohio)*. 2014;32:70–84.
- Hara T, Nakamura K, Matsui M, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006;441:885–889.
- Komatsu M, Waguri S, Chiba T, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006;441:880–884.
- Lv X, Jiang H, Li B, et al. The crucial role of Atg5 in cortical neurogenesis during early brain development. *Sci Rep*. 2014;4:6010.
- Wang C, Liang CC, Bian ZC, Zhu Y, Guan JL. FIP200 is required for maintenance and differentiation of postnatal neural stem cells. *Nat Neurosci*. 2013;16:532–542.
- Yazdankhah M, Farioli-Vecchioli S, Tonchev AB, Stoykova A, Cecconi F. The autophagy regulators Ambra1 and Beclin 1 are required for adult neurogenesis in the brain subventricular zone. *Cell Death Dis*. 2014;5:e1403.
- Valcourt JR, Lemons JM, Haley EM, Kojima M, Demuren OO, Collier HA. Staying alive: metabolic adaptations to quiescence. *Cell Cycle (Georgetown, Tex.)*. 2012;11:1680–1696.
- Llorens-Bobadilla E, Zhao S, Baser A, Saiz-Castro G, Zwadlo K, Martin-Villalba A. Single-cell transcriptomics reveals a population of dormant neural stem cells that become activated upon brain injury. *Cell Stem Cell*. 2015;17:329–340.
- Settembre C, Zoncu R, Medina DL, et al. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J*. 2012;31:1095–1108.
- Roczniak-Ferguson A, Petit CS, Froehlich F, et al. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci Signal*. 2012;5:ra42.
- Wang H, Wang R, Xu S, Lakshmana MK. Transcription factor EB is selectively reduced in the nuclear fractions of Alzheimer's and amyotrophic lateral sclerosis brains. *Neurosci J*. 2016;2016:4732837.
- Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Bjorklund A. TFEB-mediated autophagy rescues midbrain dopamine neurons from alpha-synuclein toxicity. *Proc Natl Acad Sci U S A*. 2013;110:E1817–E1826.
- Paik JH, Ding Z, Narurkar R, et al. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. *Cell Stem Cell*. 2009;5:540–553.
- Renault VM, Rafalski VA, Morgan AA, et al. FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell*. 2009;5:527–539.
- Gopinath SD, Webb AE, Brunet A, Rando TA. FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. *Stem Cell Reports*. 2014;2:414–426.
- Tothova Z, Kollipara R, Huntly BJ, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell*. 2007;128:325–339.
- Warr MR, Binnewies M, Flach J, et al. FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature*. 2013;494:323–327.
- Vilchez D, Boyer L, Morante I, et al. Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature*. 2012;489:304–308.
- Webb AE, Brunet A. FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem Sci*. 2014;39:159–169.
- Webb AE, Pollina EA, Vierbuchen T, et al. FOXO3 shares common targets with ASCL1 genome-wide and inhibits aSCL1-dependent neurogenesis. *Cell Rep*. 2013;4:477–491.
- Webb AE, Kundaje A, Brunet A. Characterization of the direct targets of FOXO transcription factors throughout evolution. *Aging Cell*. 2016;15:673–685.
- Hay N. Interplay between FOXO, TOR, and Akt. *Biochim Biophys Acta*. 2011;1813:1965–1970.