UHRF1 Licensed Self-Renewal of Active Adult Neural Stem Cells

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Key Words. Cell cycle • CreERT2 • Neural stem cells • NP95 • Proliferation • UHRF1

ABSTRACT

Adult neurogenesis in the brain continuously seeds new neurons throughout life, but how homeostasis of adult neural stem cells (NSCs) is maintained is incompletely understood. Here, we demonstrate that the DNA methylation adapter ubiquitin-like, containing PHD and RING finger domains-1 (UHRF1) is expressed in, and regulates proliferation of, the active but not quiescent pool of adult neural progenitor cells. Mice with a neural stem cell-specific deficiency in UHRF1 exhibit a massive depletion of neurogenesis resulting in a collapse of formation of new neurons. In the absence of UHRF1, NSCs unexpectedly remain in the cell cycle but with a 17-fold increased cell cycle length due to a failure of replication phase entry caused by promoter demethylation and derepression of Cdkn1a, which encodes the cyclin-dependent kinase inhibitor p21. UHRF1 does not affect the proportion progenitor cells active within the cell cycle but among these cells, UHRF1 is critical for licensing replication re-entry. Therefore, this study shows that a UHRF1-Cdkn1a axis is essential for the control of stem cell self-renewal and neurogenesis in the adult brain. Stem Cells 2018;36:1736–1751

SIGNIFICANCE STATEMENT

A precise control of adult neurogenesis is crucial and may participate in memory formation and emotional behavior, and dysregulation may participate in the pathology of neuropsychiatric disorders, such as schizophrenia and autism spectrum disorders. This study revealed that a UHRF1-Cdkn1a axis is critical for the control of adult neurogenesis. Neural stem cell-specific ablation of UHRF1 results in a massive cell depletion due to increased cell cycle length, caused by a failure of cells to enter S-phase. UHRF1 represses Cdkn1a allowing for self-renewal and, in the absence of UHRF1, ectopic re-expression of Cdkn1a rescues the deficits. Thus, these results may allow for a better understanding of mechanisms that could participate in cognition and neuropsychiatric disorders.

INTRODUCTION

The continuous renewal and integration into functional circuits of neurons in discreet areas of the adult brain contributes to brain plasticity. The importance in rodents of the constant inflow of new neurons from the subgranular zone (SGZ) in the hippocampus has been illustrated in mood regulation, learning, and memory [1, 2] and from the anterior subventricular zone (aSVZ) of the lateral ventricle for neural plasticity of olfactory information processing in the olfactory bulb [1, 3, 4]. The sustained generation of new neurons relies on the continuous self-renewal of the neural stem cells (NSCs) residing in the SGZ and SVZ [5] and without it, the stem cell niches rapidly deplete of progenitors, leading to precipitously dwindling neurogenesis. Maintenance of neural progenitors and the differentiation into functional neurons are controlled by many different signaling molecules such as growth factors and neurotransmitters, but also rely on cell intrinsic regulators [6–8].

Ubiquitin-like, containing PHD and RING finger domains-1 (UHRF1) is a unique chromatin effector protein by integrating the recognition of both histone and DNA methylation through its multidomain protein structure and hence, is involved in two major repressive epigenetic pathways. The SET and RING-associated (SRA) domain mediates UHRF1 binding to hemimethylated DNA and recruits the maintenance methyltransferase DNMT1 ensuring the faithful copying of DNA methylation patterns to the daughter strands during DNA replication [9–15] and consistently, UHRF1 depletion results in DNA hypomethylation in cell lines [11, 14, 15]. The first Tudor domain of UHRF1 engage di and trimethylated H3K9 (H3K9me2/3) in heterochro-
matin and this activity is believed to be linked to the DNA methylation maintenance function of UHRF1 [16, 17]. However, the in vivo role of UHRF1 facilitated DNA methylation and putative other functions are not fully understood.

UHRF1 is expressed in highly proliferating cells including cancer cell lines, NSCs in the embryonic brain, and bronchial epithelial stem-like cells and is downregulated during cell differentiation or quiescence [18–24]. Depletion of UHRF1 abrogates proliferation in many different cell lines as well as in epithelial and immune cells in vivo [18, 24–28]. However, conditional deletion of Uhrf1 in the embryonic cortex has minor effects on proliferation and instead causes a delayed neurodegeneration phenotype [22]. In cell types where it controls proliferation, it has been shown to be essential for S-phase entry [18] and consistently, overexpression can force S-phase entry even in terminally differentiated myotubes in vitro [18, 29]. UHRF1 can repress factors that serve to restrain S-phase entry in cell lines, such as the S-phase cell cycle entry regulator pRB [30] and the cell cycle regulator cyclin-dependent kinase inhibitor 1A (p21Cip1 or p21) which blocks cyclinD/CDK2,4 activation of pRB via its hyperphosphorylation [24, 26, 31]. Here, we show that conditional deletion of UHRF1 in neural progenitor cells leads to Cdkn1a derepression, failure of S-phase entry and as a consequence a marked decrease of self-renewal with a concomitant loss of newly born neurons in the adult brain.

**Materials and Methods**

**Isolation of Proteins on Nascent DNA**

Isolation of proteins on nascent DNA (iPOND) was previously described [32]. NSCs were pulsed for 10 minutes with 100 μM of the thymidine analog EdU. For the chase experiments, the pulse was followed by extensive washing with phosphate-buffered saline (PBS) + 100 μM thymidine (Sigma, Missouri, USA) and incubation in serum-free media with 100 μM thymidine. Subsequently, the cells were harvested, pelleted by centrifugation (2,400 × g, 10 minutes at 4 °C), and lysed in lysis buffer (ChIP Express kit, Active Motif, California, USA) for 30 minutes at 4 °C. Lysates were passed 10× through a 21-gauge needle, and the nuclei were pelleted by centrifugation (2,400g, 10 minutes at 4 °C), washed with PBS + protease inhibitor cocktail (PIC; Roche), then subjected to Click reaction for 30 minutes at RT with 0.2 mM Biotin-azide forming a covalent bond between the EdU and the biotin. The EdU is derivatized by a copper-catalyzed cycloaddition reaction by which an organic azide reacts to organic chemistry reaction by which an organic azide reacts to a concomitant loss of newly born neurons in the adult brain.

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**Methylation Sites Analysis**

After obtaining methylation data using Infinium HumanMethylation 450K BeadChip array from Illumina Inc (biological triplicate for each condition) green/red color channel bias was adjusted. For quantile normalization of pooled methylated and unmethylated signals the R-function lumiMethylC were used. Analysis of methylation data was performed on GenomeStudio software version 2011.1 (Illumina Inc., California, USA). Methylation levels were calculated as ratio of signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals (beta values). For 77,098 targeted sites signal was detected in all samples (p < .02, Supporting Information Table S1). Among those sites, 11,243 were allocineable to mouse genome (out of the total around 485,000 human sites covered by the methylation kit, 13,295 were homologous to mouse genome (alignment performed with program bowtie, mm10 mouse genome assembly, 3 mismatches allowed). 3,627 out of 11,243 sites (homological to mouse genome) were differentially methylated when compared against at least one of two control conditions (floxed cells with vehicle or WT cells with Tx; Student’s t test, Benjamini and Hochberg method for multiple testing (BH) FDR < 0.2 with total number of tests = 22,486). Out of these 3,627 sites, 3,449 hypomethylated sites were selected as hits for all further analysis. One hundred twenty five sites (hypermethylation) and 53 sites (hypomethylation and hypomethylated by comparing with the two controls) were excluded as probable false positives. To assess enrichment between different areas of genome, gene body and CpG islands, 3,449 hypomethylated sites were analyzed against all 11,243 sites homologous to mouse genome. Gene structure annotation information (5 kb and 1 kb promoter area, UTRs etc.) was obtained from UCSC genome browser, model-based CpG island annotation for mm9 was downloaded from http://rafalab.jhsph.edu/CGI/. Sites were assigned to the different elements of gene structures and CpG islands using ChiPpeakAnno package in R. After obtaining sites counts for each categories enrichment analysis was done using Chi-square test in R and p value adjusted by BH. To assess if differentially methylated sites have any bias in distribution along the genome, sites counts were binned in 1 x 10^6 bp genome spans and significance was calculated by Fisher’s exact test in R (testing both alternative hypotheses “less” and “greater,” BH adjusted p value, FDR ≤ 0.1).

**RNA Sequencing Analysis**

We performed a whole genome RNA sequencing analysis of neospheories from the aSVZ of Rosa26CreERT2: UHRF1fllox/ fllox and Rosa26CreERT2: UHRF1wt/wt mice treated either with vehicle (EtOH) or hydroxytamoxifen (OHTx) resulting in four experimental groups (wEtOH, wtOHTx, floxedEtOH, floxedOHTx, n = 3 biological replicates).

In the RNA sequencing, clustering was done by “onboard clustering” and samples were sequenced on HiSeq2500 (HiSeq Control Software 2.2.58/RTA 1.18.64) with a 1x51 setup using “HiSeq Rapid SBS Kit v2” chemistry. The Bcl to FastQ
conversion was performed using bc12fastq-1.8.4 from the CASAVA software suite. The quality scale used is Sanger / phred33 / Illumina 1.8+. The samples were analyzed as follows: (a) Mapping: Reads were mapped with TopHat/2.0.4 to the Mouse genome assembly, build NCBI-M37. (b) Merging: Bamfiles from samples run on different lanes were merged with samtools. (c) Sorting and Marking duplicates: Merged bam files were sorted and duplicates removed using picard-tools/1.29. (d) Counts for genes: Gene counts were generated using htseq/0.6.1 on bam files with duplicates included. (e) FPKMs for genes and transcripts: FPKMs for genes and transcripts were generated using cufflinks/2.1.1 on bamfiles with duplicates included.

For further analysis, the raw gene counts were analyzed with the DESeq2 package in R [33]. The counts were normalized by size factor estimation. To ensure data quality the Euclidean distance of the rlog transformed values of all 12 samples was calculated and visualized in a heatmap. To identify differentially expressed genes, the estimate of the dispersion parameter for each gene was computed, the Wald statistical test performed (nbinomWaldTest), independent filtering applied and the p-values corrected with the fdrtool package in R.

**Imaging and Statistical Analysis**

Immunohistochemistry was performed on 14 μm coronal sections and the number of immunoreactive cells was quantified in an area in a single confocal plane within a 635.4 x 635.4 μm² area. Quantification was performed using the software Imaris (v7.3) and ImageJ 1.47v. Statistical analysis was performed using GraphPad Prism software (v5.0) and data are presented as mean ± SEM; for single comparison analysis in vitro paired t test was performed, while in vivo analysis was analyzed by unpaired t test, Mann–Whitney or unpaired two-tailed tests were consistently used, for multiple comparisons one way ANOVA and Turkey’s post-test were used with an α level of 0.05 for all statistical analyses.

See Supporting Information for additional Experimental Procedures.

**RESULTS**

**Expression of UHRF1 Only in Active Adult Neural Progenitor Cells**

Immunohistochemical detection revealed UHRF1 to be highly restricted to neurogenic areas of the adult mouse brain, including the hippocampus and the aSVZ (Supporting Information Fig. S1) as well as in the rostral telencephalon where the aSVZ extends into the rostral migratory stream (RMS). Occasionally cells were also observed within the RMS in which neuroblasts migrate toward the olfactory bulb. In the aSVZ and RMS, UHRF1 was exclusively localized to the nucleus of the cells and the labeling intensity varied between cells in both the dorsal and the ventral parts of the ventricle wall and in the dorsolateral horn of the aSVZ. The aSVZ niche houses slow dividing, relatively quiescent stem cells (referred to as type B1 cells) and fast dividing transit amplifying cells (type C cells) which differentiate into neuroblasts (type A cells) migrating to the olfactory bulb [35–39]. A number of experiments were conducted in order to define which cell types express UHRF1. Colocalization was prominent in Ascl1+ (Mash1+) type C cells which additionally were in the cell cycle as observed by double staining for the cell cycle marker Ki67, a protein that is present during all cell cycle phases but absent from quiescent (G0) adult NSCs cells [40] (Supporting Information Fig. S2A–2C). The majority of MASH1+ cells in the aSVZ were UHRF1+ (83.4% ± 3.49% of MASH1+ cells, Supporting Information Fig. S2A–2C), consistent with a rapid proliferation of these transit amplifying cells [41]. Some UHRF1+ cells were neuroblasts as determined by doublecortin (DCX+);β-III tubulin staining of these cells (9.17% ± 3.12%). However, only 4.91% ± 0.24% of DCX;β-III tubulin+ cells were UHRF1+ (Supporting Information Fig. S2D), consistent with a cell cycle exit and migration of most of these cells. Targeting the tamoxifen-inducible form of the Cre recombinase (CreERT2) to the astrocyte-specific glutamate transporter GLAST (Slc1a3) locus allows inducible recombination in the astrocytic lineage as well as in NSCs of the adult mouse brain [25, 42] that can be monitored using a CAG-GFP reporter mouse line [43]. NSCs can also be monitored using transgenic hGFAP:GFP mice [44]. We therefore used hGFAP:GFP mice as well as GlastCreERT2: CAG-GFP mice treated for 2 days with Tx (3 mg/animal) to identify the relatively quiescent GFAP+ and GLAST+ adult NSCs. In both of these strains approximately 5% of the GFP+ population was labeled with UHRF1 (Fig. 1A). UHRF1 expression is highly cell cycle regulated with a presence at least in fibroblasts as cells enter S-phase and is thereafter lost in mid/late S-phase [18] and hence, the GFP+/UHRF1+ cells may represent the proportion of adult NSCs undergoing cell division. Consistent with this conclusion, GFP+ cells expressing UHRF1 were cycling as showed by Ki67 coexpression in the GFP+UHRF1+ cells (Supporting Information Fig. S2G), furthermore a brief pulse of Edu (2 hours) prior to analyzing the mice revealed that 64.9% ± 4.7% of GFP+/UHRF1+ cells incorporated Edu (Fig. 1B). Because nearly all Edu+ cells were UHRF1+ at 2 hours after a single Edu pulse (Fig. 1C), we conclude that virtually all active cells (i.e., cell in the cell cycle) that are in S-phase express UHRF1. At the population level, adult NSCs are largely quiescent and even upon an asymmetric neurogenic cell division returns to quiescence [5]. We therefore reasoned that the relationship between proliferation and UHRF1 expression could be confirmed by pulse-chase experiments. Thus, many EdU+ cells would be predicted to enter quiescence in a chase and EdU labeling should with time therefore start to dissociate with UHRF1. In agreement with this, less than half of the 24 hours chased EdU+ cells coexpressed UHRF1 and the proportion declined further with time (e.g., 36 hours and 5 days; Fig. 1C). The dissociation was even greater when characterizing label retaining cells. Animals received BrdU injections once daily for 5 days to label most NSCs and C cells in the SVZ. Analysis was performed 3 weeks later, when labeled C cells have migrated to the olfactory bulb and hence, cells retaining BrdU
largely represents B cells that were dividing at least once several weeks earlier. In this paradigm, no BrdU and UHRF1 double-positive cells were identified (Supporting Information Fig. S2F).

Unlike the GlastCreERT2:CAG-GFP mice which permanently labels NSCs and all their progeny, hGFAP-GFP mice labels cells only actively expressing GFAP supporting that UHRF1 is expressed in NSCs in the above results. However, since it cannot be excluded that GFP protein in hGFAP-GFP mice can be carried over from NSCs to transit amplifying precursors, we depleted the niche from all dividing cells (e.g., actively dividing C and B cells) and thereafter monitored expression of UHRF1 during the process of repopulation from quiescent B cells that become active in response to cell deletion [45]. In order to permanently trace progenitors arising from B cells we made use of the GlastCreERT2:CAG-GFP mice treated twice with Tp (5 mg per animal) following intracranial administration of the antimitotic drug cytotoxic α-D-arabinofuranoside (AraC), as previously described [45] (Fig. 1D). The treatment led to a loss of cells with a markedly thinner ventricle wall (Fig. 1E), loss of more than half of the GFP+ cells in both the dorsal and ventral SVZ and near complete absence of Edu incorporation (2 hours) and UHRF1+ cells (GFP: p = 7.6 × 10−2; UHRF1: p = 8.83 × 10−3; Edu 2 hours: p = 1.15 × 10−3) (Fig. 1F, 1G). Thus, both UHRF1+ cells and Edu incorporation were virtually depleted in the aSVZ upon AraC treatment, in full agreement with the presence of UHRF1 in dividing cells. Upon withdrawal of AraC, Edu incorporating cells could be observed in the SVZ at 6 hours and increased in numbers at 12 hours (Fig. 1G). The increase in Edu+ cells was paralleled by an increase of GFP+ cells and UHRF1+ cells (Fig. 1G). These results confirm that UHRF1 is expressed in B cells that repopulate the SVZ niche. Consistent with this conclusion, the proportion of UHRF1+ cells in AraC treated as compared to saline treated mice which were genetically traced from the GlastCreERT2 locus, for example, GFP+ as well as GFAP+ increased and encompassed nearly all cells following depletion of C cells (6 hours, Fig. 1H). This proportional increase in UHRF1+ cells expressing GFP and GFAP was maintained at 12 hours. The results were similar for aSVZ and dorsal subventricular zone (Fig. 1F), showing that NSCs contain UHRF1 regardless of their regional identity. We therefore conclude that UHRF1 is expressed in B cells with competence to repopulate the SVZ. In summary, UHRF1 expression is turned on in all types of actively dividing progenitors. Among all UHRF1+ cells in the SVZ approximately 9% are NSCs, 80% are Mash1+ transit amplifying cells and 5% neuroblasts. However, within the NSC population of cells only 5% are UHRF1+, within the transit amplifying cells 82% are UHRF1+ and within neuroblasts 5% are UHRF1+.

To further characterize the relation of UHRF1 to proliferation in NSCs and transit amplifying cells, we developed an in vitro strategy using GFAP-GFP transgenic mice to identify SVZ cell types, similar as previously described [44]. The SVZ was microdissected and cells expanded in culture without passaging. Because GFPS is expressed directly from the GFAP locus in this mouse strain, fluorescent cells isolated from such mice actively express GFAP and hence, should represent NSCs while GFP− cells include transit amplifying cells. Consistently, neurospheres displayed a mix of GFP+ and GFPS cells and the two cell populations were prospectively isolated using fluorescent activated cell sorting (Supporting Information Fig. S3A–3C). Quantification of Edu+ cells following a 10 minutes pulse in primary neurospheres revealed that significantly fewer percent of all cells were UHRF1+ and incorporated Edu in the GFP+ as compared to GFP− cell population (Supporting Information Fig. S3D, 3E). Thus, C cells divide more rapidly than B cells. However, close to 70% of the cells that expressed UHRF1 were Edu+ in both GFP+ and GFP− cells (Supporting Information Fig. S3F). Therefore, once entering S-phase, Uhrf1 is expressed similarly in both B and C cells. Hence, UHRF1 is expressed in the same proportion of dividing NSCs and transit amplifying cells in vitro, but a smaller proportion of all NSCs (e.g., among GFP+ cells) incorporated Edu as confirmed by quantifying Edu+;UHRF1+ double positive (e.g., among GFP+ cells compared to transit amplifying (e.g., GFP) cells (Supporting Information Fig. S3F), similar as observed in vivo, and consistent with that UHRF1 is present in a lower proportion of NSCs because these divides slower than transit amplifying cells.

**Generation of Ubiquitous and Stem Cell-Type Specific UHRF1 Deficient Mice**

**Uhrf1** novel mutant mice display early embryonic lethality. To enable studies of the biological functions of UHRF1 in NSCs, we generated mice in which the **Uhrf1** gene conditionally can be disrupted by a Cre-mediated deletion of exon 4, generating a frameshift from exon 3 to exons 5 with a premature stop codon in exon 5 (Supporting Information Fig. S3H–3J). UHRF1lox/lox mice were bred to either Rosa26CreERT2 mice or to GLAST-CreERT2 mice that also carry the floxed reporter allele CAG-GFP [43] to generate GLASTCreERT2:GFPUHRF1lox/lox mice and Rosa26CreERT2:UHRF1lox/lox mice (Fig. 2A, 2C). The resulting mouse strains allow for inducible UHRF1 loss of function in all cells of the adult (Rosa26CreERT2: UHRF1lox/lox or specifically

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**Figure 1.** Expression of UHRF1 in dividing cells of the anterior subventricular zone (aSVZ). (A): Quantification of UHRF1 among GFP+ neural stem cells (arrows) in hGFAP-GFP (n = 5) and GLAST-CreERT2-GFP (n = 6) mice. (B): UHRF1+;GFPS cells incorporates Edu in the aSVZ (n = 5). (C): Colocalization of UHRF1 in Edu at different times after EdU administration (n = 5 in all groups). Note in photomicrographs that virtually all Edu cells contain UHRF1 after 2 hours pulse (arrows), while UHRF1 do not always colocalize with Edu (arrowheads). (D): Schematic illustration of the AraC and cell lineage tracing strategy. (E): aSVZ at the end of AraC administration (DAPI). Ventricle wall (arrows), lateral dorsal horn (asterisk). (F): Recovery at indicated time points after AraC. GFP only cells (asterisk), UHRF1 only cells (arrows), UHRF1+/Edu− cells (thick arrows) and GFP−/UHRF1−/Edu− cells (arrowheads). (G): Quantification of GFP, UHRF1, and 2 hours Edu pulsed cells in the aSVZ in saline and AraC treated groups at end of AraC treatment and different times of recovery (n = 4 in all groups). Statistical analysis was performed between AraC and recovery groups: ***, p = 0.0085 AraC + 6 hours; ***, p = 0.013 AraC + 12 hours; #, p = 0.02 AraC + 6 hours, ###, p = 0.0014 AraC + 12 hours; #, p = 0.013 AraC + 6 hours, #, p = 0.025 AraC + 12 hours. (H): Quantification of the expression of GFP and GFAP in UHRF1− cells after AraC treatment and 12 hours recovery (n = 4 in all groups). V = Ventricle. Scale bars: (A, B, and D) = 15 μm; (E) = 100 μm; (F) saline and AraC = 50 μm; recovery 6 hours and recovery 12 hours = 20 μm; inset saline = 6 μm; inset AraC = 10 μm. All graphs represent the mean ± SEM. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; dSVZ, dorsal subventricular zone; UHRF1, ubiquitin-like, containing PHD and RING finger domains-1; vSVZ, ventral subventricular zone.
in cells expressing the astrocyte-specific glutamate transporter GLAST including the radial glia-like cells that contributes to neurogenesis (e.g., NSCs) [46]. UHRF1wt/wt and UHRF1wt/wt mice carrying the RosaCreERT2 or GLASTCreERT2:GFP alleles were used as controls.

Efficiency of recombination and loss of UHRF1 expression in the SVZ cells was examined in both strains: (a) GLASTCreERT2:GFPxUHRF1flox/flox mice 15 or 30 days after Tx injections (Fig. 2B); (b) Rosa26CreERT2:UHRF1flox/wt mice 48 hours and 7 days after Tx administration (Fig. 2D). The latter strain of mice with the ubiquitous ablation of UHRF1 developed progressive deterioration of health, which limited studies using this strain to 1 week after Tx administration. Nevertheless, both strains of mice displayed an efficient elimination of UHRF1 with less than 5% remaining cells at all time points analyzed (Fig. 2B, 2D).

A Depletion of Neural Progenitors and Loss of Neurogenesis in UHRF1 Deficient Mice

To determine the role of UHRF1 in adult neurogenesis, we first characterized progenitor cell numbers in vivo following UHRF1 depletion. The GLASTCreERT2:GFPxUHRF1flox/flox and GLASTCreERT2:GFPxUHRF1flox/wt mice were used to lineage trace progenies in the aSVZ 15 days after Tamoxifen (Tx) administration. Notably, the number of GFP+ traced NSCs decreased by 34.6% in the SVZ in conditionally null mice as compared to control mice (Fig. 3A, 3B), suggesting that UHRF1 is essential for maintaining the progenitor cell pool in the SVZ of adult mice.

To further characterize the role of UHRF1 for self-renewal we developed an in vitro assay using NSCs from aSVZ dissected from Rosa26CreERT2:UHRF1flox/wt mice (wild-type, WT and knock-out, KO cells, respectively, Supporting Information Fig. S4A). To examine UHRF1-dependence for self-renewal primary spheres from UHRF1 KO cells were analyzed and found to yield significantly fewer and smaller spheres compared to WT spheres (Fig. 3C–3E). Hence, UHRF1 deletion compromises self-renewal of NSCs.
neuroblasts, which migrate through the RMS to the olfactory bulb and differentiate into neurons [39]. We therefore used the GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} and GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} mice for a 30 day cell lineage tracing experiment to analyze effects of Uhrf1 ablation on the ability of NSCs to seed new cells to the olfactory bulb. A near complete loss of traced cells was observed in the olfactory bulb of mice with uhrf1 deficient NSCs as compared to heterozygous control mice, and consequently also a depletion of NeuN\textsuperscript{+} traced cells (Fig. 3F). Combined, these results show that UHRF1 is essential for self-renewal of neural progenitor cells and in its absence, production of new neurons is nearly completely lost.

Figure 3. Depletion of neurogenesis in the adult brain of UHRF1-deficient mice. (A): Depletion of the stem cell niche in Uhrf1-deficient mice. GFP\textsuperscript{+} cells are significantly diminished in the anterior subventricular zone of GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} mice compared to GLASTCreERT2:GFPxUHRF1\textsuperscript{+/+} and GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} mice (n = 4 in both groups). (B): Quantification of the number of GFP\textsuperscript{+} cells between GLASTCreERT2:GFPxUHRF1\textsuperscript{+/+} and GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} mice (n = 4 in both groups). (C): Neurosphere cultures from neural stem cells (NSCs) taken from Rosa26CreERT2:UHRF1\textsuperscript{flx/flx} mice treated with EtOH or OH-Tx 0.1 μM for 12 hours and kept 5 days in new culture media. (D): Quantification of the number of spheres after 5 days in vitro in NSCs taken from Rosa26CreERT2:UHRF1\textsuperscript{flx/flx} mice treated with EtOH or OH-Tx 0.1 μM for 12 hours (n = 3). (E): Quantification of sphere diameter of neurospheres grown from single cells isolated from Rosa26CreERT2:UHRF1\textsuperscript{flx/flx} mice and treated with either EtOH or OH-Tx for 12 hours (n = 4). (F): Cell lineage tracing shows a marked loss of new neurons in the in the granular layer of the olfactory bulb 30 days after Tx treatment in GLASTCreERT2:GFPxUHRF1\textsuperscript{flx/flx} mice, while control (GLASTCreERT2:GFPxUHRF1\textsuperscript{+/+}) mice display large numbers of neurons labeled with NeuN (n = 4, insets). Scale bars: (A): 100 μm, (B): 200 μm, (F): 100 μm and insets in (F): 15 μm. Quantifications show mean ± SEM. Abbreviations: EPL, external plexiform layer; GrL, granular layer; SEZ, subependimal zone; UHRF1, ubiquitin-like, containing PHD and RING finger domains-1.
UHRF1 Is Critical for Licensing Neural Progenitor Cell Proliferation

Because neither senescence-associated β-galactosidase nor TUNEL or cleaved Caspase-3* cells increased in the aSVZ upon Uhrf1 ablation and furthermore, less than 1% of the Mash1, DCX, and GFAP cell populations (C, A, and B cells) contained cleaved Caspase-3 (Supporting Information Fig. S5A–S5F) and based on this we conclude that the rapid loss of progenitors is not associated with increased senescence or apoptosis. We therefore examined Ki67 at 48 hours after the last Tx injection. We first analyzed the role of UHRF1 in all dividing cells in the aSVZ, hence both NSCs and transit amplifying cells, by examining differences in Ki67* cells located in the aSVZ between Rosa26CreERT2: UHRF1*+/lox/lox mice as compared to WT mice. No differences were detected between the genotypes (Fig. 4A), showing that UHRF1 does not affect the number of cells that are in the cell cycle, strengthening the conclusion that there are no effects on senescence or apoptosis. However, when we analyzed the number of cells incorporating EdU among Mash1, DCX, and GFAP cell populations we found a significant decrease in KO mice in all cell populations (Fig. 4B). Furthermore, 48 hours was not enough to significantly alter cell numbers in the aSVZ cell-types (Fig. 4C). We next performed the same analysis in Rosa26CreERT2: UHRF1*+/lox/lox mice and WT mice after 1 week of the last Tx injection. We found a similar lack on effect in the expression of Ki67 and more robust effects on incorporation of EdU in the cell populations as seen at 48 hours after Uhrf1 ablation (Fig. 4D, 4E, and 4G). Furthermore, quantification of Mash1* transit amplifying cells and DCX* neuroblasts showed a striking 42.4% and 41.2%, respectively, reduction in the total number of cells in the SVZ as compared to control mice while the GFAP population was unchanged (Fig. 4F, 4G). Since none of these cell populations showed a significant increase in cleaved Caspase-3 expression, it appears the reduction is not caused by cell death [Supporting Information Fig. S5]. Moreover, this reduction of neural progenitors was paralleled by a marked reduction of the total number of EdU* cells following a 2 hours pulse (80.1% reduction Fig. 4F, 4G). These data therefore suggest that loss of UHRF1 rapidly attenuates cell proliferation, and as a consequence also neurogenesis.

Similar to the ubiquitous deletion of Uhrf1, specifically deleting Uhrf1 in NSCs led to marked depletion of EdU incorporating cells (2 hours) in the aSVZ without any change in Ki67* cells among the GFP* NSCs (Fig. 4H, 4I), and consistently, also Ki67*:EdU* cells within the GFP* NSCs were reduced (Fig. 4J, 4K). Thus, ablation of Uhrf1 in the aSVZ cells appears not to affect the number of NSCS in the cell cycle (i.e., Ki67* cells) but prevents cell cycle progression and replication of active NSCs. This conclusion was further corroborated by in vitro neurosphere studies. NSC spheres of both WT and KO expressed largely similar percentage of Ki67* cells, but EdU incorporation and phospho-histone 3, which labels cells in M-phase, were significantly diminished (Supporting Information Fig. 5AB–5D) without effects on apoptosis (Supporting Information Fig. 5S). In addition, DNA content analysis of NSC spheres showed that upon Uhrf1 ablation, NSCs fail to progress through the cell cycle and become arrested in G1 (Fig. 5A, 5B) which explains the persistent Ki67 labeling seen in all experiments described above. Furthermore, Uhrf1 KO cells showed a significant reduction in BrdU incorporation (Fig. 5C, 5D), corroborating previous results that showed that Uhrf1 ablation leads to a cell cycle arrest specifically in the G1 to S-phase transition. Due to this alteration in the cell cycle seen in the NSC sphere cultures, we further investigated cell cycle behavior in vivo in NSCs with Uhrf1 ablation. We used the GLAST-CreERT2: GFP–UHRF1*+/lox/lox and GLAST-CreERT2: GFP–UHRF1*+/lox/lox mice to lineage trace NSC progenies in the aSVZ 30 days after Tx administration and established their cell cycle length. The protocol used was adapted from Ponti et al. [41] and takes advantage of a double nucleotide analogs method to estimate S phase (Ts) and cell cycle (Tc) lengths (Fig. 5E, see “Materials and Methods” section). We found that the ratio Ts/Tc in Uhrf1 KO animals was 10 times lower compared to the WT. This result might be due to a really short Ts or long Tc. We found that albeit of significant differences in the Ts between Uhrf1 KO and WT, the Tc was 17-fold longer in the Uhrf1 KO than in WT mice. Therefore, upon Uhrf1 ablation, cells still remained in the cell cycle but failed to progress to S-phase resulting in a marked extension of cell cycle length. Hence, because of this, proliferation of NSCs in the SVZ of mice deficient for UHRF1 substantially drops, leading to the massive reduction of self-renewal of the active NSCs, resulting in the observed reduction in total niche size (Fig. 3A).

Genome-Wide Hypomethylation in Uhrf1-Deficient NSCs

UHRF1 is important for the accurate maintenance of DNA methylation during DNA replication. Consistently, UHRF1 associates with origins of replication as well as with replication foci in embryonic stem cells and in cell lines as evidenced by immunohistochemistry and also by its association with replication associated proteins [11, 14], but has yet to be shown to associate with the replication fork in direct experiments. To examine if a failure of neural progenitors to progress into S-phase could be associated with a presence of UHRF1 at replication forks we used the ipOND method [32]. The ipOND technique takes advantage of the rapid incorporation of the thymidine analogue EdU during DNA replication, a covalent crosslinking between DNA and proteins, the addition of a biotin moiety to the incorporated EdU using mild conditions and finally streptavidin-biotin affinity to capture sheared EdU-labeled chromatin. We compared proteins isolated after a short EdU pulse of 10 minutes, when the recently replicated DNA is labeled, with proteins isolated after a short EdU pulse followed by a 2 hours chase when the replication fork is expected to progress, leaving behind the labeled DNA (i.e., labeling and isolation of mature chromatin). As shown in Fig. 6A, while Uhrf1 input is equal among the different conditions and histone H4 can be isolated by ipOND in both nascent and mature chromatin, Uhrf1 was efficiently isolated only from pulsed cells, with little or no Uhrf1 levels in pulse-chased cells. Such association provides a scaffold to maintain the DNA maintenance methylation enzyme DNMT1 to replication forks [11, 14] which methylate the new strand whose partners on the parental strand already carry a methyl group [47, 48]. If Uhrf1 mediates DNMT1 recruitment and DNA methylation at the replication fork, then Uhrf1 deficiency would be expected to lead to DNA hypomethylation. We performed a genome-wide screen of differential methylation in Uhrf1-deficient NSCs. DNA from NSCs isolated from
Figure 4. Deletion of UHRF1 blocks cell cycle progression in proliferating cells of the anterior subventricular zone (aSVZ). (A): Rosa26CreERT2:UHRF1^flo^/^fl^ and Rosa26CreERT2:UHRF1^wt^/^wt^ mice treated with Tx for 48 hours show no differences Ki67 expression among Mash1, DCX, and GFAP cell populations (n = 4). (B): EdU incorporation is decreased in Mash1, DCX, and GFAP cell populations upon 48 hours treatment of Tx (n = 4) (Mash1 p = .011, DCX p = .011, and GFAP p = .0026). (C): Numbers of Ki67, GFAP, Mash1, DCX, and EdU cells in the aSVZ upon 48 hours treatment of Tx (EdU p = .0215). (D): Rosa26CreERT2:UHRF1^flo^/^fl^ and Rosa26CreERT2:UHRF1^wt^/^wt^ mice treated with Tx for 1 week show no differences Ki67 expression among Mash1, DCX, and GFAP cell populations (n = 4). (E): EdU incorporation is decreased in all populations (Mash1 p < .0001, DCX p < .0001, and GFAP p = .0036). (F): Numbers of Ki67, GFAP, Mash1, DCX, and EdU cells in the aSVZ 1 week after Tx reveals a reduction among Mash1 (p = .0492), DCX (p = .04), and EdU (p = .0038) cell populations. (G): Images of above cell populations studied in Rosa26CreERT2:UHRF1^flo^/^fl^ and Rosa26CreERT2:UHRF1^wt^/^wt^ mice 1 week after Tx. (H): Depletion of EdU incorporating cells in the aSVZ of GLASTCreERT2:GFPxUHRF1^flo^/^fl^ mice as compared to control GLASTCreERT2:GFPxUHRF1^wt^/^wt^ mice 30 days after Tx treatment (n = 4). (I): GLASTCreERT2:GFPxUHRF1^flo^/^fl^ mice 30 days after Tx display normal numbers of Ki67^+ cells but reduction of EdU incorporating cells. (J): EdU incorporation is diminished in GFP^+ NSCs that are active for example Ki67^+ in the GLASTCreERT2:GFPxUHRF1^flo^/^fl^ mice 30 days after Tx (n = 4). (K): Images of (j). Scale bars: (G): 30 µm, (H): 20 µm, and (K): 15 µm. Quantifications show mean ± SEM. Abbreviation: UHRF1, ubiquitin-like, containing PHD and RING finger domains-1.
Rosa26CreERT2: UHRF1fl/ox and Rosa26CreERT2: UHRF1wt/wt mice that were Uhrf1-deicient for 5 days were analyzed by the Illumina 450K Methylation arrays, which in human covers 485,000 methylation sites per sample. Out of 13,295 target sites, which were homologous to the mouse genome (three mismatches allowed), signal was detected in 11,243 sites (p < .02). Out of these, 3,449 target sites were hypomethylated in experimental conditions against the two control conditions (i.e., controls were WT cells treated with Tx or floxed cells treated with vehicle; See Supporting Information Table S1 for information about signal from all analyzed probes). Thus, 30% of all analyzed sites are hypomethylated (with false discovery rate, FDR < 0.2) and this percentage reduces to 25% after subtracting the estimated false positives in this selection. Consistently, these differentially methylated sites showed highly consistent correlation of fold change when comparing against either of the two control conditions (Supporting Information Fig. S6A). 3,560 mouse genes were associated to these methylation sites using the GREAT webserver (Supporting Information Table S1). Distribution analysis of differentially methylated sites along elements of gene structure surprisingly revealed that hypomethylation observed in the absence of UHRF1 was strongly underrepresented in the vicinity of transcription start sites (TSS). Conversely, this effect was reciprocal in exons and 3’UTR regions—where hypomethylation levels were enriched (i.e., higher percentage hypomethylation than genome average) (Fig. 6B, Supporting Information Table S1). In contrast, the hypomethylated sites were not over nor underrepresented as compared to all sites within structural areas of CpG sites (islands, shores and shelves, Supporting Information Fig. S6B).

Figure 5. UHRF1 controls G1- to S-phase transition. (A): DNA content analysis by flow cytometry of neural stem cells (NSCs) isolated from the anterior subventricular zone (aSVZ) of Rosa26CreERT2:UHRF1fl/ox and Rosa26CreERT2:UHRF1wt/wt mice treated with OH-Tx for 12 hours and cultured for 4 DiV. (B): Statistical analyses of cells gated to the different cell cycle phases. Note reduction of the total number of cells in S and G2-M phases while cells in G1 is increased in uhrf1-deficient NSCs (floxed OH-Tx group, n = 4; *, p < .05, ANOVA). (C): BrdU incorporation (S-phase cells) analysis in same paradigm as (A), shows lower numbers of BrdU incorporating cells in uhrf1-deficient NSC (floxed OH-Tx compared to the other three control groups. (D): Statistical analysis of cells gating to the different cell cycle phases (n = 4, *, p < .05 and **, p < .001, Student’s t test). (E): Cell cycle length analysis comparing Ts (Time in S-phase, hours), Tc (total cell cycle time, hours), and their relation in GFP+ cells of the aSVZ from GLASTCreERT2:GFPxUHRF1fl/ox and GLASTCreERT2:GFPxUHRF1fl/ox mice (n = 3, p-values are from Student’s t test). Arrow indicates GFP+ cells that are still in the cell cycle seen mostly in wt/flox background. Scale bars: (C): 20 μm. Quantifications show mean ± SEM. Abbreviation: UHRF1, ubiquitin-like, containing PHD and RING finger domains-1.

Rosa26CreERT2: UHRF1fl/ox and Rosa26CreERT2: UHRF1wt/wt mice that were Uhf1-deficient for 5 days were analyzed by the Illumina 450K Methylation arrays, which in human covers 485,000 methylation sites per sample. Out of 13,295 target sites, which were homologous to the mouse genome (three mismatches allowed), signal was detected in 11,243 sites (p < .02). Out of these, 3,449 target sites were hypomethylated in experimental conditions against the two control conditions (i.e., controls were WT cells treated with Tx or floxed cells treated with vehicle; See Supporting Information Table S1 for information about signal from all analyzed probes). Thus, 30% of all analyzed sites are hypomethylated (with false discovery rate, FDR < 0.2) and this percentage reduces to 25% after subtracting the estimated false positives in this selection. Consistently, these differentially methylated sites showed highly consistent correlation of fold change when comparing against either of the two control conditions (Supporting Information Fig. S6A). 3,560 mouse genes were associated to these methylation sites using the GREAT webserver (Supporting Information Table S1). Distribution analysis of differentially methylated sites along elements of gene structure surprisingly revealed that hypomethylation observed in the absence of UHRF1 was strongly underrepresented in the vicinity of transcription start sites (TSS). Conversely, this effect was reciprocal in exons and 3’UTR regions—where hypomethylation levels were enriched (i.e., higher percentage hypomethylation than genome average) (Fig. 6B, Supporting Information Table S1). In contrast, the hypomethylated sites were not over nor underrepresented as compared to all sites within structural areas of CpG sites (islands, shores and shelves, Supporting Information Fig. S6B,
Figure 6.

(A) EdU 10' Chase

(B) % of slices hypermethylated in Uhr1 WT

(C) All Sites: Diff. methylated sites

(D) All H1s (1946 genes)

(E) Cell Cycle (305 genes)

(F) G1/S transition (48 genes)

(G) Downregulated Upregulated

(H) Cell Cycle DNA replication (11 genes)

(I) Regulation of mitotic nuclear division genes (50 genes)

(J) Cell Cycle arrest (26 genes)

(K) ROSa26CreERT2 x UHRF1 flox 7days

(L) Rosa26CreERT2 x UHRF1 flox 7days

(M) Relative expression of Cdkn genes

(N) WT

(O) floxed

(P) Vehicle

(Q) OH-TX

(R) vehicle

(S) OH-TX

(T) Cdkn1a sh-A

Figure 6.
Supporting Information Table S1) showing that these elements are equally susceptible to hypomethylation. Furthermore, at a macro-scale level we did not find any strong bias in the distribution of hypomethylated loci across different regions of the genome (Fig. 6C) and consistently, Gene Ontology terms of hypomethylated genes were only weakly significant and enriched in genes associated at the organism and system level (multicellular organism development, system development and more, Supporting Information Table S1). Thus, UHRF1-deficiency leads to genome-wide hypomethylation with almost no distribution bias along the genome, but areas surrounding TSS were less vulnerable than coding exons and 3'UTR regions from a loss of UHRF1.

**Derepression of Cell Cycle Inhibitors in the Absence of Uhrf1**

Variable distribution of DNA methylation is a fundamental principle governing stable gene repression [49–51]. A deficit in DNA methylation maintenance is expected to result in hypomethylation-induced derepression of transcription. To examine if genes responsible for cell cycle progression and cell cycle arrest are among the affected genes we performed whole transcriptome RNA sequencing analysis of neurospheres from the aSVZ of Rosa26CreERT2: UHRF1 flox/flox and Rosa26CreERT2: UHRF1 wt/wt mice treated either with vehicle (EtOH) or OH-Tx resulting in four experimental groups (wtEtOH, floxedEtOH, floxedEtOH, n = 3 biological replicates). To obtain an overview and to assure data quality, we first calculated the Euclidian distance between all 12 samples of the different experimental groups and thereby confirmed unique patterns of gene expression changes in the floxed Uhrf1 cells treated with OH-Tx compared to either wt cells treated with OH-Tx or Uhrf1 floxed cells treated with EtOH (Supporting Information Fig. 5G). A total of 1,946 genes were differentially expressed upon ablation of Uhrf1 in NSCs, when comparing to either one of the two controls groups (wtOHtx or floxedEtOH) (Supporting Information Table S1; FDR (p.adjusted) < 0.1). Among these, 305 genes involved in the cell cycle (gene ontology GO:0007049) were regulated either up or down, consistent with the observed deficit in cell cycle progression, as many cell cycle genes are strictly regulated in a cell cycle-dependent way. Detailed analysis of 305 differentially regulated genes in the gene ontology categories “G1/S transition,” “cell cycle DNA replication” and “regulation of mitotic nuclear division” confirmed and reinforced this result displaying up to 90% of genes downregulated (Fig. 5D, see Supporting Information Table S1 for a complete list of the genes in each category). This result confirms the previous conclusion that loss of Uhrf1 results in a failure of transition from G1 to the replication phase and consequently, expression of genes associated with these cell cycle phases are downregulated as compared to the controls simply because cells do not enter the cell cycle without Uhrf1. In contrast, cell cycle arrest genes displayed markedly more upregulated genes (Fig. 5D). Examining the Gene Ontology terms enriched in the genes that are both hypomethylated and upregulated (294 genes), “regulation of cell proliferation” was among the significant GO terms (Supporting Information Table S1; pValue corrected <0.05), however this analysis did not help identifying key genes responsible for cell cycle arrest in the absence of Uhrf1, likely because only a fraction of the genes in the genome were included in the methylation array and furthermore, did not cover all methylation sites for each gene. To obtain further insight into possible direct roles of gene products we therefore instead examined the protein–protein interacting relationships of upregulated genes using the STRING database [52] (Fig. 6E). A single core cluster included the key regulators of G1/S transition cyclin-dependent kinase 4 (Cdk4), cyclin D1 (Ccn1d1) and the Cdk4 inhibitors cyclin-dependent kinase inhibitor 1A (p21, Cdkn1a), cyclin-dependent kinase inhibitor 2B (p15INK4b, Cdkn2b) and cyclin-dependent kinase inhibitor 2C (p18, Cdkn2c). While Cdk4 and Cdkn2c were downregulated, Ccn1d1, Cdkn1a and Cdkn2b were upregulated (Fig. 6E). Based on protein function and direction of regulation by Uhrf1-deficiency, Cdkn1a and Cdkn2b appeared as possible mediators determining cell cycle arrest, because they are potent cyclin-dependent kinase inhibitors preventing G1-phase cell cycle progression [53, 54] and display increased expression, thus, consistent with a derepression-induced cell cycle arrest. We next examined expression levels of these cell cycle repressor genes in the RNA sequencing data by comparing expression of Uhrf1 floxed NSCs treated with OH-Tx with vehicle. In addition to Cdkn1a and Cdkn2b, we also examined Cdkn1 family members Cdkn1b (p27Kip1) and Cdkn1c (p57Kip2). Cdkn1a was the most abundantly expressed, and in addition, displayed an Uhrf1-dependent derepression in Uhrf1-deficient cells (Fig. 6F). To confirm these in vitro results we next analyzed the in vivo levels in microdissected SVZ from Rosa26CreERT2: Uhrf1flox/flox mice by RT-qPCR (Fig. 6G). Nonsignificant changes were observed except for Cdkn1a, which displayed a marked eightfold increase. Thus UHRF1 is critical for repression of Cdkn1a in NSCs and in its absence, Cdkn1a is derepressed. To examine if derepression was associated with hypomethylation of the Cdkn1a promoter we therefore ablated Uhrf1 in NSCs.
isolated from Rosa26CreERT2: UHRF1^flow/fox mice and subjected DNA from these and control cells to bisulphite sequencing. The CpG island located in the distal Cdkn1a promoter was markedly hypomethylated in KO, but not in any of the control NSCs (Fig. 6H). Because p21 (encoded by Cdkn1a) is a well-characterized cell cycle regulator that acts as a break for S-phase entry [55] its deregulation could represent the mechanism by which Uhrf1-deficiency causes S-phase entry failure. To explore if the dererepression of Cdkn1a caused the cell cycle arrest, we treated NSCs isolated from Rosa26CreERT2: UHRF1^flow/fox and control NSCs in vitro with OH-Tx or vehicle (EtOH), followed by treatment of the cells with short hairpin RNA (shRNAs) targeting Cdkn1a and analyzed proliferation. Knockdown of Cdkn1a which diminished Cdkn1a expression to less than half that of scrambled shRNAs (Supporting Information Fig. S6D), fully reversed the Uhrf1-dependent arrest in G1 and thus, restored the G1-S transition, as indicated by a normalization of EdU incorporation (Fig. 6I, Supporting Information Fig. S6E). From these data, we conclude that Uhrf1-dependent repression of Cdkn1a is critical for cell cycle re-entry and maintenance of NSCs in the adult niche.

DISCUSSION

The discovery that UHRF1 is a critical scaffold which links DNA and histone methylation during replication [23, 56] has prompted intensive research on its biochemical properties and functional roles. Here, we have addressed the physiological role of UHRF1 in adult brain SVZ NSCs. While a deficiency of UHRF1 only in GLAST^- cells do not compromise survival of the animals, the deletion of UHRF1 in all adult tissues in Rosa26 driver mice led to marked loss of health and death within weeks. In the brain, we find that Uhrf1 is critical for NSC self-renewal and neural progenitor cell proliferation and in the absence of Uhrf1, maintenance of NSC numbers in the niche is compromised, leading to a near complete loss of neurogenesis without affecting senescence or survival of neural progenitors.

In the normal adult brain, Uhrf1 is dynamically expressed in neural progenitor cells (summarized in Supporting Information Fig. S7). Within each cell type we localize Uhrf1 to the actively dividing cells and hence, Uhrf1 is expressed in markedly different proportions with 82% of Mash1^- cells expressing UHRF1 and obtained compelling results. We find that Ki67 which labels proliferative and recognition loops that interact with both grooves containing the methylated cytosine and the unmethylated cytosine that is everted from the double helix. However, Uhrf1 which recruits DNMT1 is the actual protein that “recognizes” hemimethylated sites through its SRA domain [9–12] and in addition, can also be recruited to replicating DNA through the replication machinery [62] In concordance, Uhrf1 has been observed by immunohistochemistry in...
replication foci [63]. By using iPOND, we place UHRF1 within the replication fork itself and furthermore show that UHRF1-deficient neural progenitors display whole genome hypomethylation illustrating its importance for maintenance methylation. Intriguingly, areas around TSS appear relatively resilient to hypomethylation while 3’UTR and gene body regions are more susceptible. Hence, either DNMTs act at least partly independent of UHRF1 specifically at promoter areas or hemimethylated DNA around TSS are more prone to de novo methylation, a possibility supported by the targeting of de novo DNMTs to TSS by transcription factor complexes [64]. Nevertheless, our results suggest that both hyper-methylated DNA as observed in Tet1 null mice and hypomethylated DNA as shown in the present study may be incompatible with neurogenesis in the adult brain.

One mechanism through which various states of DNA methylation can affect proliferation is by transcription. Consistently, UHRF1 can in this way regulate cell cycle progression via effects on expression of pRB and G1 phase cell cycle inhibitory protein expression in cultured COS-7 and HeLa cells [31]. via effects on p21 in T cells [26] and in bronchial epithelial stem-like cells at least partially through the p15 pathway [24]. Analysis of the transcriptome of normal and Uhrf1-deficient NSCs revealed significant changes in the transcriptome. Consistent with that NSCs arrest in the cell cycle unless UHRF1 is present, Uhrf1-deficient cells displayed a marked reduction of gene expression of genes participating in cell cycle progression. Thus, many cell cycle-related genes were downregulated because Uhrf1-deficient cells do not enter the cell cycle and therefore do not upregulate genes associated with the different cell cycle phases. In contrast, genes associated with cell cycle arrest were more enriched with upregulated genes. Among these, Cdkn1a was identified as a target of UHRF1 in vivo in NSCs. Intriguingly, loss of function studies has shown that p21 plays an important role determining proliferation in stem cell populations. In both hematopoietic stem cells and NSCs, p21-deficiency causes an acceleration of the rate of proliferation, which eventually becomes exhausted in aged animals [65–67]. Here, we find that Uhrf1-deficiency results in hypomethylation of the distal Cdkn1a promoter and a derepression of expression, and as a consequence a failure of S-phase entry of NSCs. Consistently, normalizing Cdkn1a levels in Uhrf1-deficient NSCs by knockdown, rescues proliferation. Thus, onset of UHRF1 expression is critical for G1- to S-phase transition in active NSCs and the mechanism by which UHRF1 controls S-phase entry is through suppression of Cdkn1a expression. This UHRF1-p21 axis contributes to the overall rate of NSC proliferation, neuronal output as well as stem cell homeostasis.

CONCLUSION

The present study demonstrates the importance of the epigenetic regulatory UHRF1 in controlling gene-regulation essential for renewal of adult NSCs. The functional axis involves a UHRF1-dependent repression of Cdkn1a expression in actively dividing progenitors, which is a prerequisite for S-phase entry and self-renewal. This mechanism has profound effects on stem cell numbers and the generation of new neurons in the adult brain.

ACKNOWLEDGMENTS

We thank Helena Samuelsson for technical support, the CLICK Imaging Facility supported by the Wallenberg Foundation and Ruani Fernando for her helpful comments regarding experimental design and manuscript writing. We thank Dr. Francois Lallemand for kindly provide the Rosa26CreERT2 mice and Dr. Magdalena Götz for sharing the GLAST-CreERT2;CAG-GFP mice. We would like to acknowledge support from Science for Life Laboratory, the National Genomics Infrastructure, NGI, and Uppmax for providing assistance in massive parallel sequencing and computational infrastructure. This work was supported by the Swedish Research Council for Medicine and Health, the Swedish Cancer Society and SFO grants, the Swedish Brain Foundation, Wallenberg Scholar, Söderberg Foundation and ERC advanced grant (740491) to P. E. This author (S.A.) is currently affiliated to Centre for Genomic Regulation Dr. Aiguader, Barcelona, Spain.

AUTHOR CONTRIBUTIONS

A.B. and A.V.N.: study design, performed experiments, data analyses, manuscript preparation and editing; N.A.L., D.U., S.A., and J.S.: performed experiments, data analyses; P.E.: study design, data analyses, manuscript preparation and editing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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