Hypoxia Inducible Factor-1 α (HIF-1 α) Is Required for Neural Stem Cell Maintenance and Vascular Stability in the Adult Mouse SVZ

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HIF-1 α is a hypoxia-inducible protein that regulates many cell and molecular processes, including those involved in angiogenesis and stem cell maintenance. Prior studies demonstrated constitutive HIF-1 α stabilization in neural stem cells (NSCs) of the adult mouse SVZ, but its role there has not been elucidated. Here, we tested the hypothesis that HIF-1 α plays an essential role in the maintenance of adult NSCs and stabilization of the SVZ vascular niche using conditional, tamoxifen-inducible *Hif1a* knock-out mice. We generated nestin-CreER^{T2}/R26R-YFP/*Hif1a*^{fl/fl} triple transgenic mice, to enable tamoxifen-inducible *Hif1a* gene inactivation in nestin-expressing NSCs within the adult SVZ. *Hif1a* gene deletion resulted in a significant loss of YFP⁺ NSCs within the SVZ by 45 d post recombination, which was preceded by significant regression of the SVZ vasculature at 14 d, and concomitant decrease of VEGF expression by NSCs. Loss of YFP⁺ NSCs following *Hif1a* gene inactivation *in vivo* was likely an indirect consequence of vascular regression, since YFP⁺ neurosphere formation over serial passage was unaffected. These results identify NSC-encoded HIF-1 α as an essential factor in the maintenance of the adult SVZ, and demonstrate that NSCs within the SVZ maintain the integrity of their vascular niche through HIF-1 α -mediated signaling mechanisms.

Key words: hypoxia; stem cell niche; vasculature

Introduction

The adult rodent SVZ harbors a reservoir of multipotent neural stem cells (NSCs) capable of generating neurons, astrocytes, and oligodendrocytes throughout life (Alvarez-Buylla and Garcia-Verdugo, 2002). Under nonpathological conditions, slowly dividing NSCs within the SVZ give rise to proliferative transit amplifying progenitors (TAPs), which then generate neuroblasts and postmitotic olfactory neurons. Under pathological conditions, SVZ-NSCs initiate multilineage regenerative responses important for aspects of plasticity, repair, and recovery from brain injury (Arvidsson et al., 2002; Li et al., 2010). Understanding the mechanisms that govern NSC maintenance within the SVZ is important for regenerative medicine in the CNS, since dysregulation can lead to imbalances in progenitor populations and depletion of the stem cell pool.

The microvasculature of the SVZ represents a critical regulator of stem cell function. NSCs reside in close proximity to blood vessels within the SVZ (Tavazoie et al., 2008) and receive signals from endothelial cells that direct stem cell renewal (Shen et al.,

2004; Ramírez-Castillejo et al., 2006) and lineage progression (Kokovay et al., 2010). Likewise, stem cells regulate endothelial cell function, indicating bidirectional signaling (Louissaint et al., 2002). The vasculature as a critical component of the stem cell niche is not unique to the CNS, but contributes to stem cell maintenance across many organ systems and in the maintenance of CNS cancer stem cells (Calabrese et al., 2007).

Hypoxic signaling also plays an important role in stem cell maintenance (Panchision, 2009). Hypoxic characteristics of stem cells include constitutive stabilization of HIF-1 α , a key transcriptional regulator of metabolism and angiogenesis. Although HIF-1 α normally undergoes proteasomal degradation under nonhypoxic conditions (Semenza, 2004), it is constitutively stabilized under aerobic conditions in a variety of stem cell types, including bone marrow hematopoietic stem cells (Nombela-Arrieta et al., 2013), mesenchymal stem cells (Palomäki et al., 2013), and NSCs (Mazumdar et al., 2010; Roitbak et al., 2011). We previously demonstrated that *Hif1a* gene deletion impairs the vasculotrophic properties of embryonic and adult NSCs in cell culture and following intracerebral transplantation (Roitbak et al., 2008), suggesting that NSC-encoded HIF-1 α may function to stabilize the vascular niche through pro-angiogenic mechanisms, although this has not been previously investigated.

Here, we tested the hypothesis that NSC-encoded HIF-1 α plays an essential role in the maintenance of adult NSCs and stabilization of SVZ vasculature *in situ* using conditional, tamoxifen-inducible HIF-1 α knock-out mice. We demonstrate that induced *Hif1a* gene deletion in adult NSCs results in their

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Figure 1. HIF-1 α is essential for maintenance of SVZ NSCs. **A**, *Hif1a* exon 2 deletion in YFP⁺ cells isolated from SVZ of HIF-1 α WT and HIF-1 α cKO mice. **B**, Experimental time line. **C**, SVZ in the coronal plane 45 d following the final tamoxifen (TAM) injection. Merged images of YFP⁺ cells (green) and DAPI (blue)-labeled nuclei. Inset shows higher power magnification of YFP⁺ cells within the SVZ. Scale bar, 50 μ m. **D**, YFP⁺ cell number within the SVZ at 14 and 45 d post-tamoxifen treatment (* p < 0.02, n = 5 mice/group/time point). **E**, TUNEL⁺ cells at 45 d post-tamoxifen treatment (n = 5 mice/group). **F**, Representative TUNEL⁺ image. LV, Lateral ventricle; STM, striatum.

gradual loss, which is preceded by regression of the SVZ vasculature. These findings identify NSC-HIF-1 α as a critical regulator of NSCs maintenance within the adult SVZ, and demonstrate that SVZ-NSCs maintain the integrity of their vascular niche through HIF-1 α -mediated signaling mechanisms.

Materials and Methods

Mice

Animal experiments were performed in accordance with protocols approved by the University of New Mexico Animal Care and Use Committee. Nestin-CreER^{T2}/R26R-YFP/*Hif1a*^{fl/fl} (HIF-1 α cKO) triple transgenic mice and nestin-CreER^{T2}/R26R-YFP/*Hif1a*^{w/w} control (HIF-1 α WT) mice were generated, maintained and genotyped as previously described (Candelario et al., 2013).

Tamoxifen administration

Tamoxifen was prepared and administered as described previously (Candelario et al., 2013). Deletion of *Hif1a* exon 2 from YFP-sorted cells isolated from SVZ was confirmed using PCR as described previously (Candelario et al., 2013; Fig. 1A). *Hif1a* exon 2 gene deletion abolishes HIF-1 α transcriptional activity within YFP⁺ NSCs, as previously demonstrated using a hypoxia response element binding assay (Harms et al., 2010). For neurosphere assays, tamoxifen was administered as a single dose at postnatal day 3 (P3; 33 mg/kg) and mice were killed at P11. For all other experiments, tamoxifen (180 mg/kg, i.p.) was administered to 6- to 8-week-old male mice daily for 5 consecutive days and mice were killed 14 d or 45 d after the final tamoxifen dose.

Immunohistochemistry. Immunofluorescent staining of histological brain sections was performed as described previously (Li et al., 2010), with the following primary antibodies: goat anti-doublecortin (DCX; 1:200; Santa Cruz Biotechnology), mouse anti-GFAP (1:500; Sigma), rabbit anti-Glut-1 (1:200; Abcam), rabbit anti-laminin (1:500; Sigma), chicken anti-GFP (1:1000; Invitrogen), rabbit anti-S100 β (1:500; DAKO), rat anti-BrdU (1:250; Accurate), rabbit anti-Ki67 (1:1000; Leica Microsystems), and rabbit anti-VEGF (1:200; Santa Cruz Biotechnology). TUNEL staining was performed using the NeuroTACS II kit

(Trevigen) according to the manufacturer's protocol as described previously (Kokovay et al., 2006). SVZ whole-mount dissection and staining were performed as described previously (Mirzadeh et al., 2010) and imaged using a Zeiss 510 laser scanning confocal microscope.

Stereology

Cell counting and phenotype analysis. The number of YFP⁺ cells within the SVZ was estimated using Optical Fractionator Stereo Investigator software (MicroBrightField) linked to an Olympus DSU spinning disk confocal microscope. The contour of the SVZ was manually outlined in each histological section used for counting, using a 20 $\times$ objective. A total of six coronal sections per mouse, spaced 240 μ m apart, located between +1.32 mm anterior and -0.22 mm posterior to bregma, were used for analysis. The counting frame size was 30 $\times$ 30 μ m and grid size was 120 $\times$ 120 μ m. For phenotype analysis, the number of YFP⁺ cells within 35 μ m from the edge of lateral ventricle that coexpressed each phenotypic marker was determined using the same method.

Vascular density. Blood vessel density was quantified as published previously (van Praag et al., 2005). A total of six coronal sections per mouse, spaced 240 μ m apart, were used for analysis. The optical fractionator (Stereo Investigator; MicroBrightField) was used to estimate blood vessel number. The counting frame was 50 $\times$ 50 μ m and the grid size was 100 $\times$ 100 μ m. In cases where blood vessels had multiple branches, each branch was counted as one additional vessel. The total number of laminin-positive blood vessels was divided by the total volume to give an estimate of density.

Serial neurosphere formation assay

SVZs were dissected from P11 mice, 8 d following tamoxifen administration, and plated in uncoated tissue culture plates as described previously (Candelario et al., 2013). Neurospheres were maintained in Neurobasal medium (Invitrogen) supplemented with B-27 (2%; Invitrogen), glutamine (2.0 mM; Sigma), penicillin (100 U/ml; Invitrogen), streptomycin (100 μ g/ml; Invitrogen), EGF (10 ng/ml; Invitrogen), and bFGF (20 ng/ml; Invitrogen). Neurospheres were passaged every 8 d by enzymatic dissociation with Accutase (Sigma) and subcultured at 1.4×10^4 cells/

Figure 2. Phenotypic analysis of YFP⁺ NSC. **A–C**, Confocal images with orthogonal views (left) and quantitative comparisons (right) for NSCs (**A**), TAPs (**B**), and neuroblasts (**C**) at 45 d post-tamoxifen treatment (* p < 0.02, ** p < 0.001, n = 5 mice/group).

cm² in 6-well tissue culture plates. Before each passage the number of YFP⁺ neurospheres 150–200 μ m in diameter were scored across 20 randomly chosen 1 mm² counting frames per well using an Olympus inverted fluorescence microscope and a 20 $\times$ objective.

Statistical analysis

Data are expressed as means $\pm$ SEM. Statistical comparisons were made using Student's t tests, with p < 0.05 considered statistically significant.

Results

HIF-1 α is required for the maintenance of neural stem and progenitor cells within the adult SVZ

To examine the effects of *Hif1a* gene deletion on NSCs within the adult SVZ, we treated adult HIF-1 α WT and HIF-1 α ciKO mice with tamoxifen for 5 consecutive days, and compared the number of YFP⁺ cells at 14 and 45 d following the final tamoxifen injection (Fig. 1). YFP⁺ cells were observed throughout the dorso-ventral extent of the anterolateral SVZ in both HIF-1 α WT and HIF-1 α ciKO mice at 45 d post tamoxifen (Fig. 1C); however, *Hif1a* gene deletion abolished the expansion of YFP⁺ cells over time in HIF-1 α ciKO mice compared with controls. As shown in Figure 1D, the number of YFP⁺ cells increased significantly ($\sim$ 4-fold) between 14 and 45 d post tamoxifen in HIF-1 α WT, but not in HIF-1 α ciKO mice. By 45 d post tamoxifen, the number of YFP⁺ cells within the SVZ of HIF-1 α ciKO mice was decreased by 50% compared with control mice (p < 0.01). To determine whether the lower number of YFP⁺ cells in HIF-1 α ciKO mice was due to increased cell death by apoptosis, we compared the number of TUNEL⁺ cells within the SVZ of both genotypes. We found no difference in the number of TUNEL⁺ cells at either 14 d (data not shown) or 45 d post tamoxifen (Fig. 1E).

Hif1a gene deletion results in gradual depletion of NSCs within adult SVZ

Nestin is expressed within primitive NSCs, TAPs, and early proliferating neuroblasts within the adult SVZ (Pastrana et al., 2009). To determine which cell type is primarily affected by *Hif1a* gene deletion, we surveyed YFP⁺ cells at 45 d post tamoxifen for coexpression of markers to identify specific cell types as described (Ables et al., 2010). NSCs were identified by the expression of GFAP in the absence of the mature astrocyte marker, S100 β ; TAPs were identified by the presence of the cell cycle marker, Ki67 and the absence of the neuroblast marker, DCX; and neuroblasts were identified using DCX. As shown in Figure 2, *Hif1a* gene deletion in nestin⁺ cells resulted in marked reduction in the number of YFP⁺ NSCs and TAPs compared with control mice.

YFP⁺ NSCs were decreased by 82% in HIF-1 α ciKO mice compared with controls (4657 ± 1960 vs $26,199 \pm 8220$, respectively; p < 0.01). The mean number of YFP⁺ TAPs was decreased by 58% (6871 ± 872 vs $16,501 \pm 6307$, p < 0.02). The approximate 25% decrease in the mean number of YFP⁺ neuroblasts at 45 d following *Hif1a* gene deletion did not reach statistical significance (p = 0.06).

Hif1a gene deletion also resulted in a significant decrease in the percentage of all YFP⁺ cells represented by NSCs at 45 d post tamoxifen (13.2 ± 2.9 vs $4.1 \pm 1.3\%$ of all YFP⁺ cells were NSCs in HIF-1 α WT vs HIF-1 α ciKO mice, respectively; $p < 0.01$). Although the percentage of YFP⁺ cells represented by neuroblasts appeared to increase by $\sim 8\%$ at 45 d following *Hif1a* gene inactivation, this increase did not reach statistical significance (32.3 ± 3.1 vs $40.9 \pm 6.2\%$, $p = 0.12$); there was no significant difference in the proportion of YFP⁺ cells represented by TAPs (8.2 ± 2.4 vs $6.2 \pm 0.1\%$ in HIF-1 α WT vs HIF-1 α ciKO mice, respectively; $p = 0.10$). The percentage of YFP⁺ cells undergoing proliferation at the time of being killed was not different between strains, as assessed by Ki67 immunofluorescence (11.8 ± 1.9 and $12.0 \pm 2.9\%$ in HIF-1 α WT vs HIF-1 α ciKO mice, $p = 0.90$). These results suggest that the gradual depletion of YFP⁺ nestin-lineage cells following *Hif1a* gene deletion is not due to an overall impairment of proliferation, but primarily involves depletion of upstream NSCs.

Hif1a gene deletion results in loss of BrdU label-retaining NSCs within the adult SVZ, but has no effect on capacity for neurosphere formation *ex vivo*

To label slowly dividing NSCs, we used a BrdU label-retaining assay whereby mice were treated with tamoxifen for 5 consecutive days followed by BrdU in their drinking water (0.8 mg/ml) continuously for 2 weeks. After a 12 d BrdU washout period, mice were analyzed for numbers of BrdU label-retaining cells situated within $35 \mu\text{m}$ of the lateral ventricle (Figure 3A). In agreement with our phenotype analysis, the number of BrdU⁺/YFP⁺ colabeled cells was significantly decreased by $\sim 60\%$ in HIF-1 α ciKO mice compared with controls (651 ± 141 vs 1601 ± 284 colabeled cells, respectively; $p < 0.01$; Fig. 3B). However, the capacity for YFP⁺ NSC self-renewal *ex vivo* was not affected by *Hif1a* gene deletion, as indicated by formation of YFP⁺ SVZ neurospheres generated from tamoxifen-treated HIF-1 α WT and HIF-1 α ciKO mice (Fig. 3C,D). These data suggest that NSC-encoded HIF-1 α may act indirectly to regulate NSC maintenance and self-renewal *in vivo*.

HIF-1 α is essential for SVZ vascular stability

HIF-1 α activates the transcription of VEGF and other angiogenic growth factor genes and is required for vasculature development. Previous studies demonstrated that NSCs support the survival of endothelial cells in culture and following transplantation through HIF-1 α -VEGF signaling pathways (Roitbak et al., 2008). To determine whether HIF-1 α gene expression by NSCs is essential for maintaining vascular stability within the adult SVZ *in situ*, we quantified microvascular density within the SVZ of both HIF-1 α ciKO and HIF-1 α WT mice using immunofluorescence for laminin or glucose transporter-1 (Glut-1). As shown in Figure 4, microvascular density was significantly decreased by $\sim 50\%$ in

Figure 3. BrdU and neurosphere analyses of NSCs. **A**, Experimental time line for BrdU label-retaining assay. **B**, Left, Dual immunofluorescence for YFP (green) and BrdU (red) within adult SVZ of HIF-1 α WT mouse. Right, Quantification of BrdU label-retaining cells within SVZ measured within $35 \mu\text{m}$ from lateral ventricle ($*p < 0.02$, $n = 5$ mice/group). **C**, YFP⁺ neurosphere in phase contrast (top) and fluorescence (bottom) microscopy at passage 2. **D**, Propagation of YFP⁺ neurospheres over five serial passages ($n = 2$ separate experiments). TAM, tamoxifen.

HIF-1 α ciKO compared with HIF-1 α WT mice at both 14 and 45 d post tamoxifen administration. At 2 weeks, the density of blood vessels within the SVZ of HIF-1 α WT mice ($1.73 \times 10^{-4} \pm 0.32 \times 10^{-4}/\mu\text{m}^3$) was significantly greater than that in HIF-1 α ciKO mice ($1.03 \times 10^{-4} \pm 0.15 \times 10^{-4}/\mu\text{m}^3$), and this difference was maintained at 45 d post tamoxifen ($p < 0.01$). The reduced vascular density at 2 weeks post tamoxifen was also apparent in whole-mount preparations of adult SVZ in which the vasculature was visualized using immunofluorescence for Glut-1 (Fig. 4B). Importantly, blood vessel density within striatum was not decreased within striatum of HIF-1 α ciKO mice ($2.4 \times 10^{-5} \pm 8.7 \times 10^{-7}$ vs $2.6 \times 10^{-5} \pm 1.8 \times 10^{-6}$ in HIF-1 α WT vs HIF-1 α ciKO, respectively; $p = 0.44$, $n = 5$ mice per group), indicating that *Hif1a* gene inactivation within NSCs does not impact the vasculature outside the SVZ.

Hif1a gene inactivation impairs VEGF expression

Based on prior studies demonstrating impaired VEGF production following *Hif1a* gene silencing in cultured NSCs (Harms et al., 2010), we compared VEGF production in YFP⁺ cells from HIF-1 α ciKO and HIF-1 α WT mice at 2 weeks following tamoxifen-induced recombination. As shown in Figure 4, E and F, robust VEGF immunofluorescence was observed within SVZ YFP⁺ NSCs in HIF-1 α WT mice, but not within the majority of YFP⁺ NSCs in HIF-1 α ciKO mice. These observations suggest that *Hif1a* gene deletion leads to a downregulation of VEGF production in adult SVZ-NSCs, which may underlie impaired vascular stability.

Figure 4. HIF-1 α is essential for maintenance of SVZ vasculature. **A**, Montage of coronal section through the SVZ demonstrating dual immunofluorescence for YFP (green), and laminin (red) from HIF-1 α WT and HIF-1 α ciKO mice at 45 d post tamoxifen, counterstained with DAPI nuclear label (blue). Scale bar, 50 μ m. **B**, Glut-1 immunofluorescence of vasculature within SVZ whole-mount preparation from HIF-1 α WT and HIF-1 α ciKO mice 45 d following tamoxifen injection. **C**, **D**, Quantification of blood vessel density within the SVZ of HIF-1 α WT versus HIF-1 α ciKO at 14 and 45 d post-tamoxifen injection (* p < 0.02, n = 5 mice/group). **E**, Dual immunofluorescence for YFP (green) and VEGF (red) within SVZ of HIF-1 α WT (left) and HIF-1 α ciKO (right) mice. **F**, Quantification of VEGF immunofluorescence within YFP⁺ cells (p < 0.05; n = 5 mice/group).

Discussion

These studies demonstrate that HIF-1 α expression is required for the maintenance of NSCs within the adult SVZ and vascularity within the stem cell niche. Conditional and inducible *Hif1a* gene deletion in nestin⁺ cells resulted in a gradual depletion of YFP⁺ NSCs and TAPs over the course of several weeks, preceded by SVZ vascular regression. That *Hif1a* gene deletion within nestin⁺ cells first resulted in vascular regression followed by depletion of NSCs and downstream TAPs, suggests a potential indirect role of NSC-encoded HIF-1 α in neural stem cell maintenance secondary to stabilization of the vascular niche.

Utilizing a combination of phenotypic fate analysis coupled with a long-term BrdU label-retaining assay, we show that the gradual loss of YFP⁺ cells over time was most likely due to initial depletion of upstream NSCs with subsequent diminishment of downstream progenitors. *Hif1a* gene deletion in nestin⁺ cells resulted in an 82% loss of YFP⁺ NSCs and a 58% loss of YFP⁺ TAPs by 45 d following tamoxifen-induced recombination. Although the number of neuroblasts was also reduced by ~25%, this did not reach statistical significance by 45 d. The reduction in the number of TAPs was likely a consequence of upstream NSC loss, since the percentage of YFP⁺ cells that represented NSCs was decreased ~3-fold, whereas the percentage of YFP⁺ cells representing TAPs and neuroblasts remained unchanged or slightly increased. *Hif1a* gene deletion also resulted in a 2- to 3-fold loss of BrdU label-retaining cells. The loss of YFP⁺ cells within the SVZ following *Hif1a* gene deletion was not due to cell death, as assessed by TUNEL, or to an overall reduction in cell proliferation, since there was no change in the percentage of

YFP⁺ cells expressing the proliferation marker Ki67. These results suggest that *Hif1a* gene deletion may slow the expansion of NSCs, possibly through altering the mode of self-renewing cell divisions, without altering overall proliferation rate or cell death. We observed no significant decrease in the number of YFP⁺ neuroblasts or YFP⁺ cells within the olfactory bulb (data not shown), which could be explained by that fact that downstream depletion of these cell populations may require several months following the gradual loss of upstream NSCs (Imayoshi et al., 2010).

Loss of NSCs within the SVZ of HIF-1 α ciKO was preceded by vascular regression, suggesting that the loss of NSCs could be secondary to destabilization of the SVZ vasculature. The importance of SVZ vasculature for maintenance of neural stem cells and lineage progression within the SVZ has been well documented (Shen et al., 2004, 2008; Tavazoie et al., 2008; Kokovay et al., 2010). Pigment epithelial-derived factor (PEDF) may mediate these effects, at least in part, since it is expressed by SVZ endothelial cells and promotes Notch-dependent self-renewal of NSCs (Andreu-Agulló et al., 2009). Furthermore, endothelial-derived PEDF maintains the NSC population within the SVZ by directing the mode of self-renewing divisions rather than regulating proliferation rate (Andreu-Agulló et al., 2009). In addition, stromal derived factor-1 is another endothelial mediator that directs NSC activation (Kokovay et al., 2010), and its receptor is a known target of HIF-1 (Zagzag et al., 2006). Thus, depletion of YFP⁺ cells within the SVZ following *Hif1a* gene deletion may be secondary to vascular regression, as opposed to a cell-autonomous

effect of *Hif1a* gene deletion within NSCs, particularly since *ex vivo* neurosphere propagation was not affected.

The paracrine signaling mechanisms by which NSC-encoded HIF-1 α maintains vascular stability within the SVZ are likely due to sustained transcriptional activation of VEGF or other angiogenic signaling molecules. Stabilized HIF-1 α forms a heterodimeric complex with HIF-1 β to create HIF-1, which is a potent transcriptional activator of VEGF and other angiogenic factors (Simon and Keith, 2008). Indeed, conditional deletion of HIF-1 α in embryonic nestin-expressing stem cells of the developing nervous system results in regression of vasculature and loss of neural cells (Tomita et al., 2003). We have previously demonstrated that NSCs are vasculotrophic and support the survival of endothelial cells in culture and following intracerebral transplantation through HIF-1 α -regulated VEGF signaling (Roitbak et al., 2008). The present studies extend those findings to show that NSCs also support the SVZ vasculature *in situ*, and that *Hif1a* gene deletion in the adult SVZ impairs VEGF expression. It is noteworthy that Yuen et al. (2014) recently demonstrated coupling of oligodendrocyte progenitor-encoded HIF function with angiogenesis and myelination in developing CNS white matter, mediated in part by HIF-regulated Wnt signaling (Yuen et al., 2014).

The mechanisms that maintain HIF-1 α stabilization and function within SVZ-NSCs are currently unclear. Within the adult rodent SVZ, HIF-1 α expression is localized to NSCs (nestin⁺, Sox-2⁺, and GFAP⁺) but is not expressed in DCX⁺ neuroblasts (Roitbak et al., 2011). Although the SVZ is highly vascularized, it should be noted that cellular oxygen levels within this niche environment have been estimated to be ~2.5–3.0% under nonpathological conditions (Santilli et al., 2010). Furthermore, high levels of ROS are maintained within NSCs, which is important for the regulation of self-renewal, and may stabilize HIF-1 activity through the mTOR pathway (Le Belle et al., 2011). Additional O₂-independent mechanisms for HIF-1 α stabilization occur under physiological conditions that can be activated by calcium-regulated signaling events, growth factors, and cytokines that are likely present within the stem cell niche (for review, see (Majmudar et al., 2010)).

Overall, our study describes a novel regulatory mechanism for the maintenance of NSCs and their perivascular SVZ niche through NSC-encoded HIF-1 α . Ultimately, small molecule regulators that target HIF-1 α may be useful in promoting the integrity of the stem cell niche and maintenance of the stem cell pool under aging and pathological conditions, thereby ensuring an adequate supply of endogenous NSCs for regenerative purposes.

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