Activation of Neuronal Gene Expression by the JMJD3 Demethylase Is Required for Postnatal and Adult Brain Neurogenesis

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SUMMARY

The epigenetic mechanisms that enable lifelong neurogenesis from neural stem cells (NSCs) in the adult mammalian brain are poorly understood. Here, we show that JMJD3, a histone H3 lysine 27 (H3K27) demethylase, acts as a critical activator of neurogenesis from adult subventricular zone (SVZ) NSCs. JMJD3 is upregulated in neuroblasts, and Jmjd3 deletion targeted to SVZ NSCs in both developing and adult mice impairs neuronal differentiation. JMJD3 regulates neurogenic gene expression via interaction at not only promoter regions but also neurogenic enhancer elements. JMJD3 localizes at neural enhancers genome-wide in embryonic brain, and in SVZ NSCs, JMJD3 regulates the I12b enhancer of Dlx2. In Jmjd3-deleted SVZ cells, I12b remains enriched with H3K27me3 and Dlx2-dependent neurogenesis fails. These findings support a model in which JMJD3 and the poised state of key transcriptional regulatory elements comprise an epigenetic mechanism that enables the activation of neurogenic gene expression in adult NSCs throughout life.

INTRODUCTION

Epigenetic regulation via histone methylation is critical for the establishment and maintenance of lineage-specific gene expression. The trimethylation of histone 3 at lysine 27 (H3K27me3) by Polycomb repressive complex 2 (PRC2) correlates with transcriptional repression (Margueron and Reinberg, 2011), and such gene repression is critical for the proper function of stem cells in both the embryonic and adult brain (Hirabayashi and Gotoh, 2010). While previous studies have highlighted the importance of H3K27me3 placement and gene repression in neural development (Hirabayashi et al., 2009; Hwang et al., 2014; Pereira et al., 2010), how H3K27me3 is removed for the activation of neurogenic gene expression is not well understood. JMJD3 (KDM6B) is an H3K27me3-specific demethylase that belongs to the family of JmjC-domain-containing proteins (Agger et al., 2007; De Santa et al., 2007). Jmjd3 null mice die at birth due to respiratory failure (Burgold et al., 2012; Satoh et al., 2010). In the embryonic mouse forebrain, Jmjd3 expression is regulated (Jepsen et al., 2007), and in embryonic stem cells (ESCs), Jmjd3 is required for neural commitment (Burgold et al., 2008). While knockdown studies in the embryonic spinal cord and retina indicate developmental roles for Jmjd3 (Akizu et al., 2010; Iida et al., 2014), the function of Jmjd3 in adult neural stem cells (NSCs) has not been reported.

The adult mammalian brain harbors NSCs in the subventricular zone (SVZ) and dentate gyrus of the hippocampus (Fuentesalba et al., 2012; Ming and Song, 2011). The epigenetic mechanisms required to maintain lifelong neurogenesis in these germinal zones are only beginning to be elucidated (Gonzales-Roybal and Lim, 2013: Ma et al., 2010). Emerging evidence indicates that dynamic changes of chromatin modifications at transcriptional enhancers are a strong determinant of gene expression (Calo and Wysocka, 2013). Like promoters, the activity of enhancers can be “poised” by the presence of repressive H3K27me3 (Rada-Iglesias et al., 2011), suggesting that transcription can be activated by the action of H3K27me3-specific...
JMJD3 is Required for Postnatal OB Neurogenesis

To study the role of JMJD3 in SVZ-OB neurogenesis, we used a conditional knockout allele of Jmjd3 in SVZ NSC precursors at embryonic day 13.5 (E13.5) (Lim et al., 2009), and SVZ cells of hGFAP-Cre; Jmjd3F/F mice were Jmjd3 negative (Figure S1J). hGFAP-Cre; Jmjd3F/F mice and their littermate controls (wild-type and hGFAP-Cre; Jmjd3F/F) were born at the expected Mendelian ratios and were similar in size, weight, and overall survival. To assess OB neurogenesis, we injected postnatal day 30 (P30) mice with 5-bromo-2-deoxyuridine (BrDU) to label a cohort of newly born SVZ neuroblasts and analyzed the OB for BrdU+ neurons 10 days later. In the hGFAP-Cre; Jmjd3F/F OBs, there were approximately 50% fewer BrdU+, NeuN+ neurons (Figures 1A–1C), which was not likely related to changes in neuronal survival, as the number of activated caspase-3+ OB cells was not increased (Figure 1D).

To evaluate the production of neuroblasts in the SVZ, we administered the thymidine analog ethynyl deoxyuridine (EdU) to mice 1 hr before being culled. In P40 hGFAP-Cre; Jmjd3F/F mice, there were 2- to 3-fold fewer DCX+, EdU+ cells in the SVZ (Figures 1H–1I). Furthermore, the expression of DLX2, a key neurogenic transcription factor, was strongly reduced (Figures 1K and 1L). Despite being fewer EdU+ cells in hGFAP-Cre; Jmjd3F/F mice, the dorsal SVZ was abnormally expanded with DCX+ cells (Figures 1F–1G and S2D–S2F). Defective neuroblast migration can result in the postnatal accumulation of DCX+ cells in the SVZ (Lim et al., 2009); in hGFAP-Cre; Jmjd3F/F mice, the neuroblast migratory pathways were highly disorganized (Figures S2K and S2L), and many SVZ cells pulse-labeled with BrdU failed to migrate from the SVZ (Figures S2G–S2I). Thus, in hGFAP-Cre; Jmjd3F/F mice, the addition of new neurons to the OB was abrogated by a decrease in SVZ neurogenesis as well as abnormal neuroblast migration.

Adult SVZ NSCs (type B1 cells) contact the ventricle with a specialized apical surface located at the center of a pinwheel-like structure composed of ependymal cells (Mirzadeh et al., 2008). Interestingly, adult hGFAP-Cre; Jmjd3F/F mice had 3- to 4-fold more SVZ cells with such ventricular contact (Figures S2M–S2O). As is characteristic of type B1 cells, these apical surfaces had solitary basal bodies and were GFAP+ (Figures S2P–S2S). This accumulation of type B1-like cells was evident by P7 (Figures S3A–S3L) and not related to cell proliferation (Figures S3M–S3R). Thus, although hGFAP-Cre; Jmjd3F/F mice had greater numbers of cells with SVZ NSC characteristics, the production of neuroblasts was reduced, suggesting that the ventricle-contacting SVZ cells in hGFAP-Cre; Jmjd3F/F mice are defective for neurogenesis.

Jmjd3 Deletion Targeted to Adult SVZ NSCs Inhibits OB Neurogenesis

To test whether Jmjd3 plays a role in adult neurogenesis independent of its potential function in postnatal SVZ NSC development, we targeted Jmjd3-deletion to SVZ NSCs in adult mice (Figures 1L–1M). Stereotactic injection of the Ad:GFAP-Cre adenovirus into the SVZ induces Cre-mediated recombination in GFAP+ NSCs (Mirzadeh et al., 2008). We injected Ad:GFAP-Cre into the SVZ of P60–90 Jmjd3F/F mice or littermate controls (Jmjd3F/F) that carry the Ai14-tdTomato Cre-reporter transgene. To control for experimental variation related to the stereotactic injection itself, the Ad:GFAP-Cre adenovirus was mixed and coinjected with a lentivirus that constitutively expresses only GFP (lenti-GFP).

We quantified the number of tdTomato+ OB neurons in Jmjd3F/F, Ai14 and control; Ai14 mice 14 days after injection (Figures 1N–1P). For each mouse, the number of tdTomato+ OB neurons was normalized to the number of GFP+ OB neurons. In control; Ai14 mice, there were 5.5 tdTomato+ cells per 10 GFP+ cells in the OB. In contrast, there were only 2.0 tdTomato+ cells per 10 GFP+ cells in the OB of Jmjd3F/F; Ai14 mice. Thus, Jmjd3-deleted SVZ NSCs produced 60% fewer OB neurons than NSCs in control mice. Furthermore, in the SVZ of Jmjd3F/F mice, there were 24% fewer DLX2+, tdTomato+ cells (Figures S2T–S2W). Taken together, these results indicate that adult NSCs require Jmjd3 for SVZ-OB neurogenesis.

Jmjd3 Regulates the Differentiation of SVZ NSCs

We next used SVZ NSCs monolayer cultures to study Jmjd3 function. During differentiation, SVZ NSCs upregulated Jmjd3, correlating with increased Dlx2 expression (Figures S4A–S4G). Short-hairpin RNA (shRNA) Jmjd3-knockdown lentiviruses (LV-DK1-GFP and LV-DK2-GFP) strongly reduced Jmjd3. Jmjd3 knockdown reduced Dlx2 expression, but not the expression of proneural Mash1, PRC2 component Ezh2, or Utx (Kdm6a), the other known H3K27me3-specific demethylase (Figures 2A and 2B).

To target shRNA knockdown to GFAP+ SVZ NSCs, we used an EnvA-pseudotyped lentivirus and tva receptor transgenic mouse strategy (Holland et al., 1998; Lewis et al., 2001) (Figure 2A). In self-renewal conditions, Jmjd3 knockdown in
Figure 1. Jmjd3 Is Required for Adult OB Neurogenesis

(A) Illustration of the experimental design for (B)–(D). The blue box indicates regions of shown in (B) and (B'). GCL, granule cell layer.
(B) Analysis of OB neurogenesis. Immunohistochemistry (IHC) for BrdU (green) and NeuN (red) in coronal OB sections of control (B) and hGFAP-Cre;Jmjd3<sup>F/F</sup> mice (B').
(C) Quantification of BrdU+, NeuN+ OB neurons (**p < 0.001; n = 4 each; error bars represent SEM).
(D) Quantification of cleaved caspase-3+ OB cells (p = 0.1933; n = 4 each; error bars represent SEM).
(E) Illustration of the experimental design for (F)–(I). EdU was injected 1 hr before analysis.
(F–I) Analysis of cell proliferation and neuroblasts in the SVZ.
(F–H) DAPI+ (white), DCX+ (green), and EdU+ (white) cells in the SVZ of control mice. (F'–H') DAPI+ (white), DCX+ (green), and EdU+ (white) cells in the SVZ of hGFAP-Cre;Jmjd3<sup>F/F</sup> mice.
(I) Quantification of EdU+, DCX+ cells in SVZ coronal sections (n = 3 each; error bars represent SEM; **p < 0.01).
(J–K) Analysis of DLX2 expression in the SVZ. IHC for DLX2 (red) in SVZ coronal sections of control (J–K) and hGFAP-Cre;Jmjd3<sup>F/F</sup> mice (J'–K'). (K) and (K') are higher-magnification views of the yellow boxed region in (J) and (J').
(L) Schematic illustration of the experimental design for (M)–(P). Ad:GFAP-Cre virus (to delete floxed alleles in GFAP+ SVZ NSCs) and GFP lentivirus (injection control) were coinjected into the adult SVZ of tdTomato;Jmjd3<sup>F+</sup> (M–O) or tdTomato;Jmjd3<sup>FF</sup> (M'–O') mice.
(M–P) Analysis of OB neurogenesis. IHC for GFP (green) and tdTomato (red) in adult SVZ coronal sections of tdTomato;Jmjd3<sup>F+</sup> (M) and tdTomato;Jmjd3<sup>FF</sup> mice (M').
(N–O) IHC for GFP (green) and tdTomato (red) in adult OB sections of tdTomato;Jmjd3<sup>F+</sup> (N and O) and tdTomato;Jmjd3<sup>FF</sup> mice (N' and O') 14 days after injection.
(P) Quantification of tdTomato+ neurons per ten GFP+ neurons in the OB (**p < 0.0001; n = 4 per group; error bars represent SEM). LV, lateral ventricle; Str, striatum; GCL, granule cell layer.
GFAP+ SVZ NSCs did not affect BrdU incorporation or cell viability and the expression of NSC marker Sox2 and cell-cycle inhibitor p16 was unaffected, suggesting that NSCs self-renewal was not impaired (Figures S4H, S4I, and S4M). After 2 days of differentiation, there were approximately 50% fewer DLX2+ cells with Jmjd3 knockdown, and the production of Tuj1+ neurons after 4 days was reduced by nearly 5-fold (Figures 2C–2G). While Jmjd3 knockdown did not reduce the number of GFAP+ astrocytes, the production of OLIG2+ cells and O4+ oligodendrocytes was impaired (Figures S4J–S4L). Thus, Jmjd3 is required for proper SVZ NSC differentiation.

The cis-regulatory regions of Jmjd3 harbor retinoic acid (RA) receptor response elements (Jepsen et al., 2007), and RA regulates postnatal SVZ neurogenesis in vivo (Wang et al., 2005). The addition of RA to SVZ NSCs increased Jmjd3 expression by 2- to 3-fold, and Dlx2 was also induced 8- to 9-fold (Figure 2H). Furthermore, transient transfection of a Jmjd3 expression vector, but not a construct in which the catalytic JmjC domain is inactivated (Jmjd3-Mut) (Burgold et al., 2008), increased the expression of both Dcx and Dlx2 (Figure 2I). Thus, in SVZ NSCs, Jmjd3 can be upregulated by RA, and increased levels of Jmjd3 induce neurogenic gene expression.

Dlx2 Overexpression Rescues Neurogenesis from Jmjd3-Deficient SVZ NSCs

To investigate whether Dlx2 is a key developmental factor for Jmjd3-dependent neurogenesis, we enforced Dlx2 expression in Jmjd3-deficient SVZ cells. GFAPp-tva SVZ cultures were first infected with LV(EnvA)-shRNA-GFP constructs. After 2 days, cultures were infected with lentiviruses that express Dlx2 (LV-Dlx2-dsRed) or control (LV-AP-dsRed) (Figure 2J). Remarkably, LV-Dlx2-dsRed rescued neuronal differentiation from Jmjd3-deficient cells (Figures 2K–2M). Furthermore, injection of LV-Dlx2-GFP into the SVZ hGFAP-Cre;Jmjd3F/F mice increased the number of DCX+ cells (Figures S4N–S4R). Taken together, these results suggest that the activation of Dlx2 is a key aspect of Jmjd3-dependent neurogenesis.

JMJD3 Is Required for Removal of H3K27me3 at Key Transcriptional Promoters in SVZ NSCs

To gain broader insight into Jmjd3-dependent gene expression, we performed microarray analysis. Treatment of SVZ

(F) ICC for GFP (green) and Tuj1 (red) with LV-sh-Jmjd3 (F) and LV-sh-luciferase (F).
(G) Quantification of Tuj1+ cells (error bars represent SEM of quadruplicate cultures; *p < 0.05, ***p < 0.001).
(H) qRT-PCR analysis of retinoic acid treatment of SVZ NSCs (***p < 0.001; *p < 0.05; n = 6 per group; error bars represent SEM).
(I) qRT-PCR analysis of Jmjd3 overexpression in SVZ NSCs (***p < 0.001; *p < 0.05; n = 3; error bars represent SEM).
(J) Illustration of the experimental design for (K)–(M).
(K–M) Analysis of neuronal differentiation in Jmjd3-deficient cells after Dlx2 overexpression.

(K–L) IHC for Tuj1 (white) in Jmjd3-deficient cells with Dlx2 overexpression (yellow in K, corresponding white cells in L) and control vector expression (yellow in K, corresponding white cells in L).
(M) Quantification of Dlx2 rescue of neurogenesis (error bars represent SEM of quadruplicate cultures; **p < 0.01).
NSCs from UBC-Cre/ERT2; Jmjd3<sup>F/F</sup> mice with 4-OHT resulted in Jmjd3 deletion, and neurogenesis was severely impaired (Figures 3A, 3B, and S5A). In nondeleted cultures, 1,050 genes were upregulated after 30 hr of differentiation (>1.5-fold; false discovery rate [FDR]-corrected < 0.05; n = 3). In differentiating Jmjd3-deleted cells, 18 of these genes, including Dlx2, failed to reach the same levels of expression (>1.5-fold decreased as compared to control cells; FDR-corrected < 0.05; n = 3; Figure 3C). Analysis of chromatin immunoprecipitation sequencing (ChIP-seq) data (Ramos et al., 2013) indicated that 12 of the 18 genes were enriched for H3K27me3 in undifferentiated SVZ NSCs (Figure 3D).
suggesting that removal of this repressive mark is required for their upregulation.

In differentiating SVZ cells, the increased expression of Myt1, Slc32a1, and Gjb6—genes involved in neurogenesis—was strongly Jmjd3 dependent (Figure 3E). In undifferentiated NSCs, these gene promoters were enriched for H3K27me3, and in control cells, H3K27me3 levels were reduced during differentiation (Figure 3F), and JMJD3 protein was detected at these promoters (Figure 3G). In contrast, in Jmjd3-deleted cells, H3K27me3 levels were not decreased with differentiation (Figure 3F). Thus, JMJD3 is required for H3K27 demethylation at specific gene promoters during SVZ NSC differentiation.

The promoter region upstream of the Dlx2 transcriptional start site (TSS) contains regulatory elements (Ghanem et al., 2012). Interestingly, in undifferentiated SVZ NSCs, the TSS and proximal promoter was not enriched with H3K27me3 (Figure S5B). Furthermore, this low level of H3K27me3 did not change during differentiation, indicating that H3K27me3 levels at the promoter do not correlate with Dlx2 upregulation.

**JMJD3 Is Enriched at Neural Transcriptional Enhancers**

Some transcriptional enhancers in stem cells exist in a poised state, which includes H3K27me3 enrichment. To investigate whether JMJD3 localizes at enhancers in neural development, we analyzed JMJD3 ChIP-seq data from NSCs derived from E12.5 mouse cortex (Estarás et al., 2012) in conjunction with p300 localization in E11.5 cortex (Visel et al., 2013). Of the 4,425 potential enhancer regions bound by p300, 2,611 (59%) exhibited JMJD3 enrichment (Figures 4A and 4C). These JMJD3-enriched enhancers correlated with brain development (McLean et al., 2010), including differentiation toward neuronal lineages (Figure 4B). Together, these data suggest that JMJD3 can regulate the chromatin state of neural-specific enhancers genome-wide. We therefore hypothesized that JMJD3 regulates Dlx2 expression in SVZ NSCs through interactions at a key enhancer.

**JMJD3 Is Required for H3K27me3 Demethylation of a Dlx2 Enhancer**

I12b is an enhancer that regulates the expression of Dlx2 (Poitras et al., 2007) (Figure 5A). In SVZ NSC cultures, I12b had a poised chromatin state, which included high levels of H3K27me3 (Figures S5C and S5D). To analyze I12b chromatin state changes in the SVZ neurogenic lineage, we used fluorescence-activated cell sorting (FACS) to isolate GFAP+, NESTIN+ SVZ NSCs in self-renewal conditions and Tuj1+ neuroblasts 3 days after differentiation (Figures 5B–5E). In NSCs, I12b was enriched with H3K27me3. In the neuroblasts, H3K27me3 was decreased ~12-fold (Figure 5C). This reduction in H3K27me3 correlated with JMJD3 enrichment at I12b (Figure 5D). Furthermore, in neuroblasts, H3K27 acetylation (H3K27ac) was increased (Figure 5E). Thus, JMJD3 enrichment at I12b correlated with the chromatin state activation of this Dlx2 enhancer. Another Dlx1/2 enhancer, URE2, had lower levels of H3K27me3 that did not decrease during neuronal differentiation (Figures S5C and S5E). We therefore focused our analysis upon I12b.

We next studied SVZ NSCs with and without acute Jmjd3 deletion. During differentiation, H3K27me3 at I12b was...
decreased in control cells. In contrast, in Jmjd3-deleted cells, H3K27me3 did not decrease at the I12b enhancer (Figures 5F and 5F'), correlating with the lack of Dlx2 upregulation (Figure 3C). Thus, Jmjd3 is required for H3K27 demethylation at the I12b enhancer.

In differentiating Mll1-deficient SVZ cells, the Dlx2 locus is enriched with H3K27me3 and its transcription remains repressed (Lim et al., 2009). We therefore investigated a potential molecular-genetic relationship between Mll1 and Jmjd3 in the regulation of Dlx2. In differentiating SVZ cells, MLL1 was localized at both I12b and the Dlx2 promoter (Figure 5H). Interestingly, in Mll1-deleted NSCs, Jmjd3 was not enriched at I12b (Figure 5I) and Dlx2 is not properly expressed. However, in Jmjd3-deleted cells, MLL1 remained enriched at I12b (Figure 5J). Taken together, these data indicate that Mll1 is required for the enrichment of Jmjd3 at I12b.

While multiple studies have indicated the critical nature of Polycomb-mediated transcriptional repression in neural development (Hirabayashi and Gotoh, 2010; Hwang et al., 2014), the role of active H3K27me3 demethylation has been less clear. How do H3K27me3-repressed genes normally become activated? While loss of H3K27me3 at specific loci could potentially be passive (e.g., through decreased EZH2 activity), EZH2 expression is not downregulated during SVZ neurogenesis (Hwang et al., 2014). In this study, we show that active H3K27me3 demethylation via Jmjd3 is required for efficient expression of lineage-specific genes.

Without Jmjd3, SVZ NSCs appear to be “stalled” in a precursor cell state. Jmjd3-deficient SVZ NSCs did not efficiently activate Dlx2 expression and were defective for neurogenesis. However, Jmjd3 did not appear to be required for NSC identity, suggesting that Jmjd3 does not play a key role in SVZ NSC maintenance. Interestingly, adult hGFAP-Cre;Jmjd3F/F mice had...
greater numbers of cells with type B1 cell characteristics. Given that we did not observe increased cell proliferation with Jmjd3 deficiency, it is possible that the accumulation of type B1-like cells in hGFAP-Cre;Jmjd3<sup>F/F</sup> mice relates to an increase in the proportion of symmetric, self-renewing divisions, as compared to asymmetric NSC divisions that lead to the generation of differentiated cell types.

In the developing neocortex (Estarás et al., 2012; Visel et al., 2013), we found that 59% of the mapped enhancers exhibited Jmjd3 enrichment. Notably, these enhancers corresponded strongly to neuron differentiation, forebrain development, regulation of neurogenesis, and other activities consistent with NSC differentiation and cortical development. These data support a role for Jmjd3 at neural enhancers genome-wide in neural precursor cells, including those in the developing brain.

The requirement of Jmjd3 for H3K27me3 demethylation at the I12b enhancer does not diminish the function of Jmjd3 at gene promoter regions. Indeed, Jmjd3 enrichment has been mapped to 7,170 promoter regions in NSCs cultured from E12.5 cortex (Estarás et al., 2012), and Jmjd3-dependent H3K27me3 demethylation occurs at a number of different neural gene promoters (Akizu et al., 2010; Burgold et al., 2008; Iida et al., 2014). In this study, we found that the promoter regions of Myt1, Slc32a1, and Gnb6 required Jmjd3 for H3K27me3 demethylation and transcriptional activation. Taken together, these data suggest that Jmjd3 acts at both promoters and enhancers in NSC populations.

Although UTX (KDM6A)—the other known H3K27me3-specific demethylase (Lan et al., 2007)—is required to activate gene expression in the developing heart, Utx null ESCs can efficiently differentiate into neurons, suggesting that UTX is not necessary for neuronal differentiation per se (Lee et al., 2012). In SVZ NSCs, Jmjd3 deficiency did not affect the expression of Utx, indicating that Utx alone is not sufficient to activate Dlk2 expression, and RNAi knockdown of Utx in SVZ cells did not inhibit neurogenesis (data not shown). Thus, different developmental lineages may utilize UTX and Jmjd3 in a nonredundant manner.

How Jmjd3 becomes localized at specific genomic regions is not well known, but emerging evidence indicates that transcription factors such as SMAD3 and HES1 are involved (Dai et al., 2010; Estarás et al., 2012). Jmjd3 has also been found in tri-thorax group (trxG) chromatin remodeling complexes (De Santa et al., 2007), and trxG family member Mll1 is required for SVZ neurogenesis (Lim et al., 2009). In SVZ cells, we found both Mll1 and Jmjd3 proteins localized to I12b, and Jmjd3 enrichment at this enhancer was lost in Mll1-deleted cells. While these data indicate that Jmjd3 requires Mll1 for localization to a key neurogenic enhancer, it remains to be determined whether Mll1 and Jmjd3 physically interact at I12b. Alternatively, Mll1 may be required to mediate local chromatin modifications that enable the subsequent recruitment of Jmjd3 to specific genomic regions. The discovery of this genetic interaction may provide insights into how mutations in MLL genes and H3K27me3-specific demethylases contribute to the transcriptional dysregulation of medulloblastomas (Jones et al., 2012; Parsons et al., 2011).

Finally, our studies establish Jmjd3 as a central player for the activation of neurogenesis from a neural stem cell population, which may help inform methods of neuronal production for therapeutic purposes.

**EXPERIMENTAL PROCEDURES**

**Generation of Jmjd3 Conditional Knockout Mice and Mouse Studies**

Jmjd3<sup>F/F</sup> mice were generated, maintained, and genotyped as described previously (Iwamori et al., 2013). BrdU (50 μg/g body weight, Sigma) or EdU (10<sup>−2</sup> μmol/g body weight, Invitrogen) was injected intraperitoneally. Stereotactic SVZ injections were performed essentially as described previously (Mizadeh et al., 2008). Briefly, for adult Jmjd3-deletion studies, we injected 100 nl of adenovirus (AdiGFAP-Cre) and LV-GFP lentivirus. For Dlk2 overexpression, we injected 100 nl of LV-Dlk2-GFP and LV-Ap-DsRed into the SVZ. Stereotactic SVZ coordinates were 0.5 mm anterior, 1.3 mm lateral (relative to bregma), and 1.76 mm deep to the pia. Brains were fixed by intracardiac perfusion and sectioned on a cryostat (Leica) at 12 or 16 μm thickness. After blocking, primary antibodies were incubated at 4°C overnight. Antibody dilutions are listed in the Supplemental Experimental Procedures. Whole-mount dissection and immunostaining was performed as described elsewhere (Mizadeh et al., 2008). From each animal, we analyzed at least three separate tissue sections, and from each section, we collected more than three nonoverlapping confocal images (Leica TCS SP5X) with 20× or 63× oil objectives for quantification (ImageJ, NIH). Experiments were performed in accordance to protocols approved by Institutional Animal Care and Use Committee at University of California, San Francisco.

**Cell-Culture Studies**

SVZ NSC monolayer cultures were generated and analyzed as in Lim et al. (2009). High-titer pSicoR lentiviruses were produced in human embryonic kidney 293T cells as described previously (Lewis et al., 2001). For Jmjd3 knockdown, we derived SVZ NSCs from transgenic mice that express the tva receptor under the control of the GFAP promoter (GFAPp-tva) (Holland et al., 1999). To delete Jmjd3 in SVZ NSCs, cultures from UBC-Cre/Ert<sup>2</sup>;Jmjd3<sup>F/F</sup> mice were treated with 4-OHT (50 nM, Sigma) for 96 hr. For RA treatment and Jmjd3 overexpression, SVZ NSC monolayer cultures were established as described previously (De Santa et al., 2008). For Jmjd3 overexpression, pCDNA-Flag-Jmjd3 and pCDNA-Flag Jmjd3 mutant (H1388A) (Burgold et al., 2008) were transfected using JetPRIME (Polyplus) and analyzed after 48 hr. For each culture well, we captured more than 13 nonoverlapping fields (Leica DMi4000B) with a 20× objective and analyzed them with ImageJ (NIH). Primary and secondary antibodies are listed in the Supplemental Experimental Procedures. SYTOX red (Invitrogen) was used per the manufacturer’s protocol and quantified on a fluorescent flow cytometer (BD FACSaria).

**ChIP, qRT-PCR, Microarray, and Bioinformatic Analysis**

Quantitative ChIP (qChIP) and quantitative RT-PCR was performed essentially as previously described (Lim et al., 2009). qChIP analysis of FACS-isolated cells was performed as described previously (Hwang et al., 2014). ChIP antibodies and primer sequences and other methodological details are in the Supplemental Experimental Procedures and Table S1. For microarray analysis, biological replicates were prepared as in (Ramos et al., 2013) and hybridized to MouseRef-8 v2.0 Expression BeadChip arrays (Illumina). Array data were normalized with IlluminaNormalizer v.2 and analyzed with Cyber-T (http://cybert.ics.uci.edu). Gene Ontology analysis was performed with DAVID. SVZ RNA-seq and SVZ NSC ChIP-seq data were analyzed as in Ramos et al. (2013). ChIP-seq data from Gene Expression Omnibus (GEO) series GSE36673, GSE42881, and GSE13845 were analyzed as follows: FASTQ reads were aligned to mm9 using Bowtie2 2.1.0 with default parameters. Tracks and peaks were generated using MACS 1.4 with default parameters. Co-occupancy analysis was performed using BEDTools against published peaks. Genomic Regions Enrichment of Annotations Tool (GREAT) analysis was performed as described previously (McLean et al., 2013). Analysis parameters and references are in the Supplemental Experimental Procedures.
ACCESSION NUMBERS

The NCBI Gene Expression Omnibus accession number for the microarray analysis reported in this paper is GSE59888.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.07.060.

AUTHOR CONTRIBUTIONS

D.H.P. and D.A.L. conceived of the project, designed experiments, analyzed data, and wrote the manuscript. D.H.P., S.J.H., R.D.S., S.J.L., and S.W.S. performed experiments and contributed to statistical analysis. J.S. and G.T. performed preliminary ChIP experiments and contributed antibodies. M.M.M. and N.I. contributed transgenic mice. All authors helped write and edit the manuscript.

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