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CHROMATIN REMODELING FACTOR BRG1 SUPPORTS THE EARLY MAINTENANCE AND LATE RESPONSIVENESS OF NESTIN-LINEAGE ADULT NEURAL STEM AND PROGENITOR CELLS

David Petrik¹, Sarah E. Latchney^{1,*}, Irene Masiulis^{1,*}, Sanghee Yun^{1,*}, Zilai Zhang^{2,*}, Jiang I. Wu^{2,^}, and Amelia J. Eisch^{1,^}

¹Department of Psychiatry, The University of Texas Southwestern Medical Center, Dallas, Texas, 75390, U.S.A.

²Departments of Physiology and Developmental Biology, The University of Texas Southwestern Medical Center, Dallas, Texas, 75390, U.S.A.

Abstract

Insights from embryonic development suggest chromatin remodeling is important in adult neural stem cells (aNSCs) maintenance and self-renewal, but this concept has not been fully explored in the adult brain. To assess the role of chromatin remodeling in adult neurogenesis, we inducibly deleted Brg1 – the core subunit of SWI/SNF-like BAF chromatin remodeling complexes – in nestin-expressing aNSCs and their progeny *in vivo* and in culture. This resulted in abnormal adult neurogenesis in the hippocampus, which initially reduced hippocampal aNSCs and progenitor maintenance, and later reduced its responsiveness to physiological stimulation. Mechanistically, deletion of Brg1 appeared to impair cell cycle progression, which is partially due to elevated p53 pathway and *p21* expression. Knockdown of p53 rescued the neurosphere growth defects caused by Brg1 deletion. Our results show that epigenetic chromatin remodeling (via a Brg1 and p53/p21-dependent process) determines the aNSCs and progenitor maintenance and responsiveness of neurogenesis.

[^]co-corresponding authors, **CONTACT (CO-CORRESPONDING AUTHORS)**, Amelia J. Eisch, amelia.eisch@utsouthwestern.edu, Telephone: 01-214-648-5549, Fax: 01-214-645-9549, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX, USA 75390-9070, Jiang I. Wu, jiang9.wu@utsouthwestern.edu, Telephone: 01-214-648-1824, Fax: 01-214-648-1960, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX, USA 75390-9133.

*authors contributed equally and are listed alphabetically

AUTHOR CONTRIBUTIONS

David Petrik: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript

Sarah E. Latchney: Collection and/or assembly of data, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Irene Masiulis: Collection and/or assembly of data, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Sanghee Yun: Collection and/or assembly of data, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Zilai Zhang: Collection and/or assembly of data, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Jiang I. Wu: Conception and design, financial support, provision of study material or patients, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Amelia J. Eisch: Conception and design, financial support, provision of study material or patients, data analysis and/or interpretation, manuscript writing, final approval of manuscript

Keywords

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INTRODUCTION

Adult neurogenesis in the hippocampal subgranular zone (SGZ) depends on proper proliferation and maintenance of adult neural stem cells (aNSCs) and progenitors to sustain active adult neurogenesis [1]. Understanding the molecular and genetic basis of aNSCs self-renewal and maintenance is an area of great interest [2–4]. Much is known about how a range of factors influence aNSCs [3, 5], including signaling molecules like Notch1 [6], transcriptional factors like Sox2 [7] and transcriptional repressors such as Bmi1 [8] and NRSF [9]. It is also clear aNSCs are influenced by epigenetic mechanisms [10], including histone modification [11] and non-coding RNAs [12]. Notably, it is not clear how a third group of epigenetic modifications – chromatin remodeling (CR) – influences adult neurogenesis in the hippocampus. This knowledge gap is striking, since ATP-dependent CR complexes (CRC) are critical for many developmental processes [13, 14].

The prototypical ATP-dependent CRC is the SWI/SNF complex, and the functional equivalents in mammals are the BAF (Brg1/Brm-associated factor) complexes [15–17]. The core ATPase of the BAF complex is Brg1 (Brm-related gene 1), which acts with 10 other subunits as a CRC to modify DNA accessibility to transcription factors and co-factors [18–20]. During embryogenesis, BAF complexes play extensive roles in maintaining stem cell pluripotency, differentiation, and cell survival [21, 22]. As the enzymatic subunit of BAF complexes, Brg1 is particularly critical for BAF complex function. Brg1 knockouts are embryonic lethal [18], and Brg1 is required for embryonic stem cell self-renewal and proliferation [23–25]. The molecular mechanisms underlying Brg1's regulation of cellular processes like proliferation and differentiation are beginning to be understood [21, 26]. However, the role of Brg1 is highly cell- and context-dependent [21, 26–30]. Therefore, it will be interesting to determine the specific function of Brg1 in adult neurogenesis. A recent study investigated the role of Brg1 in neurogenesis from the adult subventricular zone (SVZ) and found that Brg1, together with the transcription factor Pax6, directs aNSCs in the SVZ towards neuronal instead oligodendrocyte lineage [31]. Since dramatic changes in fate choice towards oligodendrocyte lineage do not occur in SGZ, it motivated us to consider the possibility that Brg1 may serve dissimilar functions in the adult hippocampus [32].

As the role of BAF complexes in aNSC maintenance and responsiveness to environmental stimuli in the adult hippocampus remains unclear, we inducibly deleted Brg1 in nestin-expressing aNSCs *in vivo* and in culture. Our results suggest that, similar to embryonic neurogenesis, Brg1 is critical for the proliferation and maintenance of aNSCs and progenitors. Specifically, we show that Brg1 deletion appears to disrupt progression of aNSCs/progenitors. This leads to fewer cells being generated soon after Brg1 deletion. Mechanistically, we show the Brg1 deletion phenotype is dependent on p53-p21 pathways. Brg1 deletion also impairs the neurogenesis response to physiological stimulation. Our

results reveal for the first time that chromatin remodeling factor Brg1 supports the early maintenance and late responsiveness of nestin-lineage aNSCs/progenitors in the adult hippocampus.

MATERIALS AND METHODS

Animals

The Institutional Animal Use and Care Committee at UT Southwestern (UTSW) Medical Center approved all experiments in this study. Mice were housed at UTSW in an ALAAC-accredited vivarium. Two different strains of mice were used. For *in vivo* studies, we generated transgenic mice that allowed temporal and tissue-specific control of Brg1 expression. We crossed nestin-CreER^{T2}/R26R-YFP mice [33] with Brg1^{flox/flox} mice [34] to obtain CreER^{T2}/R26R-YFP/Brg1^{wt/flox} (Ctrl) and CreER^{T2}/R26R-YFP/Brg1^{flox/flox} (iBrg1) mice. Administration of i.p. Tamoxifen (Tam) at around 5 weeks of age induces CreER^{T2} translocation to the nucleus, which excises exons in Brg1 gene and the stop signal from YFP cassette resulting in Brg1 deletion and YFP expression in nestin-expressing cells and their progeny [33]. In each group and time point, 7–12 mice were used.

For voluntary running, mice were single-housed and allowed 24h access to running wheels (Coulbourn Instruments, Whitehall, PA) for 30 days, with the activity monitored by ClockLab software (ActiMetrics, Wilmette, IL) [6].

For neurosphere experiments, we crossed CAGG-CreER mice [35] with Brg1^{flox/flox} mice [34] to generate Brg1^{flox/flox} and CAGG-CreER/Brg1^{flox/flox} pups. Application of 1 μ M 4-hydroxy-Tam (4OH-Tam) to the culture media induced LoxP sites recombination and deletion of Brg1 only in CAGG-CreER/Brg1^{flox/flox} cells. Alternatively, neurospheres cultured from Brg1^{flox/flox} mice were infected with Cre expressing lentiviruses to delete Brg1.

Drug Administration

Adult mice were given Tam (Sigma; 150 mg/kg i.p.) for 5d to induce Cre-mediated recombination [33] and were killed 14, 30, 60 or 90d post-Tam.

Immunohistochemistry

Brains were immersion-fixed (2d) in 4% paraformaldehyde and sunk in 30% sucrose [6, 33]. 30 μ m coronal sections (entire hippocampus) were cut in serial sets of 9 for stereological evaluation. Slide-mounted or free-floating IHC was performed and immunoreactive(+) SGZ cells were quantified [6, 33]. IHC details, including primary antibodies used, are provided in supplement.

Cell quantification and phenotypic analyses

Quantification of YFP+ SGZ cells was performed stereologically [33, 36]. Confocal phenotyping was performed as described previously [6, 37] and is detailed in the supplement. Unpaired t-test was used for statistical analysis.

Neurosphere assay

Micro-dissected hippocampi from P10 CAGG-CreER/ Brg1^{flox/flox} or Brg1^{flox/flox} pups [9, 38] was dissociated and resulting neurospheres were cultured in non-differentiating media with EGF and FGF [39]. Adult neurospheres were cultured from the SVZ and SGZ areas of P28 CAGG-CreER/ Brg1^{flox/flox} mice. 4OH-Tam was added to induce Brg1 deletion, whereas ETOH solvent was added as controls. After 3 days, spheres were dissociated and cells were counted. The number of cells/well was quantified by hemacytometer or per visual fields as previously described [9]. Additional details provided in supplement.

CFSE dye staining and FACS

Following the last passage, neurospheres were labeled with CFSE dye [40, 41] and treated with 4OH-Tam. Dissociated cells were incubated with anti-CD15 antibody for FACS [42]. CFSE+ live cells were separated from dead cells using standard parameters of forward and side-scattering [43]. CFSE+ live cells were gated into CD15+ and CD15- cells to distinguish neural stem/progenitor vs. differentiated cells. The CD15+ cells were separated into two groups by levels of CFSE fluorescence intensity: low CFSE (1st log decade of CFSE fluorescence scale) and high CFSE (2nd log decade and higher). Additional details provided in supplement.

For cell cycle and cell apoptosis analyses, P10 Brg1^{flox/flox} neurospheres were infected with Cre-expressing lentiviruses or control empty lentiviral vector. After three days, dissociated cells were analyzed with 7AAD for cell cycle and Annexin V for apoptosis using a FACSaria (BD Bioscience).

RT-qPCR

Neurosphere RNA was isolated, cDNA was synthesized, and qPCR was performed using standard approaches as described in Supplemental Information. Results were analyzed following the 2^{-CT} method [44] and normalized to expression levels of GAPDH. Primer sequences are provided in the supplement. Unpaired t-tests were used for statistical analysis.

Preparation and infection with lentivirus

Lentiviruses expressing Cre or the control empty pSin vector as well as the p53 shRNA or the PLKO.1 empty shRNA lentiviral vectors were prepared in cultured HEK 293T cells. After 14d (and 3 passages), same number of Brg1^{flox/flox} neurospheres (above) were infected for 24hrs with control virus (PLKO.1 or pSin), p53 shRNA virus, Cre virus, or Cre virus together with p53 shRNA virus. After infection, cells were re-plated in virus-free media, and cell number was quantified 3d later. A parallel experiment with neurospheres utilized standard western blotting techniques to assess levels of Brg1, p53, and GAPDH. Unpaired t-tests were used for statistical analysis. Additional details provided in supplement.

RESULTS

Brg1 deletion in nestin-expressing aNSCs and their progeny initially results in fewer hippocampal aNSCs and progenitor cells

We produced an inducible, conditional knockout of Brg1 in mice by crossing nestin-CreER^{T2}/R26R-YFP mice [33] with Brg1^{flox/flox} mice [34] to obtain CreER^{T2}/R26R-YFP/Brg1^{wt/flox} (control, Ctrl) and CreER^{T2}/R26R-YFP/Brg1^{flox/flox} (iBrg1) mice. Injections of Tamoxifen (Tam, Fig.1A) caused Brg1 deletion and YFP expression only in nestin-expressing aNSCs and their progeny, and YFP-immunoreactive (YFP+) cells were restricted to regions of the adult brain which host aNSCs and adult-generated neurons, such as the hippocampal SGZ (Fig.1B, sFig.1) [45].

Stereological quantification of the number of YFP+ SGZ cells 14 days (14d) post-Tam revealed ~40% fewer cells in iBrg1 vs. Ctrl mice (Fig.1B–D). Confocal phenotypic analysis of the discrete developmental stages of YFP+ cells [6, 46] revealed fewer aNSCs (Type-1; GFAP+Sox2+YFP+) in iBrg1 vs. Ctrl mice (Fig.1E,F) but no change in Sox2+YFP+GFAP- early-stage progenitor cells [7]. Later-stage progenitors/immature neurons appeared unaffected, as there was no difference in doublecortin (DCX)+YFP+Ki67- cell number in iBrg1 vs. Ctrl mice (Fig.1G,H). However, there were fewer DCX+YFP+ cells also positive for the endogenous proliferation marker, Ki67 [47] (Fig. 1G, 1H) in iBrg1 vs. Ctrl mice. This proliferation impairment was restricted to DCX+ cells, as there was no significant difference in Ki67+YFP+DCX- cell number (Fig.1H). While there were fewer aNSCs and progenitors, there was no difference in YFP+NeuN+ mature granule cell number [6, 48] (Fig.1I,J). Thus, at an early timepoint post-Tam, iBrg1 mice have fewer SGZ aNSCs and progenitors, yet produce similar number of adult-generated neurons.

Brg1 deletion reduces number and size of neurospheres in culture

The reduction in aNSCs and progenitors in iBrg1 mice *in vivo* (Fig.1) suggested altered cell maintenance and/or proliferation. To probe cellular mechanisms of this iBrg1 phenotype, we prepared primary cell cultures of postnatal NSCs using the neurosphere assay. Cells prepared from hippocampi of P10 CAGG-CreER/Brg1^{flox/flox} or Brg1^{flox/flox} mice (Fig.2A–F) were kept in non-differentiating media. After 14d in culture for expansion, cells were exposed to 4OH-Tam to induce Brg1 gene deletion. While neurospheres from both genotypes grew with similar efficiency before 4OH-Tam (Fig.2B,C), deletion of Brg1 in neurospheres derived from CAGG-CreER/Brg1^{flox/flox} mice resulted in fewer neurospheres (Fig.2D) with smaller diameter (Fig.2E) and reduced total cell numbers (Fig.2F) compared to those derived from Brg1^{flox/flox} mice. Neurospheres cultured from SVZ and SGZ of adult (P28) CAGG-CreER/Brg1^{flox/flox} mice displayed similar growth defects after 4OH-Tam induced Brg1 deletion. There is a significant decrease in total cell numbers in 4OH-Tam treated cultures compared to control samples treated with ETOH (sFig. 2A). Thus, Brg1 deletion *in vivo* (Fig.1) and in culture (Fig.2, sFig.2A) similarly reduces NSCs and the proliferation of their progeny.

Brg1 deletion appears to impair cell cycle progression in neurospheres

The *in vitro* and *in vivo* phenotypes described above could be caused by multiple mechanisms, including slower progression through the cell cycle and/or elevated cell quiescence. To test whether Brg1 deletion reduces number of cell divisions over time, we used fluorescence activated cell sorting (FACS) to analyze neurospheres labeled by CFSE fluorescent dye, in which the fluorescence intensity is reduced by half after each division [41, 49]. The neurospheres were labeled by CFSE just before the addition of 4OH-Tam, and 72hrs later the cells were sorted for CD15, a marker of NSCs [42](Fig.2G–I). Analysis of CFSE fluorescence intensity in CD15+ cells revealed a greater proportion of cells with high CFSE intensity in CAGG-CreER/Brg1^{flox/flox} vs. Brg1^{flox/flox} cells (Fig.2H,I). As CFSE fluorescence is halved with each cell division and thus higher percentage of cells with higher CFSE intensity means fewer cell divisions, these results suggest Brg1 deletion leads to slower cell cycle progression in CD15+ neural stem/progenitor cells.

To directly analyze the effects of Brg1 deletion on cell cycle progression, we performed cell cycle analyses with 7AAD staining of dissociated neurosphere culture to determine the DNA content. Neurospheres cultured from P10 Brg1^{flox/flox} hippocampi were infected with Cre-expressing lentiviruses or empty vectors. Cre-induced Brg1 deletion resulted in increased percentage of cells in G1 phase accompanied with decreased cell numbers in G2/M phase, suggesting a defect in cell cycle progression, likely a G1 arrest (Fig.2J). In addition, Annexin V staining of the same cells indicated that Brg1-deleted sphere cultures have increased apoptosis (Fig. 2K). Taken together, these data suggest the decreased growth of Brg1-deleted neural stem/progenitor cells result from a combination of defected cell cycle progression, fewer cell division, and increased cell death. *In vivo*, we observed no difference in the number of AC3+ cells in SGZ in both the control and iBrg1 mice (sFig.3), which may be rapidly removed via microglia engulfment [50].

Brg1 deletion results in increased p21 expression in neurospheres

To identify molecular mechanisms responsible for Brg1-induced impairment of cell cycle progression, we explored relative gene expression using RT-qPCR from postnatal neurospheres focusing on genes known to be involved in stem cell regulation[24, 51, 52]. After 72hrs in 4OH-Tam (Fig.3A), there was no difference in CAGG-CreER/Brg1^{flox/flox} vs. Brg1^{flox/flox} cells in expression of stemness gene Cdk6 and Sox2 (Fig.3B). We also investigated the expression of cyclin-dependent kinase inhibitors from the Cip/Kip family that regulate transition from G1 to S-phase [53]. While there was no change in expression of p27 or p57, Brg1 deletion led to increased expression in p21/Cip1/Waf1 in neurospheres cultured from both P10 and P28 brain (Fig. 3B and sFig. 2B).

Knockdown of p53 rescues the Brg1 deletion phenotype in neurospheres

As it has been reported that deleting Brg1 or other BAF subunits in normal cells including specific stem cells may activate p53 pathway and stabilize p53 protein [28, 54, 55], we next explored whether this increase in p21 was related to activation by the tumor suppressor gene p53 [30]. The activations of the p53 pathways by many signals converge to the stabilization of p53 proteins through p53 posttranslational modifications. The activated p53 could interact with transcription co-factors and activate the expression of target genes such as p21 [56].

We tested the connection between Brg1 and p53 in neurosphere cultures using lentiviral delivery of p53 shRNA. We hypothesized that Brg1 deletion activates the p53 pathway by increasing p53 protein levels, and p53 knockdown would rescue the Brg1 deletion phenotype. Because the treatment of 4OH-Tam and viral infection together caused high cellular toxicity (data not shown), we opted for recombination induction by incubating the Brg1^{flox/flox} neurospheres with Cre-expressing lentivirus. In combination with Cre or empty vector control, these Brg1^{flox/flox} neurospheres were co-infected with either PLKO.1 control RNAi lentiviruses or lentiviruses expressing shRNA targeting p53. (Fig.3C–I). Brg1 deletion by viral-mediated Cre expression replicated the phenotype observed after the Brg1 deletion induced by 4OH-Tam (Fig.2F), as there were fewer cells in the Cre vs. control virus infected cultures (Fig.3I). In addition, western blotting of parallel-prepared neurospheres confirmed the viral-mediated efficacy of Brg1 deletion (Fig.3H). Indeed, Brg1 deletion elevated p53 protein levels (Fig.3H). Importantly, although transfection with lentiviral p53 shRNA did not affect cell number in control cells (Fig.3E, 3I), in Brg1 deleted neurospheres, reducing p53 protein levels by p53 shRNA (Fig.3H) restored cell number (Fig.3G, 3I). These data show that knockdown of p53 rescues the Brg1 deletion phenotype, suggesting that elevated p53 pathway may be a mechanism that impairs cell cycle in stem cells lacking Brg1.

Brg1 deletion extends active adult neurogenesis with age

Our results suggest that deletion of Brg1 slows the cell cycle or halts its progression in aNSCs and progenitors. We hypothesized that slower cycle may cause the cells to apparently proliferate less, which may extend their lifespan and consequently augment neurogenesis in later life. To test this, we prepared mice similar to our 14d post-Tam experiment (Fig.1) and examined YFP+ cells 30, 60, and 90d post-Tam (Fig.4A). There was no significant difference in the number of YFP+ cells between Ctrl and iBrg1 mice 30d and 60d post-Tam (data not shown). However, in contrast to the fewer YFP+ SGZ cells seen 14d post-Tam in iBrg1 vs. Ctrl mice (Fig.1), there were no decrease, but a trend towards more YFP+ SGZ cells and more GFAP+Sox2+YFP+ aNSCs 90d post-Tam in iBrg1 vs. Ctrl mice (Fig.4B–E). There was also no change in immature or mature neuron number (Fig.4F–G). These data suggest that there may be altered maintenance of basal adult neurogenesis in iBrg1 mice.

Impaired running-induced neurogenesis after Brg1 deletion

One specific feature of hippocampal neurogenesis is the ability to respond to many external stimuli such as exercise. We determined whether YFP+ cells lacking Brg1 may have impaired responsiveness to neurogenic stimuli, such as running [57]. We prepared mice similar to our 14d post-Tam experiment (Fig.1) but gave mice free access to a running wheel 60 to 90d post-Tam (Fig.4H). Cell quantification 90d post-Tam revealed the expected running-induced increase in YFP+ SGZ cells in Ctrl mice (Fig.4D vs. 4M). However, there were significantly more YFP+ cells after running in Ctrl mice vs. iBrg1 mice (Fig.4H–M). Confocal phenotypic analysis further revealed that in the pool of YFP+ cells, there was a trend towards fewer GFAP+Sox2+YFP+ aNSCs and significantly fewer NeuN+YFP+ neurons in iBrg1 vs. Ctrl runners (Fig.4N–O). These results suggest that while running can upregulate adult hippocampal neurogenesis even in iBrg1 mice, this upregulation is less

potent than in Ctrl. Taken together, the data in Fig.4 suggest that although Brg1 deletion does not dramatically affect aNSC numbers with age, it impairs the neurogenic responsiveness of these cells to physiological stimulation.

DISCUSSION

The BAF complexes and the core subunit Brg1 regulate many cellular processes, including the cell cycle progression [13]. From studies *in vivo* and in cultured malignant and non-malignant cells [28], it is clear that Brg1 regulates the cell cycle via diverse mechanisms and in a context-dependent manner. A recent report on Brg1 function in adult SVZ neurogenesis revealed a critical role of Brg1 in neuronal fate specification by cooperating with PAX6 [31]. However, it remains unclear whether Brg1 affect neural stem cell maintenance and responsiveness to environmental stimuli in the adult hippocampus. Here we addressed this major knowledge gap, and our findings suggest that Brg1 supports the early maintenance and late responsiveness of nestin-lineage aNSC/progenitors without dramatically altering the fate choice. We show that acute Brg1 deletion in nestin-lineage cells leads to fewer aNSC/progenitors, perhaps due to a slower cell cycle caused by activated p53/p21 pathway. Brg1 deletion also leads to impaired response to neurogenic stimulation. Together these data show for the first time the integral role that CRC play in regulating neurogenesis in the adult hippocampus. Below we discuss our three major findings, and how these data influence our understanding of the regulation of adult hippocampal neurogenesis by CRCs.

p53/p21 activation contributes to stem cell maintenance defects caused by Brg1 deletion

Various embryonic stem and progenitor cells lacking Brg1 have impaired proliferation, arrest, and cell death [13, 21]. Similarly, here we show aNSCs lacking Brg1 have impaired maintenance and slowed cell cycle. However, BAF complexes are not likely essential for cell proliferation or survival *per se*, since many cancer cells lack different BAF subunits and yet display extensive proliferation capacity [58–60]. Thus a puzzling paradox persists: removal of a tumor suppressor like Brg1 actually decreased proliferation of stem cells.

One possible explanation is that deletion of Brg1 triggers the activation of cell surveillance pathways and causes cell cycle arrest and increased quiescence indirectly. Indeed, p53 is activated after loss of Brg1 or another BAF complex subunit, SNF5, in MEF cells and specific cancer cells [28, 55]. In human bone marrow mesenchyme stem cells, Brg1 deletion leads to senescence through the activation of RB-p53 pathways. These reports agree with our present results, where Brg1 deletion in hippocampal neural stem cells increased p53 protein levels and p53 target genes are upregulated. Our present work further confirms this Brg1/p53 link in stem cells, as knockdown of p53 in neurospheres lacking Brg1 rescues the reduction in cell number. The link between Brg1 and p53/p21 in regulating stem cells is plausible, as loss of p21 reduces the quiescence and accelerates the exhaustion of aNSC proliferative capacity [61]. Moreover, loss of p53 in aNSCs leads to increased proliferation and dramatically reduced levels of p21 [62], while increased p53 activity reduces proliferation [63, 64]. Since our results show that Brg1 deletion increases p53 protein and p21 expression in neurospheres, we speculate that iBrg1 stem cells *in vivo* have increased cell quiescence. It is also possible that p53 protein is stabilized following Brg1 deletion [28,

55], or there exists p53 post-transcriptional regulatory mechanisms [56]. Given the challenges of visualizing increases in p53+ or p21+ cells in the healthy adult mouse hippocampus [65, 66], this hypothesis is ripe for testing using other approaches, such as cell cycle reporter mice [67, 68].

Impaired cell maintenance and quiescence caused by Brg1 deletion is part of a multilayered phenotype

The function of Brg1 in aNSC/progenitors is likely to be multilayered, because Brg1 regulates not only stem cell self-renewal and maintenance [23–25] but also differentiation and maturation of neurons [31, 69]. Several aspects of our data support the notion of a complex, multilayered phenotype in the SGZ of iBrg1 mice. First, in addition to iBrg1 mice having fewer aNSCs and progenitors, they also have fewer proliferating Ki67+DCX+ neuroblasts. Second, iBrg1 mice display no difference in the basal number of YFP+ mature neurons either early or late after Tam-induced recombination. This multilayered phenotype suggests a compensatory mechanism in maturation or survival of YFP+ neurons, which may also explain the diminishment of the phenotype at late post-Tam.

The lack of change in mature neurons at early or late post-Tam deserves further discussion. We interpret our data from the early post-Tam timepoint (Fig. 1) as indication that Brg1 deletion in nestin-expressing aNSCs and their progeny initially results in fewer hippocampal aNSCs and progenitor cells, but does not change the number of adult-generated granule cell neurons. This timepoint may seem too early to assess differentiation, as it is widely stated that it takes ~30 days for an adult-generated hippocampal neuron to achieve terminal differentiation and thus express proteins like NeuN [1, 46]. However, numerous publications show mature neuron protein expression much sooner than 30 days [48, 70]. In fact, NeuN expression can be detected as early as a few days after division [38]. Therefore, we feel our interpretation remains valid: Brg1 deletion in nestin-expressing aNSCs and their progeny initially decreased the number of hippocampal aNSCs and progenitor cells without changing adult-generated granule cell neuron number. However, we do not know if the terminal differentiation of adult-born neurons is affected at intermediate timepoints between 14 and 90 days post-Tam. While our late post-Tam timepoint shows that terminal differentiation of adult-born neurons is not affected (Fig. 4G), this lack of change may reflect a compensatory mechanism in maturation or survival of YFP+ neurons, as a lower number of aNSCs and progenitors still produce similar number of neurons in both iBrg1 and Ctrl SGZ. It is interesting, though, that when exposed to a proneurogenic stimuli such as running [57] the terminal differentiation of adult-generated neurons is actually decreased (Fig. 4O). This suggests that terminal differentiation may be vulnerable under certain physiological or pathological conditions.

The initial reduction in number of aNSC/progenitors early after Brg1 deletion was observed both *in vivo* and in culture, suggesting a cell-intrinsic effect. We cannot exclude the possibility that the initial decrease in aNSCs and progenitors after Brg1 deletion is not just due to slower progression through cell cycle or increased cell quiescence but also due to reduced number of proliferating cells or increased cell death. However, we did not observe a

change in number of Ki67+YFP+ cells at 14d post-Tam, suggesting no change in proliferation *per se*.

It is also important to consider that recombination efficiency (or lack thereof) is an important issue in inducible transgenic mouse models. Some of our results may be complicated by limited recombination efficiency of Brg1 in our inducible mouse model. However, determining recombination efficiency in our iBrg1 mice is challenging since Brg1 expression in aNSC is relatively low and it is difficult to distinguish it from the highly similar homolog Brm (75% identical at the protein level). We do provide evidence of a greater proportion of YFP+ cells that were Brg1- in iBrg1 vs. control mice (sFig.1). More compellingly, most of these Brg1- cells were NSC-like, Type-1, or Type-2 cells, fitting with our concept that Brg1 expression are higher in mature YFP+ neurons than in Type-1 or Type-2 progenitors.

aNSC/progenitors lacking Brg1 are less responsive to physiological stimuli with age

The third major finding presented here is the mitigation of the KO phenotype with time. The reduction in neurogenesis in iBrg1 KO mice 14d post Tam is replaced by a trend towards increased neurogenesis at 90 days. While we recognize that this switch may be partially a consequence of incomplete deletion of Brg1 in the recombined population, we suggest that the trend towards the increase in number of hippocampal aNSC/progenitors in iBrg1 mice over time is a paradoxical consequence of their initial decrease. Our *in vitro* results show that neurospheres lacking Brg1 progress through cell cycle slower, which would extend their active lifespan if aNSCs have a limited number of cell divisions [4]. Alternatively, Brg1 deletion would increase stem cell quiescence as a consequence of increased p21 expression [61] leading to extended lifespan of aNSCs.

Interestingly, while there may be unaltered or even increased neurogenesis in aged iBrg1 KO animals, their YFP+ population in SGZ is less responsive to environmental and physiological stimuli such as running [57]. This suggests that although impact of Brg1 deletion in aNSCs and their progeny may be compensated with age, the responsiveness of the aNSCs and progenitors in aged KO animals is impaired. Since running increases hippocampal adult neurogenesis mainly via promoting proliferation [57, 71, 72], we may speculate that cells lacking Brg1 are either less likely to be activated or the number and cell cycle progression may be affected.

In summary, our results not only reveal the importance of Brg1 for proliferation and maintenance of aNSCs and progenitors in adult hippocampus but also highlight the innate differences between the two adult neurogenic niches. Deletion of Brg1 driven by Nestin-CreER^{T2} leads to different consequences in the lateral ventricles versus in adult hippocampus. In SVZ, Brg1 coordinates with Pax6 to keep the aNSCs and their progeny in the neurogenic lineage [31], which is important for the Type B aNSCs SVZ because they have strong multipotency and can generate both glia and neurons [73, 74]. The limited multipotency of Type-1 aNSCs in adult hippocampus directs them towards generating neurons [4] and thus Brg1 seems to assume a different role in their biology, regulation of proliferation and maintenance of aNSCs and their progeny. The differential development of the Brg1 KO phenotype in SGZ and SVZ also underlines the differences in cellular

dynamics between the two neurogenic niches with respect to epigenetic regulation by CRC complexes and the ability to compensate impaired neurogenesis [57]. This supports the idea that the proliferative potential of aNSC/progenitors that lack Brg1 is altered in time, and that their responsiveness to physiological stimuli is restricted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance Statement

Our results reveal the importance of a chromatin remodeling factor, Brg1, in regulation of stem and other dividing cells in the hippocampus, a region in the adult brain important for memory and mood regulation. Notably, although new neurons are generated in the hippocampus and another brain region, termed the SVZ, Brg1 has a distinct role in the hippocampus than has been shown in the SVZ. This work is significant in its advancement of our understanding of how stem and dividing cells are regulated in the body, and these findings have relevance for understanding brain structure and function in regards to development as well as in the adult brain.

Figure 1. Brg1 deletion in nestin-expressing aNSCs and their progeny 14d post-TAM decreases the number of Ki67+DCX+ progenitors in the subgranular zone (SGZ)

(A) Experimental paradigm of giving tamoxifen (Tam) to control (Ctrl) and iBrg1 mice. (B,C) Photomicrographs of Ctrl (B) and iBrg1 (C) mice dentate gyri displaying YFP+ SGZ cells 14d post-Tam (scale bar, sb=50 μ m). (D) Quantification of YFP+ SGZ cells 14d post-Tam. (E) Confocal photomicrographs of a Ctrl mouse dentate gyrus 14d post-Tam. Arrowheads: GFAP+Sox2+YFP+ aNSCs with radial glia morphology (sb=20 μ m). (F) Confocal phenotyping of YFP+ cells for GFAP+ and Sox2+. (G) Photomicrographs of a Ctrl mouse dentate gyrus 14d post-Tam. Arrowhead: DCX+Ki67+YFP+ cell; Arrows: DCX+YFP+ cells (sb=20 μ m). (H) Cell phenotyping of YFP+ cells for DCX+ and Ki67+. (I) Photomicrograph of a Ctrl mouse dentate gyrus 14d post-Tam. Arrowheads: NeuN+YFP+ cells (sb=20 μ m). (J) Quantification of NeuN+YFP+ SGZ cells. Data are mean $\pm$ SEM. Unpaired T-Test (*= p <0.05; #= p <0.1).

Figure 2. Brg1 deletion in postnatal neurospheres reduces number and slows cell division
 (A) Experimental paradigm of neurosphere preparation and gene recombination by 4OH-Tam in culture. (B) Photomicrographs of neurospheres from *Brg1^{flox/flox}* (B, top) and *CreER/Brg1^{flox/flox}* (B, bottom) (sb=50 μ m). (C) Cell number before 4OH-Tam. (D) Spheres/field during 4OH-Tam. (E) Sphere diameter during 4OH-Tam. (F) Cell number after 4OH-Tam. (G) Experimental paradigm of neurospheres staining with CFSE dye and CD15 antibody for subsequent FACS. (H) Abnormal cell cycle progression/Representative FACS scatterplots of sorted cells showing the CD15+ fluorescence intensity as a function of

CFSE fluorescence intensity in Brg-1^{flox/flox} (left) CAGG-CreER/Brg1^{flox/flox} (right). (I) CD15+ cell numbers in the high CFSE fluorescence intensity bracket as a percent of Brg1^{flox/flox}. Data are mean±SEM. Un-paired T-Test (*=p<0.05; **=p<0.01; #=p<0.1). (J) Representative histograms of sorted cells showing cell cycle arrest in Brg1^{flox/flox} neurosphere cells infected with Cre versus control viruses. (K) Representative Annexin V histograms showing increased cell death in Cre virus infected Brg1^{flox/flox} neurosphere cells.

Figure 3. *Brg1* deletion phenotype in postnatal neurospheres is rescued by p53 knockdown
 (A) Experimental paradigm of neurosphere preparation, Cre induction, and RT-qPCR. (B) Comparison of relative gene expression between neurospheres from *Brg1^{flox/flox}* and CAGG-CreER/*Brg1^{flox/flox}* mice. (C) Experimental paradigm of lentiviral-mediated expression of Cre and p53 shRNA in P10 neurospheres. (D–G) Photomicrographs of neurospheres 72hrs post-transfection. Neurospheres transfected with control viruses (D), p53 shRNA virus (E), Cre virus (F), and viruses for both Cre and p53 shRNA (G; sb=50 μ m). (H) Western blot of P10 neurospheres transfected with control, Cre or Cre + p53 shRNA

viruses and stained with antibodies against Brg1, p53 and GAPDH. (I) Quantification of cells 72hrs after viral transfection. Data are mean $\pm$ SEM. Un-paired T-Test (*= $p<0.05$).

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Figure 4. Brg1 deletion 90d post-TAM does not decrease nestin-lineage neurogenesis, but impairs running-induced nestin-lineage neurogenesis

(A) Experimental paradigm for 90d post-Tam. (B,C) Photomicrographs of YFP+ cells in dentate gyrus of Ctrl (B) and iBrg1 (C) 90d post-Tam (sb=50 μ m). (D) Cell quantification and confocal phenotypic analysis in iBrg1 vs. Ctrl mice. (E) Cell phenotyping of YFP+ cells for GFAP+ and Sox2+. (F) Cell phenotyping of YFP+ cells for Ki67+ and DCX+. (G) Cell phenotyping of YFP+ cells for NeuN+. (H) Experimental paradigm for running post-Tam. (I,J) Photomicrographs of dentate gyrus from Ctrl (I) and iBrg1 mice (J) stained for YFP (sb=50 μ m). (K,L) Confocal photomicrographs of dentate gyri. (K) Arrow: GFAP+Sox2+YFP+ cells; Open arrowhead: Sox2+YFP+; Closed arrowheads: GFAP+YFP+ cells. (L) Arrowheads: NeuN+YFP+ cells [s.b. (K,L)=20 μ m]. (M) YFP+ SGZ cell number in iBrg1 vs. Ctrl mice after running. (N) Cell phenotyping of YFP+ cells for GFAP+ and

Sox2+. (O) Cell phenotyping of YFP+ cells for NeuN+. Data are mean±SEM. Un-paired T-Test (*=p<0.05).

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