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STATE-OF-THE-ART REVIEW

Lysosomes and signaling pathways for maintenance of quiescence in adult neural stem cells

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Quiescence is a cellular strategy for maintaining somatic stem cells in a specific niche in a low metabolic state without senescence for a long period of time. During development, neural stem cells (NSCs) actively proliferate and self-renew, and their progeny differentiate into both neurons and glial cells to form mature brain tissues. On the other hand, most NSCs in the adult brain are quiescent and arrested in G0/G1 phase of the cell cycle. Quiescence is essential in order to avoid the precocious exhaustion of NSCs, ensuring a sustainable source of available stem cells in the brain throughout the lifespan. After receiving activation signals, quiescent NSCs reenter the cell cycle and generate new neurons. This switching between quiescence and proliferation is tightly regulated by diverse signaling pathways. Recent studies suggest significant involvement of cellular proteostasis (homeostasis of the proteome) in the quiescent state of NSCs. Proteostasis is the result of integrated regulation of protein synthesis, folding, and degradation. In this review, we discuss regulation of quiescence by multiple signaling pathways, especially bone morphogenetic protein and Notch signaling, and focus on the functional involvement of the lysosome, an organelle governing cellular degradation, in quiescence of adult NSCs.

Introduction

Neural stem cells (NSCs) actively proliferate and give rise to all of the neurons and glial cells necessary to constitute the embryonic brain. Although NSCs decrease in number after production of mature brain tissues is complete, they persist and maintain multipotency in small areas of the adult brain. In the rodent brain, adult NSCs reside in the subventricular zone (SVZ) of the lateral ventricle and the hippocampal

Abbreviations
Ambra, activating molecule in Beclin-1-regulated autophagy; Ascl, achaete-scute homolog; Atg, autophagy-related gene; BMP, bone morphogenetic protein; BMPR, BMP receptor; CSF, cerebrospinal fluid; DG, dentate gyrus; Dll1, delta-like 1; Dpp, decapentaplegic; EGF, epidermal growth factor; FGF, fibroblast growth factor; FIP 200, FAK family kinase-interacting protein 200; FOXO, forkhead box O; GABA, gamma amino butyric acid; GFAP, glial fibrillary acidic protein; GLAST, glutamate aspartate transporter; Hes, hairy and enhancer of spirit; HSC, hematopoietic stem cell; Id, inhibitor of DNA binding; IFN, interferon; MiT-TFE, microphthalmia-transcription factor E; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; Ngn, neurogenin; NICD, Notch intracellular domain; NSC, neural stem cell; OP-1, osteogenic protein 1; PTEN, phosphatase and tensin homolog; RBPJ, recombination signal-binding protein for immunoglobulin kappa J region; ROS, reactive oxygen species; SVZ, subventricular zone; TFEB, transcriptional factor EB; V-ATPase, vacuolar ATPase.

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dentate gyrus (DG) (Fig. 1A,B) [1–3]. Adult NSCs are mainly quiescent; however, some adult NSCs, called active NSCs, proliferate and differentiate into mature neurons, which then integrate into the pre-existing brain network [4–7]. Quiescent NSCs become active NSCs after receiving activation signals, and the transition from quiescent to active is reversible rather than unidirectional [8,9]. Quiescence of adult NSCs is maintained by extrinsic and intrinsic factors, and diverse signaling from local NSC niches is involved in this process (reviewed in Ref. [6]) (Fig. 1C,D). For example, bone morphogenetic protein (BMP) and Notch signaling, which are activated by ligands secreted or presented by neighbor cells, regulate adult NSC quiescence in both the SVZ and the DG. Here, we discuss how NSCs are regulated by these signaling pathways to maintain quiescence. The protein functions and stabilities of these signaling molecules influence the downstream outputs and their diverse responses. Recently, proteostasis (protein homeostasis [10]) was reported as a significant regulator of the maintenance of adult NSCs. Proteostasis is a consequence of integrated regulation of protein synthesis, proper folding, and protein degradation. We also discuss proteostasis in the context of quiescence, focusing especially on the function of lysosomes, an organelle involved in degradation of cellular components.

Signals that control quiescent NSCs

Bone morphogenetic protein signaling in adult NSCs has a long history of research. Previous studies revealed that BMP is a dominant inducer of quiescence in NSCs *in vitro* and *in vivo* [5,8,11]. This canonical BMP signaling upregulates the SMAD target factors Id1-Id4 (Fig. 2A) as well as Hes1 and decreases cell proliferation [11,12]. The SVZ exhibits enriched expression of BMP components including the BMP receptors BMPR Ia and BMPR II, BMP ligands, and the BMP inhibitor Noggin [13,14]. Noggin is secreted from the ependymal cells, ciliated glial cells in the surface of lateral ventricle, in the adult brain [13]. In the DG, NSCs express the BMPR BMPR1a and BMPR2. These BMPR are activated by BMP2/4, members of the decapentaplegic (Dpp) subfamily of BMP ligands, which are secreted from dentate granule cells in the hippocampus [12]. Genetic deletion of *Bmpr1a* and *Smad4*, an effector of the canonical BMP pathway, using lentivirus-mediated KO in $Sox2^{+}$ cells in the DG immediately, induces NSC proliferation, followed by marked reduction in active NSCs and doublecortin $(DCX)^{+}$ immature neurons in the DG within 3 weeks [12]. Blockade of BMP by Noggin recruits quiescent NSCs into the cell cycle in the DG [12,15]. Thus, BMP signaling plays an essential role in the maintenance of quiescent NSCs. However, differential regulation of BMP ligand and receptor subtypes may cause adult NSCs to be differentially responsive to the BMP signal. For example, the cerebrospinal fluid (CSF) delivers many extrinsic factors to NSCs in the SVZ [13,16]. One of these factors, BMP5, a member of the osteogenic protein 1 (OP-1) subfamily of BMPs that is enriched in the CSF of young mice, was identified as a factor that enhances *in vitro* activation of quiescent NSCs from the SVZ in association with other growth factors [epidermal growth factor (EGF) and basic fibroblast growth factor] [17]. Thus, the Dpp subfamily and the OP-1 subfamily of BMP ligands have opposing functions in regulating NSCs. Furthermore, post-translational modification of BMPR1a receptor by palmitoylation modulates NSC function through receptor localization and signal transductions; different palmitoylated positions in BMPR1a differentially affect canonical and noncanonical BMP signaling, which lead to SMAD activation and extracellular signal-regulated kinase activation, respectively [18]. In addition, age-dependent different expression of BMP ligands, such as BMP2/4 and BMP6, in NSC niches, might affect the maintenance of quiescence in adult NSCs [19].

The Notch signaling pathway includes four Notch receptors (Notch1–Notch4), which are cleaved upon binding of Notch ligands (members of the delta or jagged families) expressed in neighboring differentiating cells (Fig. 2B). The intracellular domain of Notch (NICD) is transferred into the nucleus, where it binds to the transcriptional factor CBF-1, suppressor of hairless, Lag-2 [recombination signal-binding protein for immunoglobulin kappa J region (RBPJ) in mice] to activate downstream genes such as *Hes* (reviewed in Ref. [20]). Notch1–Notch3 are expressed in the neurogenic niche of the adult mouse brain [21]. Significant involvement of Notch signaling in quiescence was demonstrated using Hes5-GFP reporter mice, which express GFP under the control of Notch signaling. Both the SVZ [22] and DG [23] exhibit activated Notch signaling in heterogeneous cell populations, including active and quiescent NSCs. In canonical Notch signaling, RBPJ is the common downstream effector of all Notch receptors. Conditional knockout of RBPJ in the DG of GLAST-Cre-ER^{T2} Tg mice [24] and in the SVZ of Nestin-Cre-ER^{T2} Tg mice [25] or Hes5-Cre-ER^{T2} mice [26] results in enhanced generation of new neurons within a few weeks, mediated by NSC activation, followed by severe depletion of NSCs and the loss of neurogenesis within a few months.

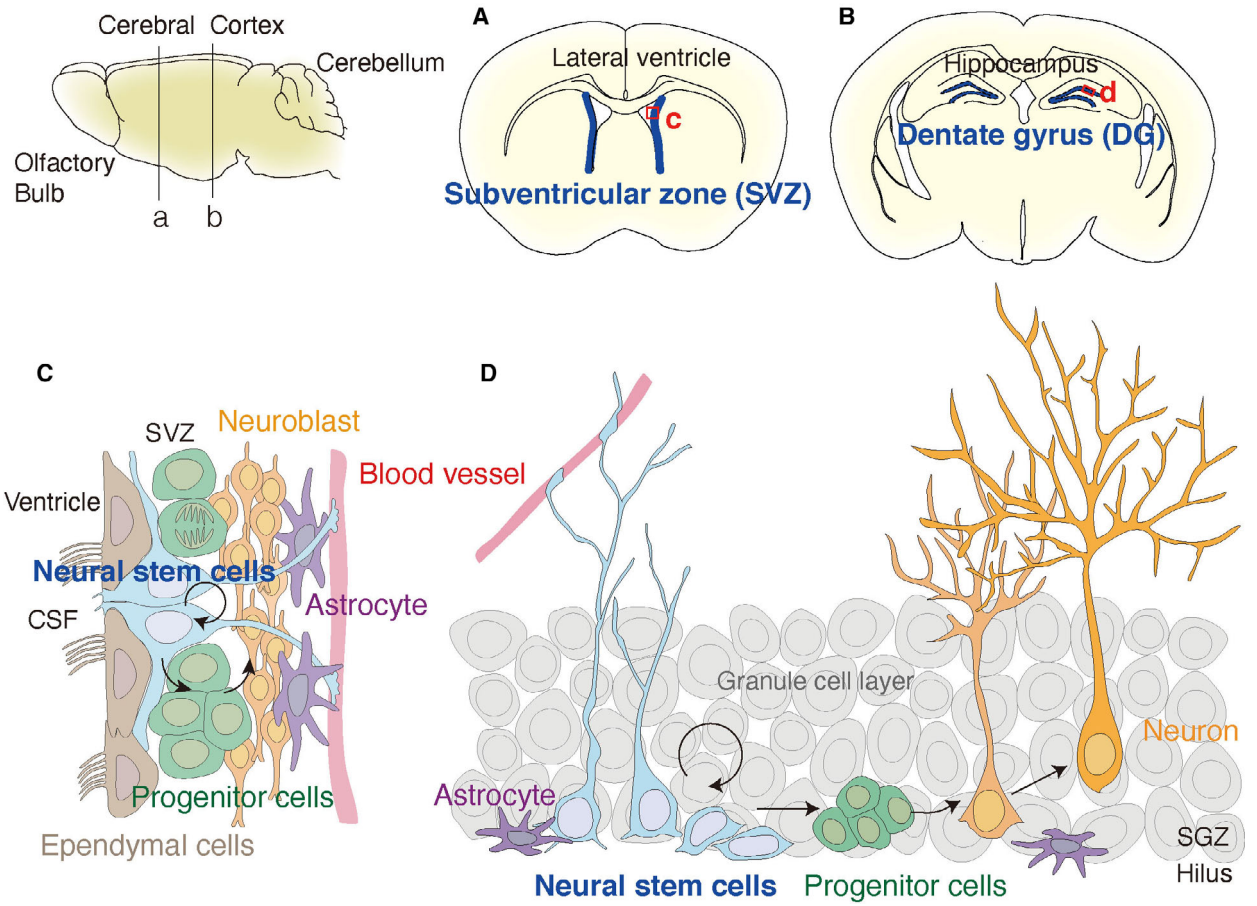

Fig. 1. Two NSC niches, the SVZ and DG, in the adult mouse brain. Schematic representation of a lateral view of the whole adult mouse brain from the olfactory bulb (left) to cerebellum (right). Coronal planes dissected at lines (a) and (b) are shown in panels (A) and (B), respectively, on the right. The two NSC niches are labeled in blue lines, highlighting the SVZ near the lateral ventricle (A) and the DG in the hippocampus (B). Ventricles in the brain sections appear in white in panels (A) and (B). The detailed compositions of niches are represented in panels (C) and (D) as enlarged views marked by red squares (c in A) and (d in B), respectively. Panel (C) displays the SVZ niche, which is located near the lateral sides of the lateral ventricles. NSCs (blue) face the CSF in the lateral ventricle together with ependymal cells and elongate their projection to blood vessels (red). Panel (D) displays the DG niche, surrounded by the granule cell layers (gray) and hilus. NSCs are located in the subgranular zone next to the granule cell layer and elongate radial fibers, resulting in a radial glial morphology. NSCs in both niches self-renew, differentiate into progenitor cells (green), and give rise to mature neurons (orange).

These reports suggest that Notch signaling regulates the maintenance of both active and quiescent NSCs in both the SVZ and DG. Importantly, previous studies showed that Notch1 and Notch2 play distinct roles in adult NSCs. Conditional KO of Notch1 decreased proliferation of neurogenic NSCs in the DG (using different Cre drivers: Nestin-Cre-ER^{T2} [27] and human GFAP-Cre-ER^{T2} [28]) and the SVZ (using Nestin-Cre-ER^{T2} [29] and Hes5-Cre-ER^{T2} Tg mice [26]). On the other hand, conditional KO of Notch2 induced abnormal activation of quiescent NSCs, resulting in exhaustion of the NSC pool in both the SVZ and DG of Hes5-Cre-ER^{T2} mice [26,30]. These differential outputs were confirmed by the expression of active forms of the Notch receptors. Noch1ICD and Notch2ICD using a Cre-inducible expression system in hGFAP-Cre-ER^{T2} Tg mice [28] and Hes5-Cre-ER ^{T2} Tg mice [30] enhanced and decreased proliferation of NSCs, respectively. These results indicated that although Notch1 and Notch2 have distinct roles in adult NSCs, both Notch1 cKO and Notch2 cKO caused aging phenotypes by decreasing neurogenesis as well as reducing the NSC pool [26,29,30]. Another Notch receptor, Notch3, which is enriched in quiescent NSCs, affects NSC maintenance [31]. Depletion of Notch3 expression induced proliferation of adult NSCs in the SVZ

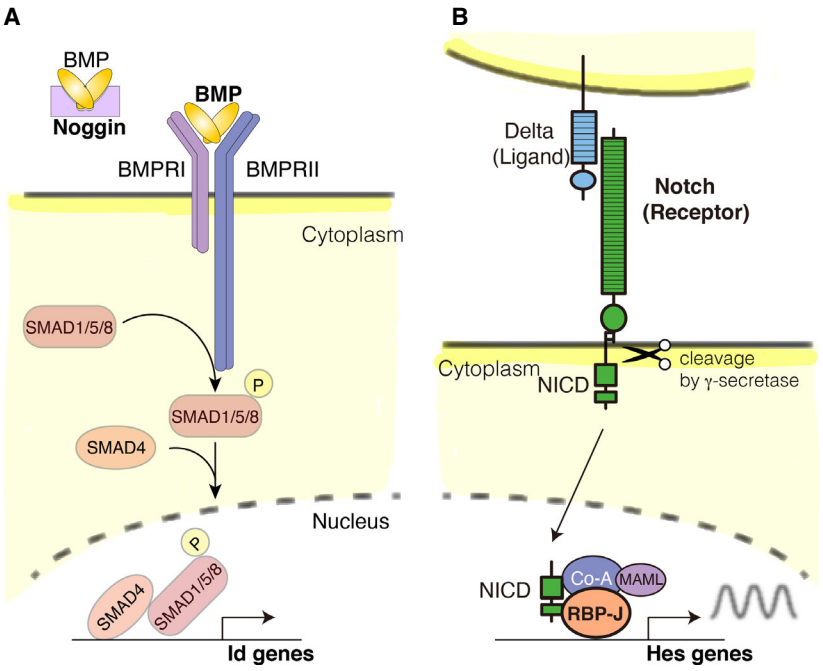

Fig. 2. BMP and Notch signaling cascades. (A) Canonical pathway of BMP signaling. BMPR bind to BMP ligands, and transduce signals via SMAD molecules, which ultimately enhance *Id* gene expression. Noggin sequesters BMP and antagonizes BMP signaling. (B) Canonical pathway of Notch signaling. Notch receptors bind to ligands on neighboring cells, inducing gamma-secretase-mediated cleavage in the signal-receiving cells (lower). Cleaved Notch receptor (NICD) translocates into the nucleus and activates the expression of *Hes* genes.

[31], whereas ectopic Notch3 expression decreased proliferation of NSCs derived from the DG [32]. The downstream factors of these Notch receptors might shed light on the diverse functions of Notch signaling in adult NSCs. One example is Id4, originally identified as a target molecule of BMP signaling. Proteins in the inhibitor of DNA binding (Id) family, members of the HLH transcription factor family that lack a DNA-binding motif, form heterodimers with other bHLH factors that sequester the partner protein in non-DNA-binding dimers (reviewed in Ref. [33]). Quiescent NSCs derived from the DG express high levels of Id4, and Id4 is a major effector of the Notch2 receptor and a quiescence-inducing factor in NSCs [30,34]. Id4 decreases the protein stability of achaete-scute homolog (Ascl)1, a proneural bHLH factor expressed in active NSCs, by sequestering E protein, the binding partner for transcriptional activator function [34]. Another example of the differential output of Notch signaling is Hes1, a canonical Notch effector and bHLH transcriptional repressor that is expressed at a higher level in quiescent NSCs [35]. Oscillatory expression of Hes1 regulates the expression dynamics of the target genes, *Dll1, Ngn2*, and *Ascl1,* which contribute to the switching from proliferation to differentiation of embryonic NSCs [36,37]. In adult NSCs, higher expression of Hes1 continuously inhibits *Ascl1* expression and maintains NSCs in the quiescent state [35]. These reports suggest that the expression dynamics and protein stability of bHLH factors might contribute to differential outputs of Notch signaling.

The quiescent state of adult NSCs is also regulated by other signaling receptors, for example, activation of gamma-aminobutyric acid (GABA) receptors by GABA secreted from NSC niche cells in the SVZ and DG [38,39] and activation of integrin receptors by binding of Mfge8, a phagocytosis factor, which is secreted from quiescent NSCs of the DG [40]. Adult NSCs express $GABA_A$ receptors and tonically respond to GABA from niche cells, which are parvalbumin-expressing (PV^+) interneurons in the DG or neuroblasts in the SVZ that can dictate the NSC choice between quiescence and activation through nonsynaptic GABA signaling [39,41]. On the other hand, quiescent NSCs secrete Mfeg8 and promote quiescence via suppression of the PTEN–Akt–mTOR1 pathway through binding to the integrin receptor [40]. Tropomyosin receptor kinase C (TrkC) receptor activation by binding of neurotrophin-3 from the CSF or the nearby vasculature promotes NSC quiescence in the SVZ [42]. Together, these observations indicate the importance of combinations of multiple membrane receptors for maintaining NSC quiescence. The activation of these signaling pathways is ligand-induced upon binding to membrane receptors, most of which induce the endocytic trafficking of receptors to lysosomes or recycling back to the cell surface, suggesting the important roles of lysosomes in NSC

states. In the next part, we will discuss the involvement of lysosomes in quiescent NSCs.

The lysosome: a digestive organelle and a hub of nutrition signaling

Subsequent to ligand-induced activation, membrane receptors are internalized in endosomes and ultimately degraded in lysosomes after endolysosomal trafficking or recycled into membrane surface (Fig. 3A). Lysosomes are membrane-enclosed cytoplasmic organelles that degrade a variety of biological macromolecules, including proteins, lipids, carbohydrates, and nucleic acids (reviewed in Ref. [43,44]). The lysosomal lumen is highly acidic; the low pH is maintained by a vacuolar ATPase (V-ATPase) on the lysosomal membrane. Cells contain between 50 and 1000 lysosomes, in which more than 60 acidic hydrolases digest macromolecules delivered from the endolysosomal and autophagic pathways. Lysosomes were once thought of as static organelles involved in the waste disposal system. However, recent reports have shown that lysosomes act as regulatory hubs for cellular homeostasis by switching the metabolic state between catabolism and anabolism. In nutrient-rich environments, multiple sensors of amino acid levels cause Rag GTPases to interact with the Ragulator–protein complex [45]. Then, the Rag–Ragulator complex recruits mTORC1 and other factors including transcriptional factor EB (TFEB), a master transcriptional regulator of lysosomal components, to the lysosomal membrane [46,47]. TFEB is phosphorylated by mTORC1, resulting in inhibition of nuclear translocation of TFEB (Fig. 3B) [47]. TFEB, a member of the microphthalmia-transcription factor E (MiT-TFE) family of HLH leucine zipper transcription factors [48], activates lysosome-related genes under the coordinated lysosomal expression and regulation (CLEAR) gene network [49]. Nutrient starvation promotes dephosphorylation of TFEB, leading to its nuclear translocation. In the nucleus, TFEB upregulates genes involved in lysosomal function and autophagy, resulting in the recycling and clearance of biomolecules inside cells. Thus, lysosomes control their functions to adapt to environmental cues. TFE3, another member of the MiT-TFE family, is also involved in this process. Moreover, lysosomes regulate extracellular conditions by lysosomal exocytosis after fusion to the plasma membrane [50] and by the degradation of extracellular matrix protein for extracellular remodeling [51]. Lysosomes are also involved in a broad range of cellular functions such as lipid homeostasis and transfer to other organelles (reviewed in Ref. [52]), calcium signaling by lysosomal calcium channels (reviewed in Ref. [53]), and responses to stress such as proteostatic dysfunction (reviewed in Ref. [54]). Next, we will discuss the role of lysosomal functions in proteostatic regulation in quiescent stem cells.

Lysosomes in quiescent NSCs

Lysosomes are involved in the regulation of proteostasis (protein homeostasis). Proteostasis is maintained by the protein quality control machinery, which consists of protein synthesis on ribosome, proper protein folding assisted by molecular chaperones, and proteolysis [55]. Proteolysis is mainly mediated by the ubiquitin–proteasome and autophagy–lysosome pathways. Differential regulation of these two proteolytic pathways was recently reported in adult NSCs [56,57] (Fig. 4). Transcriptome analyses of fluorescence-activated cell sorting-sorted active and quiescent NSCs derived from the SVZ revealed higher expression of lysosomal genes, including TFEB, and lower expression of both proteasomal and ribosomal genes in quiescent vs. active NSCs [56]. In regard to molecular chaperones, quiescent NSCs express higher levels of ER stress–related genes and lower levels of chaperonin TCP-1 ring complex/chaperonin-containing TCP-1 subunits than active NSCs [56]. Quiescent NSCs exhibit much higher lysosomal proteolytic activity and lower proteasomal activity than active NSCs *in vitro* [57]. These results suggest that quiescent NSCs alter proteostasis, shifting the predominant sites of proteolysis from proteasomes to lysosomes in order to adapt to their environment. In *in vitro* cultures, BMP is a strong inducer of quiescence in proliferating NSCs [8]. BMP-induced quiescent NSCs derived from the SVZ [56] and the DG [58] contain detergent-insoluble aggregates, which might concentrate both proteasomes and their substrates for the rapid reactivation into active NSCs [58]. In this regard, these aggregates might be similar to proteasome storage granules observed in quiescent yeast cells [59]. BMP treatment of NSCs induces dephosphorylation and activation of TFEB, followed by nuclear localization and increased gene expression, thereby increasing lysosomal activity [57]. TFEB-KO NSCs exhibit delayed entry into the quiescent state after BMP treatment with higher levels of activated membrane receptors [57]. Consistent with the *in vitro* results, conditional knockout of TFEB in adult NSCs (in GLAST-Cre-ER^{T2} mice) increases the number of active NSCs in the DG, concomitant with the accumulation of activated membrane receptors [57]. Ectopic expression of a constitutively active mutant of TFEB (caTFEB) or TFEB activation by mTORC1 inhibitors,

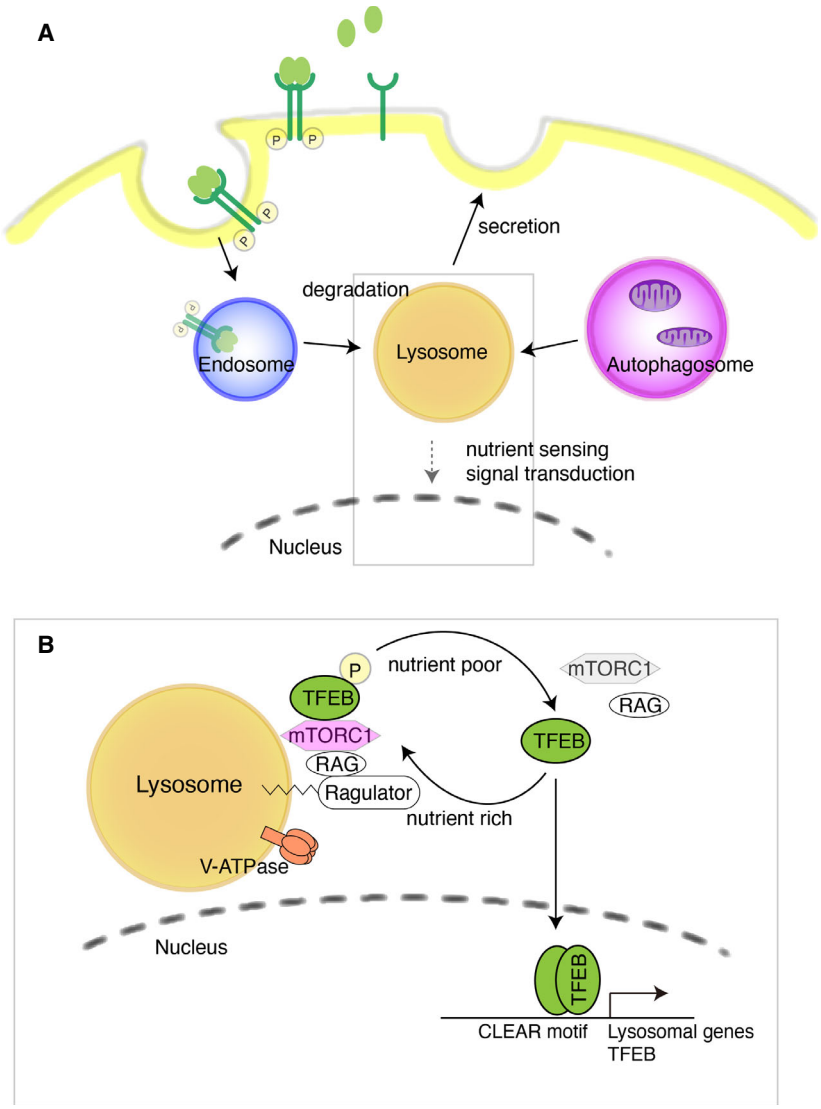

Fig. 3. The lysosome functions as a degradative organelle and a signaling hub. (A) Lysosomes digest cargo from endosomes and autophagosomes in the acidic lumen. The endolysosomal pathway degrades biomolecules, including membrane receptors, in lysosomes following their internalization by endocytosis. Autophagy encloses cytoplasmic materials, including organelles, into autophagosomes, which fuse with lysosomes where their contents are digested. Lysosomes secrete their contents via lysosomal exocytosis. Lysosomes function as a signaling hub for nutrient sensing. The gray square is enlarged in panel B. (B) The lysosome is a hub where signaling molecules localize and transduce their signals. v-ATPase maintains a low pH by pumping protons. Nutrient-rich conditions activate Ragulator-RAG, which recruits and activates mammalian target of rapamycin complex 1 (mTORC1) to lysosomes. Activated mTORC1 (pink) inhibits lysosomal biogenesis through inhibitory phosphorylation of TFEB. Low nutrient concentration results in inactivation of mTORC1 and activation of TFEB, thereby inducing expression of lysosomal genes.

rapamycin and Torin1, decreases NSC proliferation *in vitro* [57]. *In vivo*, ectopic caTFEB expression in NSCs of the DG by injection of lentivirus under the control of the *Hes5* or *GFAP* promoter decreases the number of active NSCs in young adult mice [57]. These results suggest that lysosomal activation by TFEB leads to quiescence in young NSCs and maintains their quiescence in the DG. On the other hand, aged quiescent NSCs from the SVZ express less of the lysosomal protein Lamp-1 than young quiescent NSCs, at levels similar to those in active NSCs [56]. Ectopic expression of caTFEB *in vitro* decreases the abundance of aggregates in primary NSC cultures from the SVZ in aged mice and promotes reactivation of quiescent NSCs in the presence of the growth factors EGF and FGF [56]. Rapamycin increases abundance of active NSCs in the SVZ of old mice by its dietary supplementation [56]. These results demonstrate that proteostatic regulation by lysosomes differs between young and aged NSCs (Fig. 4), and imply differential regulation of lysosomes in the SVZ and the DG. Differential outputs by TFEB activation in NSCs, including induction of quiescence in young NSCs of the DG [57] and enhancement of reactivation in aged NSCs of the SVZ [56], suggest an additional role of lysosomes in aged NSCs in alleviating severe damage caused by aging. Aged quiescent NSCs become more resistant to activation than young quiescent NSCs, a process in which inflammatory signals from niche cells are involved [60, 61]. Interferon (IFN)-γ decreases NSC proliferation *in vitro* and inhibition of IFN response through deletion of IFN-α and IFN-γ leads to a similar

fraction of active NSCs in young and old NSCs in the SVZ [61], implying an additional role for lysosomes in inflammatory responses in aged NSCs.

The autophagy–lysosomal pathway degrades cellular proteins and organelles. There are three types of autophagy in mammals: macroautophagy, microautophagy, and chaperone-mediated autophagy [62]. Macroautophagy is a major lysosomal catabolic process induced by cellular stresses such as starvation and abnormal protein accumulation. Damaged cellular components and organelles are engulfed into autophagosomes and degraded after fusion to lysosomes. This process is called macroautophagy. Microautophagy and chaperone-mediated autophagy directly incorporate cellular components and proteins into lysosomes without the use of autophagosomes. Several reports have shown that the autophagy–lysosomal pathway is involved in the adult NSC maintenance, progenitor cell differentiation, and neuronal maturation. Removal of the autophagy-related gene (Atg) FIP200, an essential gene for autophagic induction, in NSCs of hGFAP-Cre cKO mice, induced progressive loss of NSCs and defects in neurogenesis in the adult brain, concomitant with increases in mitochondria and reactive oxygen species (ROS) [63]. Deletion of *Atg5*, a gene important for autophagosome formation, in dividing neural progenitor cells, was analyzed using a retrovirus encoding Cre recombinase in the SGZ of adult mouse brain. *Atg5* deletion decreases survival of NSCs and delays neuronal maturation [64].

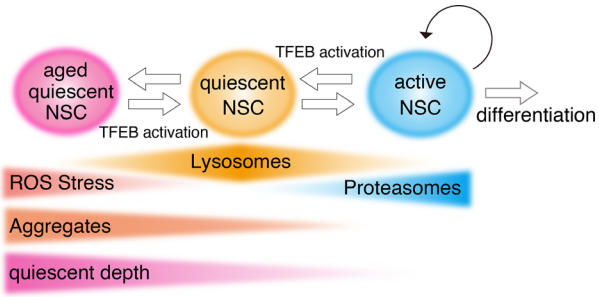

Fig. 4. Lysosomal regulation of NSC quiescence. To maintain proteostasis, active NSCs have higher proteasomal activity and lower lysosomal activity, while quiescent NSCs have lower proteasomal activity and higher lysosomal activity. Quiescent NSCs contain more lysosomes, but lysosomal abundance decreases over the course of the aging process. On the other hand, the level of protein aggregates and ROS increases with age, in turn affecting the depth of quiescence. In active NSCs, TFEB activation induces quiescence, whereas in quiescent NSCs, it rejuvenates the cells and decreases the abundance of aggregates. Thus, lysosomes serve as a switch for maintaining NSC quiescence.

Hypomorphic mutation of *Atg16L1*, another important gene for autophagosome formation, decreases autophagy and proliferation but increases Notch1ICD level in the SVZ of adult mouse brain [65]. Reduced expression of Beclin1 or activating molecule in Beclin-1-regulated autophagy (Ambra)1, both of which are involved in the initial step of autophagosome formation, decreases proliferation and increases apoptosis in the adult SVZ in heterozygote mice relative to wild-type mice [66]. These reports suggest that autophagic flux exists in adult NSCs and maintains the survival and differentiation of these cells, as well as their progeny, in both the SVZ and DG. Recent reports demonstrated that upstream factors regulating autophagic genes are regulators of adult neurogenesis. For example, *let-7*, an miRNA for cell-cycle exit, affects migration of newly generated neurons and their morphology, both of which depend on autophagic activity [67]. Forkhead box O (FOXO) transcriptional factors are critical regulators for autophagic flux and proteostasis in the adult NSCs [68–71]. Knockout of FoxO1, FoxO3, and FoxO4 in hGFAP-Cre [68] and GLAST-CreERT2 [70] mice induced an initial increase in proliferating NSCs and progenitor cells and a subsequent severe decline in the NSC pool associated with abnormal accumulation of autophagosomes. FoxO3 directly binds to numerous Atg, regulates proteostasis, and avoids protein aggregates via autophagic clearance in cultured neural stem and progenitor cells derived from the adult SVZ [71,72].

Proteostatic regulation has also been reported in stem cell differentiation; NSC differentiation is associated with rewiring of chaperone networks [73], while embryonic stem cell differentiation decreases proteasomal activity [74]. Asymmetric cell division causes selective delivery of the cellular degradation machinery and damaged proteins, and contributes to cell fate decisions through proteostatic regulation in neural and hematopoietic stem cells (HSC) [58,75,76]. Proteomic approaches are essential for understanding such proteostatic regulations in detail. For example, recent proteomic studies revealed specific regulation of extracellular matrix in the SVZ niches in comparison with other brain regions; these differences were not identified by transcriptomic analysis. Detergent extraction methods for tissue sections enabled fractionation of proteins depending on their association strength with extracellular matrix. Several core matrix proteins are more detergent-soluble in the neurogenic niches, suggesting the existence of mechanical regulation such as stiffness in the stem cell niche [77].

Lysosomes in other quiescent cells

Quiescence is a fundamental mechanism by which various types of cells, such as HSC and fibroblasts, maintain themselves in a low-metabolism state for long periods of time [78]. Recent reports revealed the importance of lysosomes in the quiescent state. HSCs, the major source of multilineage hematopoietic cells, mostly remain quiescent in order to protect themselves from metabolic stresses, while their entry into the cell cycle is accompanied by an increase in mitochondrial activity (reviewed in Ref. [79]). Interestingly, deeply quiescent HSCs, which contain small punctate mitochondria with low mitochondrial activity, express elevated levels of lysosomal genes; however, this causes not the degradation of mitochondria, but rather their transient sequestration into enlarged lysosomes [80]. Autophagy, including mitophagy, involves many regulatory steps prior to fusion with lysosomes, but the mechanisms by which HSCs regulate lysosomal activity in the enlarged lysosomes remain unknown. Further investigation is required to elucidate the degradation of cargos such as membrane receptors [81]. In the adults, mitochondrial activity (as indicated by mitochondrial membrane potential) does not significantly differ between BMP4-induced quiescent NSCs and active NSCs, whereas neuronally differentiated progenitor cells have higher levels of mitochondrial respiration than NSCs [82]. Lysosomes might affect the differentiation of neural progenitor cells by regulating mitochondrial activity. In addition, lysosomes play a role in cell division of HSCs [76]. As in NSCs, lysosomes are asymmetrically segregated in daughter cells during cell division of HSCs. This controls the fate of daughter cells, as cells receiving fewer lysosomes are prone to differentiate [76]. Lysosomes are co-inherited with other factors, including autophagosomes, mitophagosomes, and Notch factors such as Numb and Notch1, which might act together.

Rat embryonic fibroblasts can be induced to undergo quiescence by serum starvation for 2 days [83]. In a transcriptome analysis aimed at identifying factors governing long-term quiescence, lysosomal genes exponentially increased their expression 2 days after serum starvation and then increased continuously for 2 weeks [83]. Endosomal genes also exponentially increased their expression for 2 days but kept the same expression level at later time points. Lysosomal inhibition by chemicals increased the abundance of mitochondrial ROS and induced deeper quiescence, a state associated with lower responsiveness to serum stimulation. On the other hand, lysosomal activation by ectopic expression of Mitf, a member of the MiT/TFE family, in quiescent cells, reduced ROS levels and allowed more efficient reactivation by serum stimulation. These observations suggest that lysosomes maintain a quiescent state between shallow and deep quiescence that is associated with cellular metabolism and stresses, implying important links with cellular senescence and aging.

Conclusion

Here, we discussed recent findings related to adult NSC quiescence, ranging from signaling pathways to lysosomal regulation of quiescence. Recent reports have suggested that lysosomes are associated with quiescence in many types of cells. In a long-lived quiescent state of *Caenorhabditis elegans*, which can survive for months without food, TFEB is a master regulator of reproductive quiescence that is required for entry into quiescence as well as survival and recovery [84]. Further investigations, using both proteomic approaches and protein analysis at the molecular level, are required to reveal the role of lysosomes in quiescence. Imaging in live cells with fluorescent probes for lysosomal activity could also be used to monitor lysosomal function and dynamics [51,85–87]. Because the lysosome is a multifunctional organelle, the inhibition or activation of lysosomes alters multiple aspects of cellular functions, including metabolic changes and environmental stresses associated with aging. Lysosomes also regulate lipid metabolism in cells [52,88,89], suggesting that they are involved in lipid regulation in adult NSCs [90]. Taken together, these observations suggest that further studies of lysosomes will provide deep insights into stem cell quiescence.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

TK and RK wrote the manuscript.

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