**Name of journal: World Journal of Stem Cells**

**ESPS Manuscript NO: 14739**

**Columns: REVIEW**

**Epigenetic regulation of stemness maintenance in the neurogenic niches**

Montalbán-Loro R *et al*. Neural stem cells and epigenetics

Raquel Montalbán-Loro, Ana Domingo-Muelas, Alex Bizy, Sacri R Ferrón

**Raquel Montalbán-Loro, Ana Domingo-Muelas, Alex Bizy, Sacri R Ferrón,** Departamento de Biología Celular, Facultad de Biología, Universidad de Valencia, 46100 Burjassot, Spain

**Author contributions:** Montalbán-Loro R, Domingo-Muelas A, Bizy A and Ferrón SR solely contributed to this paper.

**Supported by** Ministerio de Ciencia e Innovación (SAF Program) to Sacri R Ferrón; Raquel Montalbán-Loro was funded by a Spanish FPI fellowship; Ana Domingo-Muelas by a Spanish FPU fellowship from the Ministerio de Educación y Ciencia; and Sacri R Ferrón is a Ramón y Cajal investigator.

**Conflict-of-interest:** The authors declare no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to: Sacri R Ferrón, SR PhD,** Departamento de Biología Celular, Facultad de Biología, Universidad de Valencia, C/ Dr. Moliner, 50, 46100 Burjassot, Spain. sacramento.rodriguez@uv.es

**Telephone:** +34-963-543784

**Fax:**  +34-963-543404

**Received:** October 22, 2014

**Peer-review started:** October 22, 2014

**First decision:** November 27, 2014

**Revised:** January 30, 2015

**Accepted:** March 18, 2015

**Article in press:**

**Published online:**

**Abstract**

In the adult mouse brain, the subventricular zone lining the lateral ventricles and the subgranular zone in the dentate gyrus of the hippocampus are two zones that contain neural stem cells (NSCs) with the capacity to give rise to neurons and glia during the entire life of the animal. Spatial and temporal regulation of gene expression in the NSCs population is established and maintained by the coordinated interaction between transcription factors and epigenetic regulators which control stem cell fate. Epigenetic mechanisms are heritable alterations in genome function that do not involve changes in DNA sequence itself but that modulate gene expression, acting as mediators between the environment and the genome. At the molecular level, those epigenetic mechanisms comprise chemical modifications of DNA such as methylation, hydroxymethylation and histone modifications needed for the maintenance of NSC identity. Genomic imprinting is another normal epigenetic process leading to parental-specific expression of a gene, known to be implicated in the control of gene dosage in the neurogenic niches. The generation of induced pluripotent stem cells from NSCs by expression of defined transcription factors, provide key insights into fundamental principles of stem cell biology. Epigenetic modifications can also occur during reprogramming of NSCs to pluripotency and a better understanding of this process will help to elucidate the mechanisms required for stem cell maintenance. This review takes advantage of recent studies from the epigenetic field to report knowledge regarding the mechanisms of stemness maintenance of neural stem cells in the neurogenic niches.

**Key words:** Neurogenesis; Neural stem cell; Epigenetics; Gene expression regulation; Chromatin modifications; DNA methylation

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Neural stem cells (NSCs) are capable of extensive self-renewal while preserving the ability to generate cell progeny that can differentiate into different cell types from the nervous system. Intrinsic mediators as well as extrinsic cues provided by the neurogenic niche (microenvironment where NSCs reside *in vivo*) are important for stem cell self-renewal and differentiation. Epigenetic changes, including alterations in DNA methylation, histone modifications and imprinting alter the way a gene interacts with the cell transcribing machinery, turning genes “on” or “off”. These heritable changes must be reversible and context-dependent being responsible of stem cell plasticity.

Montalbán-Loro R, Domingo-Muelas A, Bizy A, Ferrón SR. Epigenetic regulation of stemness maintenance in the neurogenic niches. *World J Stem Cells* 2015; In press

**NEURAL STEM CELLS AND THE NEUROGENIC NICHES**

Adult stem cells have the ability to divide, self-renew and generate functional differentiated cells that replace lost cells throughout an organism’s lifetime. The existence of adult stem cells was first described in tissues with high proliferation rates, such as the hematopoietic system and the intestine. Since then, stem cells have been found in almost all adult tissues including the nervous system[[1](#_ENREF_1)]. In the adult mouse brain two main regions continue to generate new neurons throughout adulthood: the subventricular zone (SVZ) in the walls of the lateral ventricles[[2](#_ENREF_2)] (Figure 1A-C) and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus[[3](#_ENREF_3)] (Figure 1D-F). Adult neurogenesis is supported by multipotent neural stem cells (NSCs) deriving from embryonic radial-glia and thus expressing astroglial characteristics[[4](#_ENREF_4),[5](#_ENREF_5)]. Astrocytic-like stem cells are relatively quiescent and can be identified by the expression of the glial fibrillary acidic protein (GFAP), the stemness-related transcription factor Sox2 (Sex determining region Y (SRY)-box 2), and the neural progenitor marker Nestin[[2](#_ENREF_2),[6](#_ENREF_6),[7](#_ENREF_7)]. Moreover, their slow division rate can be detected by the label retention of thymidine analogs incorporated during DNA replication[[6](#_ENREF_6),[8](#_ENREF_8),[9](#_ENREF_9)]. NSCs can also be isolated from their natural niche and cultured *in vitro* in the presence of the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) mitogens. In culture, NSCs form free-floating aggregates called “*neurospheres*” (Figure 1C). Self-renewal and multipotency characteristics of NSCs are assessed *in vitro* by clonal analysis in which single cells give rise to neurospheres[[10](#_ENREF_10),[11](#_ENREF_11)] (Figure 1C).

***The SVZ and the olfactory bulb system***

The SVZ is located lining the walls of the lateral ventricles and constitutes a complex microenvironment or niche in which proliferation and self-renewal of NSCs are strongly regulated by multiple extracellular factors such as EGF, bFGF, bone morphogenetic protein (BMP) and pigment epithelium derived factor (PEDF)[[12-14](#_ENREF_12)]. This significant extrinsic signaling is possible because of the special cytoarchitecture of the niche that allows NSCs to be in direct contact with the cerebrospinal fluid (CSF) produced by the choroid plexus in the ventricles, with the vasculature and with other cells from the niche like astrocytes or microglia[[15](#_ENREF_15),[16](#_ENREF_16)]. Subventricular NSCs (also known as type B1 cells) present a radial glia-like morphology, with an apical primary cilium contacting the ventricular lumen and a basal process reaching the basal lamina and the vascular structures[[17](#_ENREF_17),[18](#_ENREF_18)] (Figure 1A). The walls of the lateral ventricles show a typical organization where the small apical process of one or more type B1 cells are surrounded by a rosette of epithelial ependymal cells that form structures known as pinwheels at the surface[[19](#_ENREF_19)]. There is another astrocyte-like type B cell that is more frequently located close to the underlying striatal parenchyma known as type B2 cells[[20](#_ENREF_20)]. When activated, these slowly dividing NSCs give rise to fast cycling cells called transit-amplifying progenitors (TAP or type C cells). TAP cells contribute to reducing the number of cell division rounds that NSCs have to undergo to preserve their genome integrity. Mash1-positive type C cells generate neuroblasts (type A cells) that migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB) where they differentiate and integrate into interneurons (Figure 1B). These chains of PSA-NCAM (polysialylated neural cell adhesion molecule) and DCX (doublecortin) positive neuroblasts reach the core of the OB, where they detach from the RMS and migrate radially into the granular and periglomerular layers[[21-23](#_ENREF_21" \o "Lois, 1994 #15)]. These immature neurons then integrate and differentiate into inhibitory interneurons, playing an important role in rodent olfaction. In addition of being a neurogenic region, the SVZ can serve as a niche of oligodendrocytes although generated in much lower numbers than neuroblasts. Thereby, Olig2-positive transit amplifying cells give rise to oligodendroblasts that migrate to the corpus callosum and striatum while tightly associated with blood vessels[[24](#_ENREF_24)], where they differentiate into myelinating and nonmyelinating oligodendrocytes[[25](#_ENREF_25)].

***The SGZ of the hippocampus***

Along with the SVZ, the subgranular zone in the dentate gyrus of the hippocampus constitutes the other main neurogenic niche in the adult mouse brain[[26-28](#_ENREF_26" \o "Bonaguidi, 2012 #148)]. The SGZ is also a complex microenvironment in which the vasculature plays an important role. Dividing stem cells in the SGZ are in close proximity to an extensive network of interconnected blood vessels and parenchymal astrocytes that can regulate their proliferation and differentiation *via* paracrine signaling[[29](#_ENREF_29)]. The SGZ is located between the granular layer and the hilus of the DG and the SGZ NSCs constitute a subpopulation of GFAP-positive cells that are analogous to subventricular type B1 cells[[30](#_ENREF_30)]. In this region, two types of neural progenitors can be identified according to different expression of molecular markers and their morphologies[[23](#_ENREF_23)] (Figure 1D, E). Type I progenitors exhibit a radial process spanning the granule cell layer and arborizing profusely in the molecular layer[[27](#_ENREF_27)]. These cells express nestin, GFAP, and Sox2[[31](#_ENREF_31" \o "Suh, 2007 #45)]. Type II hippocampal progenitors have short processes and contrary to type I cells, express TRB2 but not GFAP (Figure 1F). There is evidence suggesting that type II cells may derive from type I cells but a lineage relationship study is still lacking[[31](#_ENREF_31" \o "Suh, 2007 #45)]. In the adult SGZ, precursors give rise by asymmetrical divisions to intermediate neuronal lineage-restricted progenitor cells and in a minor number, to glial lineage-restricted progenitor cells (both of them are GFAP-negative cells). Compared to the SVZ, few oligodendrocytes are generated in the SGZ. Type II cells generate in turn type III cells, which are neuronal precursors that express markers of immature migrating neurons, such DCX and PSA-NCAM (Figure 1F). These differentiated cells integrate neuronal circuits into the hippocampal CA3 region forming dendrites and spreading their axons[[22](#_ENREF_22)]. In addition to the production of granular neurons, a low percentage of activated NSCs divide asymmetrically to give rise to astrocytes. The latter migrate into the hilus and the molecular layer where they lose their stem cell identity and cause the depletion of the pool of NSCs[[32](#_ENREF_32),[33](#_ENREF_33)] thus explaining the possible decrease in hippocampal neurogenesis associated with ageing.

**EPIGENETIC REGULATION OF GENE EXPRESSION IN THE NEUROGENIC NICHES**

Epigenetic is defined as the study of heritable alterations in genome function that do not involve changes in DNA sequence itself[[34](#_ENREF_34),[35](#_ENREF_35)]. These epigenetic marks modulate gene expression either by directly altering the chromatin structure or by creating bindings sites for chromatin and transcription regulatory subunits. Two general classes of epigenetic regulation can be defined: covalent modifications to DNA and post-translational covalent modifications to the histones (H) around which the DNA is bound, influencing whether DNA is accessible or not for gene transcription[[36](#_ENREF_36),[37](#_ENREF_37)] (Figure 2A). Moreover, the three-dimensional structure and arrangement of chromatin within the nucleus are both regulated by and contribute to the establishment and maintenance of epigenetic states[[34](#_ENREF_34)]. These different classes of epigenetic modifications are intimately related, resulting in multiple layers of control allowing cells to maintain their identity over time[[34](#_ENREF_34),[38](#_ENREF_38)]. Dysregulation of these mechanisms leads to new cellular phenotypes by causing altered gene expression without a change in genotype. In the neurogenic niches, epigenetic regulators and the associated transcription factors play an important role in the control and maintenance of NSC stemness.

***DNA methylation***

DNA methylation involves the addition of a methyl group to the fifth carbon in the cytosine pyrimidine ring (Figure 2B). In most mammalian genes, CpG dinucleotides are methylated and concentrated in clusters called “*CpG islands*” which often have regulatory functions and tend to be found in the promoter and first exon regions of genes[[39](#_ENREF_39)] where it promotes a closed chromatin structure and aids the prevention of expression[[34](#_ENREF_34),[40](#_ENREF_40)]. DNA methylation marks repress gene expression either by attracting DNA methyl-binding domain proteins (MBDs) such as methyl-CpG binding protein 2 (MeCP2) which recruit repressors and chromatin remodeling molecules to generate an inactive chromatin environment or by directly inhibiting transcription factor binding[[41-43](#_ENREF_41" \o "Bird, 2002 #57)]. MBD proteins have been suggested to play a role in neurogenesis. For example, mice deficient in MBD1 show decreased neurogenesis and hippocampus-related behaviour defects. Indeed, *Mbd1-*deficient NSCs generate a reduced number of neurons when compared to wild-type cells, suggesting a role for MBD1 in neuronal fate commitment[[44](#_ENREF_44" \o "Zhao, 2003 #80)].

There are two types of methylation reactions both mediated by DNA methyltransferases (DNMTs). One is *de novo* methylation catalyzed by DNMT3a and DNMT3b, important for normal embryogenesis and development and responsible for the establishment of methylation patterns. The other type is maintenance methylation mediated by DNMT1 that effectively maintains CpG methylation upon DNA replication and provides the heritable “*memory*” of the methylation state of the parent cell[[45](#_ENREF_45),[46](#_ENREF_46)]. DNMT1 is highly expressed in the embryonic, perinatal and adult CNS in both dividing neural progenitors and mature neurons where it maintains DNA methylation[[47-49](#_ENREF_47" \o "Brooks, 1996 #62)]. A lack of DNMT1 alters neuronal excitability and increases apoptosis in post-mitotic cortical neurons[[50](#_ENREF_50" \o "Hutnick, 2009 #65)]. In support of this, mice deficient for *Dnmt1* specifically in neural progenitors at embryonic stages exhibit deficits in neuronal function and die postnatally,suggesting a requirement for methylation in brain development[[51](#_ENREF_51)]. DNMT3a and DNMT3b are highly expressed in postnatal NSCs and are required for neurogenesis and neuronal maturation[[48](#_ENREF_48),[49](#_ENREF_49),[52](#_ENREF_52),[53](#_ENREF_53)]. Loss of DNMT3a results in gene silencing[[53](#_ENREF_53" \o "Wu, 2010 #69)] and depletion of DNMT3b leads to deficient neuronal differentiation *in vitro*[[54](#_ENREF_54)].

DNA is hypomethylated in neural progenitor cells and methylation is progressively increased during lineage commitment[[55](#_ENREF_55" \o "Sikorska, 2008 #71)]. The suppression of astrogliogenesis during neuronal specification is also associated with changes in DNA methylation[[56](#_ENREF_56),[57](#_ENREF_57)]. This silencing is attenuated later in development resulting in the generation of astrocytes which correlates with the suppression of neurogenesis. Demethylation and expression of the genes coding for the astrocytic markers *Gfap* and the calcium binding protein *S100β* during astrocytic maturation, correlates with methylation and downregulation of neurogenic genes such as Neurogenin 1[[58-60](#_ENREF_58)]. Activation of the *Gfap* promoter requires binding of the signal transducer and activator of transcription 3 (STAT3) to a consensus sequence. Early progenitors are refractory to astrocyte differentiation due to methylation of the STAT3 binding site. At later development stages, loss of STAT3-binding element methylation is associated with *Gfap* promoter activation[[60](#_ENREF_60),[61](#_ENREF_61)]. A similar alteration in methylation pattern occurs at another STAT3 binding site in the *S100β* promoter[[58](#_ENREF_58" \o "Namihira, 2004 #75)].

***Hydroxymethylation***

DNA methylation marks are reversible through both passive replication-dependent demethylation and active demethylation which probably involve the recently characterized 5-hydroxymethyl (5hmC) intermediate[[62](#_ENREF_62" \o "Seisenberger, 2013 #82)] (Figure 2B). In mammals, three members of the ten-eleven translocation (TET) family of enzymes have been identified: TET1, TET2 and TET3[[63](#_ENREF_63),[64](#_ENREF_64)]. TET hydroxylases may catalyze active DNA demethylation by oxidation of 5mC to 5hmC[[65-67](#_ENREF_65)] (Figure 2B). 5hmC is relatively abundant in mouse embryonic stem cells (ESCs), the early embryo and in adult brain[[68](#_ENREF_68),[69](#_ENREF_69)]. In the brain, 5hmC is enriched at active genes, associated with the strong depletion of 5mC from these regions[[70](#_ENREF_70)]. It has been proposed that TET enzymes in the blastocyst and ESCs are involved in pluripotency by maintaining the hypomethylated state of key regulatory regions[[69](#_ENREF_69),[71](#_ENREF_71)]. Recent studies have also shown that TET1 is involved in the epigenetic regulation of neural progenitor cell proliferation in the adult hippocampus[[72-74](#_ENREF_72" \o "Kaas, 2013 #120)]. Mice lacking *Tet1* exhibit impaired hippocampal neurogenesis accompanied by poor learning and memory[[72-74](#_ENREF_72" \o "Kaas, 2013 #120)]. However, the full role and importance of hydroxymethylation in the brain remains to be elucidated.

***Histone modifications as regulators of adult neurogenesis***

In eukaryotic cells, a histone octamer including two H2A-H2B dimers and a H3-H4 tetramer acts as a scaffold around which DNA is wrapped to form a nucleosome[[75](#_ENREF_75),[76](#_ENREF_76)]. The interaction between histones and DNA is mediated by an N-terminal tail of histone proteins available for post-translational modifications that control the chromatin structure[[75](#_ENREF_75)] (Figure 2C). These covalent modifications in the histone tails alter the interaction between adjacent nucleosomes and/or between histones and the DNA, changing the three-dimensional chromatin structure. Modifications in the body of histones have also been shown to alter chromatin structure influencing gene expression[[77](#_ENREF_77)]. Histone modifications are divided into repressive and active marks according to how they correlate with levels of transcriptional activity. For example, histone acetylation of lysine residues of histones, catalyzed by histone acetyltransferases (HATs) enhances the recruitment and activation of the transcriptional machinery and is generally associated with areas of active gene transcription[[78](#_ENREF_78)]. However, histone deacetylases (HDACs) remove acetyl groups promoting the condensation of chromatin[[79](#_ENREF_79)] (Figure 2A). HDAC1 is expressed by GFAP-positive cells within the SVZ whereas HDAC2 is found in migrating neuroblasts and in TAP cells within the SVZ[[80](#_ENREF_80" \o "Foti, 2013 #123)]. Deletion of HDAC2 in the SVZ results in a defective neurogenesis to the OB[[81](#_ENREF_81)] and neurospheres treated with class I and II HDAC inhibitors promotes neuronal differentiation[[82](#_ENREF_82)] suggesting a role for this enzyme in neuronal fate determination. Furthermore, oligodendrocyte fate commitment is accompanied by a decrease in histone deacetylation at transcriptional repressors of oligodendrocytic differentiation such as *Sox11*[[83](#_ENREF_83)] and at neuronal genes such as *Sox2*[[84](#_ENREF_84)].

Histone methylation is associated with both active and silent chromatin and is catalyzed by histone methyltransferases (HMTs). Trimethylation of lysine (K)-27 and lysine 9 of histone H3 (H3K27me3 and H3K9me3) tends to associate with regions of inactive gene transcription, whereas H3K4, H3K36 and H3K79 methylations are associated with active transcription[[85](#_ENREF_85)]. Histone demethylases (HDMs) also have a key role in regulating neural development[[86](#_ENREF_86" \o "Tsukada, 2010 #142)]. During neural stem cell commitment, H3K27 methylation decreases in key developmental genes following downregulation of the HMT *Enhancer of Zest homolog 2* (EZH2) and upregulation of the HDM Jumonji domain containing-3 (JMJD3). Indeed, deletion of *Ezh2* in SGZ progenitor cells results in cell proliferation restriction leading to a reduced number of neurons that ultimately leads to impairment in spatial learning and memory[[87](#_ENREF_87)]. Additionally, 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 such as Dlx2[[88](#_ENREF_88" \o "Park, 2014 #128)]. Moreover, H3K9me3 is enriched in the adult murine SVZ and it has been recently shown that its repression in undifferentiated cells is engaged in the maintenance of cell type integrity in this neurogenic niche[[89](#_ENREF_89)]. MLL1, another HMT that methylates H3K4, has been associated with the trithorax group of transcription factors. In mice where *Mll1* is knocked out in NSCs, neurogenesis is impaired. *Mll1* is associated with the promoter of the homeobox transcription factor *Dlx2* and although loss of *Mll1* does not affect the methylation of H3K4, it does increase H3K27me3 on the promoter indicating that *Mll1* is recruiting a H3K27 demethylase[[90](#_ENREF_90" \o "Lim, 2009 #129)]. In summary, the above studies indicate that different chromatin modifiers have a critical role in adult neurogenesis[[91](#_ENREF_91)].

***Genomic imprinting and control of gene dosage***

Imprinted genes are expressed predominantly from one chromosome in a parental-origin dependent manner. While most genes are expressed from both alleles, imprinted genes are functionally monoallelic and are expressed from either the maternally or the paternally inherited chromosome[[92](#_ENREF_92)]. In mammals, this affects around 100 genes that are found in clusters. Imprinting control regions (ICRs) regulate the parental allele-specific pattern of gene expression and have differentially methylated regions (DMRs) on the two parental chromosomes. ICRs can be divided into those which are methylated on the paternally inherited copy and those with maternally inherited methylation[[93](#_ENREF_93)]. DMRs are also characterized by the asymmetrical accumulation of different histone modifications on the two parental chromosomes and the recent identification of a *“tri-mark”*, comprising the trimethylation of H3K4 and H3K9 and the trimethylation of H4K20 at all known ICRs[[94](#_ENREF_94)]. The majority of imprinted genes are expressed in the brain and several exhibit brain-specific imprinting. Their monoallelic expression makes these *loci* very vulnerable as mutation of the expressed allele can compromise expression and lead to severe developmental defects. For example, human congenital imprinting syndromes including Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are all characterized by neurological and behavioral impairments and learning difficulties[[95](#_ENREF_95)]. Evidence is suggesting that selective regulation of imprinting is a normal mechanism of modulating gene dosage and is associated with the control of stem cell potential in the neurogenic niche. For instance, relaxation of imprinting of the gene for the atypical NOTCH ligand delta-like homologue 1 (*Dlk1*) usually expressed from the paternally inherited chromosome has been shown in the neural stem cells and niche astrocytes within the SVZ[[96](#_ENREF_96" \o "Ferron, 2011 #132)]. Notably, this selective absence of *Dlk1* imprinting is associated with acquisition of DNA methylation at the germline-derived imprinting control region[[96](#_ENREF_96" \o "Ferron, 2011 #132)]. *Igf2* is also an imprinted gene expressed only from the paternally-inherited allele although it is specifically biallelically expressed in postnatal human and mouse choroid plexus epithelium and leptomeninges[[97](#_ENREF_97),[98](#_ENREF_98)]. Thus, CSF produced from the choroid plexus and blood vessels is a biallelic source of neurogenesis-promoting IGF2[[99](#_ENREF_99" \o "Lehtinen, 2011 #133)].

***Epigenetic changes during NSCs reprogramming to iPSCs***

Epigenetic reprogramming consists in the transition from one cell type to another, permitted by the loss of the molecular characteristics of the cell of origin and the acquisition of an entirely new molecular identity without changing the genomic sequence[[100](#_ENREF_100)]. Reprogramming involves changes in the transcriptome and chromatin state of the reprogrammed cell type to that of a pluripotent cell[[101-103](#_ENREF_101" \o "Mikkelsen, 2008 #135)]. This implicates different levels of changes in DNA factor binding, transcription and chromatin state[[103](#_ENREF_103)]. Since the discovery by Takahashi and Yamanaka in 2006 that the introduction of four transcription factors, *Oct3/4*, *Klf4*, *c-Myc*, *Sox2* (known as OKMS) could reprogram mouse embryonic and adult fibroblasts into induced pluripotent stem cells (iPSCs)[[104](#_ENREF_104)], the field of reprogramming has considerably evolved and several studies have reported the use of sets of these transcription factors in various combinations to reprogram mouse and human somatic cells[[105-108](#_ENREF_105)]. More recently, murine B lymphocytes, liver, stomach and pancreatic β-cells were showed to reprogram into iPSCs using the combination of factors OKMS[109-111]. In 2008, Eminli *et al*[112] reported the generation of iPSCs from murine NSCs by retroviral infection of the same combination of factors. Since neurosphere cultures express *Sox2* and *c-myc*, a considerable advance consisted in showing that they could be reprogrammed only with Oct4 and Klf4 at similar efficiency to the reprogramming rate of murine fibroblasts with the original four factors[112-114]. Finally, the forced expression of *Oct4* alone was shown sufficient to reprogram murine NSCs, albeit with a ten-fold lower efficiency than with two factors[113]. Because NSCs are originally closer to the pluripotency state than somatic cells and require fewer factors to be reprogrammed, they constitute a more simple and attractive system to study epigenetic mechanisms occurring during the acquisition of pluripotency. Importantly, iPSCs derived from human and murine NSCs exhibited markers of ESCs, showed demethylation of pluripotency genes, formed teratomas, and contributed to viable chimeras[112-114].

***Reprogramming factors and Epigenetic mechanisms***

Reprogramming of somatic cells is a stochastic event[115]. However, in NSCs, *Oct4* only seems sufficient to repress genes responsible for NSCs molecular identity and activate the pluripotency genes, suggesting that epigenetic of NSCs renders them easier to reprogram and that the combination of factors necessary for reprogramming is dependent on cellular context[112]. iPSCs have lower levels of methylation than somatic cells, suggesting that demethylation is an important chromatin feature to achieve pluripotency[116]. During reprogramming, it is stipulated that reprogramming factors interfere with methylation of the newly synthetized DNA by binding to specific promoters or enhancer regions leading to demethylation and activation of the pluripotency genes. In addition, active DNA demethylation mechanisms could be required for the reactivation of pluripotency genes[117]. Recent studies in NSCs have shown the importance of methylation level in the context of reprogramming. Undifferentiated neurospheres highly express DNMT1 and contain methylated chromatin suggesting the role of methylation for the maintenance of the quiescent or undifferentiated state of NSCs[118]. It is then probable that NSC chromatin is dynamically remodelled and that DNA methylation modification is essential for reprogramming to a pluripotent state. For instance, histone methyltransferase G9a is responsible for the downregulation of Oct4 during NSC differentiation and its inhibition results in iPSC formation after overexpression of exogenous Klf4 and c-myc only[119]. In addition, interference with DNMT1 promotes iPSC formation, also supporting that DNA methylation is a feature limiting reprogramming to pluripotency[101]. All reprogramming techniques involve demethylation of the genome thus appearing as a crucial process for successfully achieving pluripotency[120,121].

***Loss of epigenetic memory***

During reprogramming, NSCs downregulate specific genes like *Nestin* and progressively express the markers of pluripotency *Oct4*, *Nanog*, *Fgf4*, *Zfp42*[113,114]. In addition, efficiency and timing of reprogramming highly depends on the differentiation state of the initial cell type. Importantly, comparative studies with ESCs reported that efficiently reprogrammed iPSCs showtranscriptional pattern and epigenetic marks highly similar to ESCs. For instance, Oct4 and Nanog promoters are demethylated and histones H3 lysine 4 (K4) and lysine 27 (K27) mostly exhibit patterns of trimethylation[101,106,122]. However, reprogramming of NSCs into iPSCs is often incomplete and leaves epigenetic marks including DNA methylation, chromatin modification and transcriptional regulation in the resulting iPSC genome[123,124] known as epigenetic “memory”. Partially reprogrammed cell lines are characterized by an absence of complete downregulation of the exogenous reprogramming factors and partial demethylation and reactivation of pluripotency genes[101,104]. During reprogramming, somatic markers get progressively downregulated demonstrating the importance of silencing its differentiation program as a step towards pluripotency. Treatment of partially reprogrammed iPSCs with inhibitors of ERK1/2 and GSK3b signaling[125], induced genome demethylation of 30% explained by decreased levels of DNMT3a/b and their targeting factor DNMT3L[126-128]. The two inhibitors repress DNMT3A/B expression inducing demethylation of certain genomic regions in ESCs. Thus, DNA demethylation of the reprogrammed cell type as a way to remove epigenetic marks is important for complete reprogramming into iPSCs. Reprogrammed iPSCs often present the limitation of not being fully reprogrammed thus keeping epigenetic traces of the tissue of origin. Future generation of iPSCs without epigenetic memory is an important challenge in the field to ensure that differentiation decisions are not affected by events from the past[116].

**FUTURE PERSPECTIVES**

Determining the mechanisms by which neural stem cells maintain self-renewal capacity and at the same time generate differentiated progeny is a central challenge in stem cell biology. Several recent studies have demonstrated that epigenetic gene regulation plays a crucial role in the control of stem cell behaviour. Epigenetic mechanisms include changes in chromatin structure that provides a way for coordinately activating or repressing genes during proliferation and differentiation. Extracellular signaling from the microenvironment or niche in which NSCs reside *in vivo* interacts with these diverse epigenetic mechanisms, thus regulating transcription factors and intracellular pathways. These changes in gene expression are often heritable and reversible, features that support stem cell plasticity such as the ability to dedifferentiate or become reprogrammed under certain conditions. Finally, aberrant epigenetic mechanisms are known to be involved in the development of many neurological diseases. Characterizing epigenetic changes associated with a particular neural pathology may be used as biomarkers of disease and the manipulation of those epigenetic mechanisms holds great promise as a potential therapeutic strategy.

**REFERENCES**

1 **Li L**, Xie T. Stem cell niche: structure and function. *Annu Rev Cell Dev Biol* 2005; **21**: 605-631 [PMID: 16212509 DOI: 10.1146/annurev.cellbio.21.012704.131525]

2 **Doetsch F**, García-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 1997; **17**: 5046-5061 [PMID: 9185542]

3 **Gage FH**, Kempermann G, Palmer TD, Peterson DA, Ray J. Multipotent progenitor cells in the adult dentate gyrus. *J Neurobiol* 1998; **36**: 249-266 [PMID: 9712308]

4 **Doetsch F**. The glial identity of neural stem cells. *Nat Neurosci* 2003; **6**: 1127-1134 [PMID: 14583753 DOI: 10.1038/nn1144]

5 **Merkle FT**, Tramontin AD, García-Verdugo JM, Alvarez-Buylla A. Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci USA* 2004; **101**: 17528-17532 [PMID: 15574494 DOI: 10.1073/pnas.0407893101]

6 **Ferri AL**, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, Ottolenghi S, Pandolfi PP, Sala M, DeBiasi S, Nicolis SK. Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development* 2004; **131**: 3805-3819 [PMID: 15240551 DOI: 10.1242/dev.01204]

7 **Morshead CM**, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* 1994; **13**: 1071-1082 [PMID: 7946346]

8 **Nam HS**, Benezra R. High levels of Id1 expression define B1 type adult neural stem cells. *Cell Stem Cell* 2009; **5**: 515-526 [PMID: 19896442 DOI: 10.1016/j.stem.2009.08.017]

9 **Ponti G**, Obernier K, Guinto C, Jose L, Bonfanti L, Alvarez-Buylla A. Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. *Proc Natl Acad Sci USA* 2013; **110**: E1045-E1054 [PMID: 23431204 DOI: 10.1073/pnas.1219563110]

10 **Ferron SR**, Andreu-Agullo C, Mira H, Sanchez P, Marques-Torrejon MA, Farinas I. A combined ex/in vivo assay to detect effects of exogenously added factors in neural stem cells. *Nat Protoc* 2007; **2**: 849-859 [PMID: 17474182]

11 **Reynolds BA**, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992; **255**: 1707-1710 [PMID: 1553558]

12 **Faigle R**, Song H. Signaling mechanisms regulating adult neural stem cells and neurogenesis. *Biochim Biophys Acta* 2013; **1830**: 2435-2448 [PMID: 22982587 DOI: 10.1016/j.bbagen.2012.09.002]

13 **Porlan E**, Perez-Villalba A, Delgado AC, Ferrón SR. Paracrine regulation of neural stem cells in the subependymal zone. *Arch Biochem Biophys* 2013; **534**: 11-19 [PMID: 23073070 DOI: 10.1016/j.abb.2012.10.001]

14 **Ramírez-Castillejo C**, Sánchez-Sánchez F, Andreu-Agulló C, Ferrón SR, Aroca-Aguilar JD, Sánchez P, Mira H, Escribano J, Fariñas I. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 2006; **9**: 331-339 [PMID: 16491078 DOI: 10.1038/nn1657]

15 **Shen Q**, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 2004; **304**: 1338-1340 [PMID: 15060285 DOI: 10.1126/science.1095505]

16 **Silva-Vargas V**, Crouch EE, Doetsch F. Adult neural stem cells and their niche: a dynamic duo during homeostasis, regeneration, and aging. *Curr Opin Neurobiol* 2013; **23**: 935-942 [PMID: 24090877 DOI: 10.1016/j.conb.2013.09.004]

17 **Doetsch F**, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999; **97**: 703-716 [PMID: 10380923]

18 **Tavazoie M**, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* 2008; **3**: 279-288 [PMID: 18786415 DOI: 10.1016/j.stem.2008.07.025]

19 **Mirzadeh Z**, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* 2008; **3**: 265-278 [PMID: 18786414 DOI: 10.1016/j.stem.2008.07.004]

20 **Ihrie RA**, Alvarez-Buylla A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* 2011; **70**: 674-686 [PMID: 21609824 DOI: 10.1016/j.neuron.2011.05.004]

21 **Lois C**, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. *Science* 1994; **264**: 1145-1148 [PMID: 8178174]

22 **Ming GL**, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* 2011; **70**: 687-702 [PMID: 21609825 DOI: 10.1016/j.neuron.2011.05.001]

23 **Zhao C**, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008; **132**: 645-660 [PMID: 18295581 DOI: 10.1016/j.cell.2008.01.033]

24 **Cayre M**, Courtès S, Martineau F, Giordano M, Arnaud K, Zamaron A, Durbec P. Netrin 1 contributes to vascular remodeling in the subventricular zone and promotes progenitor emigration after demyelination. *Development* 2013; **140**: 3107-3117 [PMID: 23824572 DOI: 10.1242/dev.092999]

25 **Menn B**, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* 2006; **26**: 7907-7918 [PMID: 16870736 DOI: 10.1523/JNEUROSCI.1299-06.2006]

26 **Bonaguidi MA**, Song J, Ming GL, Song H. A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus. *Curr Opin Neurobiol* 2012; **22**: 754-761 [PMID: 22503352 DOI: 10.1016/j.conb.2012.03.013]

27 **Encinas JM**, Sierra A, Valcárcel-Martín R, Martín-Suárez S. A developmental perspective on adult hippocampal neurogenesis. *Int J Dev Neurosci* 2013; **31**: 640-645 [PMID: 23588197 DOI: 10.1016/j.ijdevneu.2013.04.001]

28 **Gage FH**. Mammalian neural stem cells. *Science* 2000; **287**: 1433-1438 [PMID: 10688783]

29 **Palmer TD**, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 2000; **425**: 479-494 [PMID: 10975875]

30 **Seri B**, García-Verdugo JM, McEwen BS, Alvarez-Buylla A. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J Neurosci* 2001; **21**: 7153-7160 [PMID: 11549726]

31 **Suh H**, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH. In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. *Cell Stem Cell* 2007; **1**: 515-528 [PMID: 18371391 DOI: 10.1016/j.stem.2007.09.002]

32 **Bonaguidi MA**, Wheeler MA, Shapiro JS, Stadel RP, Sun GJ, Ming GL, Song H. In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. *Cell* 2011; **145**: 1142-1155 [PMID: 21664664 DOI: 10.1016/j.cell.2011.05.024]

33 **Encinas JM**, Michurina TV, Peunova N, Park JH, Tordo J, Peterson DA, Fishell G, Koulakov A, Enikolopov G. Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. *Cell Stem Cell* 2011; **8**: 566-579 [PMID: 21549330 DOI: 10.1016/j.stem.2011.03.010]

34 **Bird A**. Perceptions of epigenetics. *Nature* 2007; **447**: 396-398 [PMID: 17522671 DOI: 10.1038/nature05913]

35 **Jaenisch R**, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003; **33** Suppl: 245-254 [PMID: 12610534 DOI: 10.1038/ng1089]

36 **Kouzarides T**. Chromatin modifications and their function. *Cell* 2007; **128**: 693-705 [PMID: 17320507 DOI: 10.1016/j.cell.2007.02.005]

37 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]

38 **Valk-Lingbeek ME**, Bruggeman SW, van Lohuizen M. Stem cells and cancer; the polycomb connection. *Cell* 2004; **118**: 409-418 [PMID: 15315754 DOI: 10.1016/j.cell.2004.08.005]

39 **Schilling E**, Rehli M. Global, comparative analysis of tissue-specific promoter CpG methylation. *Genomics* 2007; **90**: 314-323 [PMID: 17582736 DOI: 10.1016/j.ygeno.2007.04.011]

40 **Shen L**, Kondo Y, Guo Y, Zhang J, Zhang L, Ahmed S, Shu J, Chen X, Waterland RA, Issa JP. Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet* 2007; **3**: 2023-2036 [PMID: 17967063 DOI: 10.1371/journal.pgen.0030181]

41 **Bird A**. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; **16**: 6-21 [PMID: 11782440 DOI: 10.1101/gad.947102]

42 **Hendrich B**, Tweedie S. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends Genet* 2003; **19**: 269-277 [PMID: 12711219 DOI: 10.1016/S0168-9525(03)00080-5]

43 **Tost J**. DNA methylation: an introduction to the biology and the disease-associated changes of a promising biomarker. *Methods Mol Biol* 2009; **507**: 3-20 [PMID: 18987802 DOI: 10.1007/978-1-59745-522-0\_1]

44 **Zhao X**, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K, Lein ES, Eadie BD, Willhoite AR, Muotri AR, Summers RG, Chun J, Lee KF, Gage FH. Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc Natl Acad Sci U S A* 2003; **100**: 6777-6782 [PMID: 12748381 DOI: 10.1073/pnas.1131928100]

45 **Bestor TH**. Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. *EMBO J* 1992; **11**: 2611-2617 [PMID: 1628623]

46 **Pradhan S**, Bacolla A, Wells RD, Roberts RJ. Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. *J Biol Chem* 1999; **274**: 33002-33010 [PMID: 10551868]

47 **Brooks PJ**, Marietta C, Goldman D. DNA mismatch repair and DNA methylation in adult brain neurons. *J Neurosci* 1996; **16**: 939-945 [PMID: 8558262]

48 **Feng J**, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat Neurosci* 2010; **13**: 423-430 [PMID: 20228804 DOI: 10.1038/nn.2514]

49 **Goto K**, Numata M, Komura JI, Ono T, Bestor TH, Kondo H. Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* 1994; **56**: 39-44 [PMID: 8026645]

50 **Hutnick LK**, Golshani P, Namihira M, Xue Z, Matynia A, Yang XW, Silva AJ, Schweizer FE, Fan G. DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. *Hum Mol Genet* 2009; **18**: 2875-2888 [PMID: 19433415 DOI: 10.1093/hmg/ddp222]

51 **Fan G**, Beard C, Chen RZ, Csankovszki G, Sun Y, Siniaia M, Biniszkiewicz D, Bates B, Lee PP, Kuhn R, Trumpp A, Poon C, Wilson CB, Jaenisch R. DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *J Neurosci* 2001; **21**: 788-797 [PMID: 11157065]

52 **Okano M**, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; **99**: 247-257 [PMID: 10555141]

53 **Wu H**, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K, Li E, Zhang Y, Sun YE. Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science* 2010; **329**: 444-448 [PMID: 20651149 DOI: 10.1126/science.1190485]

54 **Bai S**, Ghoshal K, Datta J, Majumder S, Yoon SO, Jacob ST. DNA methyltransferase 3b regulates nerve growth factor-induced differentiation of PC12 cells by recruiting histone deacetylase 2. *Mol Cell Biol* 2005; **25**: 751-766 [PMID: 15632075 DOI: 10.1128/MCB.25.2.751-766.2005]

55 **Sikorska M**, Sandhu JK, Deb-Rinker P, Jezierski A, Leblanc J, Charlebois C, Ribecco-Lutkiewicz M, Bani-Yaghoub M, Walker PR. Epigenetic modifications of SOX2 enhancers, SRR1 and SRR2, correlate with in vitro neural differentiation. *J Neurosci Res* 2008; **86**: 1680-1693 [PMID: 18293417 DOI: 10.1002/jnr.21635]

56 **Ballas N**, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 2005; **121**: 645-657 [PMID: 15907476]

57 **Lunyak VV**, Burgess R, Prefontaine GG, Nelson C, Sze SH, Chenoweth J, Schwartz P, Pevzner PA, Glass C, Mandel G, Rosenfeld MG. Corepressor-dependent silencing of chromosomal regions encoding neuronal genes. *Science* 2002; **298**: 1747-1752 [PMID: 12399542 DOI: 10.1126/science.1076469]

58 **Namihira M**, Nakashima K, Taga T. Developmental stage dependent regulation of DNA methylation and chromatin modification in a immature astrocyte specific gene promoter. *FEBS Lett* 2004; **572**: 184-188 [PMID: 15304345 DOI: 10.1016/j.febslet.2004.07.029]

59 **Sun Y**, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X, Fan G, Greenberg ME. Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. *Cell* 2001; **104**: 365-376 [PMID: 11239394]

60 **Takizawa T**, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, Fujita N, Nakao M, Taga T. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev Cell* 2001; **1**: 749-758 [PMID: 11740937]

61 **Fan G**, Martinowich K, Chin MH, He F, Fouse SD, Hutnick L, Hattori D, Ge W, Shen Y, Wu H, ten Hoeve J, Shuai K, Sun YE. DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. *Development* 2005; **132**: 3345-3356 [PMID: 16014513 DOI: 10.1242/dev.01912]

62 **Seisenberger S**, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Philos Trans R Soc Lond B Biol Sci* 2013; **368**: 20110330 [PMID: 23166394 DOI: 10.1098/rstb.2011.0330]

63 **Ito T**. Role of histone modification in chromatin dynamics. *J Biochem* 2007; **141**: 609-614 [PMID: 17405795 DOI: 10.1093/jb/mvm091]

64 **Tahiliani M**, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; **324**: 930-935 [PMID: 19372391 DOI: 10.1126/science.1170116]

65 **Cortellino S**, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, Le Coz M, Devarajan K, Wessels A, Soprano D, Abramowitz LK, Bartolomei MS, Rambow F, Bassi MR, Bruno T, Fanciulli M, Renner C, Klein-Szanto AJ, Matsumoto Y, Kobi D, Davidson I, Alberti C, Larue L, Bellacosa A. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. *Cell* 2011; **146**: 67-79 [PMID: 21722948 DOI: 10.1016/j.cell.2011.06.020]

66 **He YF**, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, Xu GL. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* 2011; **333**: 1303-1307 [PMID: 21817016 DOI: 10.1126/science.1210944]

67 **Loenarz C**, Schofield CJ. Oxygenase catalyzed 5-methylcytosine hydroxylation. *Chem Biol* 2009; **16**: 580-583 [PMID: 19549596 DOI: 10.1016/j.chembiol.2009.06.002]

68 **Iqbal K**, Jin SG, Pfeifer GP, Szabó PE. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc Natl Acad Sci U S A* 2011; **108**: 3642-3647 [PMID: 21321204 DOI: 10.1073/pnas.1014033108]

69 **Ito S**, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* 2010; **466**: 1129-1133 [PMID: 20639862 DOI: 10.1038/nature09303]

70 **Mellén M**, Ayata P, Dewell S, Kriaucionis S, Heintz N. MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. *Cell* 2012; **151**: 1417-1430 [PMID: 23260135 DOI: 10.1016/j.cell.2012.11.022]

71 **Koh KP**, Yabuuchi A, Rao S, Huang Y, Cunniff K, Nardone J, Laiho A, Tahiliani M, Sommer CA, Mostoslavsky G, Lahesmaa R, Orkin SH, Rodig SJ, Daley GQ, Rao A. Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. *Cell Stem Cell* 2011; **8**: 200-213 [PMID: 21295276 DOI: 10.1016/j.stem.2011.01.008]

72 **Kaas GA**, Zhong C, Eason DE, Ross DL, Vachhani RV, Ming GL, King JR, Song H, Sweatt JD. TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation. *Neuron* 2013; **79**: 1086-1093 [PMID: 24050399 DOI: 10.1016/j.neuron.2013.08.032]

73 **Rudenko A**, Dawlaty MM, Seo J, Cheng AW, Meng J, Le T, Faull KF, Jaenisch R, Tsai LH. Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. *Neuron* 2013; **79**: 1109-1122 [PMID: 24050401 DOI: 10.1016/j.neuron.2013.08.003]

74 **Zhang RR**, Cui QY, Murai K, Lim YC, Smith ZD, Jin S, Ye P, Rosa L, Lee YK, Wu HP, Liu W, Xu ZM, Yang L, Ding YQ, Tang F, Meissner A, Ding C, Shi Y, Xu GL. Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell* 2013; **13**: 237-245 [PMID: 23770080 DOI: 10.1016/j.stem.2013.05.006]

75 **Luger K**, Richmond TJ. DNA binding within the nucleosome core. *Curr Opin Struct Biol* 1998; **8**: 33-40 [PMID: 9519294]

76 **Olins DE**, Olins AL. Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol* 2003; **4**: 809-814 [PMID: 14570061 DOI: 10.1038/nrm1225]

77 **Tropberger P**, Schneider R. Scratching the (lateral) surface of chromatin regulation by histone modifications. *Nat Struct Mol Biol* 2013; **20**: 657-661 [PMID: 23739170 DOI: 10.1038/nsmb.2581]

78 **Marmorstein R**, Trievel RC. Histone modifying enzymes: structures, mechanisms, and specificities. *Biochim Biophys Acta* 2009; **1789**: 58-68 [PMID: 18722564 DOI: 10.1016/j.bbagrm.2008.07.009]

79 **Bannister AJ**, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011; **21**: 381-395 [PMID: 21321607 DOI: 10.1038/cr.2011.22]

80 **Foti SB**, Chou A, Moll AD, Roskams AJ. HDAC inhibitors dysregulate neural stem cell activity in the postnatal mouse brain. *Int J Dev Neurosci* 2013; **31**: 434-447 [PMID: 23542004 DOI: 10.1016/j.ijdevneu.2013.03.008]

81 **Jawerka M**, Colak D, Dimou L, Spiller C, Lagger S, Montgomery RL, Olson EN, Wurst W, Göttlicher M, Götz M. The specific role of histone deacetylase 2 in adult neurogenesis. *Neuron Glia Biol* 2010; **6**: 93-107 [PMID: 20388229 DOI: 10.1017/S1740925X10000049]

82 **Siebzehnrubl FA**, Buslei R, Eyupoglu IY, Seufert S, Hahnen E, Blumcke I. Histone deacetylase inhibitors increase neuronal differentiation in adult forebrain precursor cells. *Exp Brain Res* 2007; **176**: 672-678 [PMID: 17216146 DOI: 10.1007/s00221-006-0831-x]

83 **He Y**, Sandoval J, Casaccia-Bonnefil P. Events at the transition between cell cycle exit and oligodendrocyte progenitor differentiation: the role of HDAC and YY1. *Neuron Glia Biol* 2007; **3**: 221-231 [PMID: 18634613 DOI: 10.1017/S1740925X08000057]

84 **Lyssiotis CA**, Walker J, Wu C, Kondo T, Schultz PG, Wu X. Inhibition of histone deacetylase activity induces developmental plasticity in oligodendrocyte precursor cells. *Proc Natl Acad Sci USA* 2007; **104**: 14982-14987 [PMID: 17855562 DOI: 10.1073/pnas.0707044104]

85 **Li B**, Carey M, Workman JL. The role of chromatin during transcription. *Cell* 2007; **128**: 707-719 [PMID: 17320508 DOI: 10.1016/j.cell.2007.01.015]

86 **Tsukada Y**, Ishitani T, Nakayama KI. KDM7 is a dual demethylase for histone H3 Lys 9 and Lys 27 and functions in brain development. *Genes Dev* 2010; **24**: 432-437 [PMID: 20194436 DOI: 10.1101/gad.1864410]

87 **Zhang J**, Ji F, Liu Y, Lei X, Li H, Ji G, Yuan Z, Jiao J. Ezh2 regulates adult hippocampal neurogenesis and memory. *J Neurosci* 2014; **34**: 5184-5199 [PMID: 24719098 DOI: 10.1523/JNEUROSCI.4129-13.2014]

88 **Park DH**, Hong SJ, Salinas RD, Liu SJ, Sun SW, Sgualdino J, Testa G, Matzuk MM, Iwamori N, Lim DA. Activation of neuronal gene expression by the JMJD3 demethylase is required for postnatal and adult brain neurogenesis. *Cell Rep* 2014; **8**: 1290-1299 [PMID: 25176653 DOI: 10.1016/j.celrep.2014.07.060]

89 **Foret MR**, Sandstrom RS, Rhodes CT, Wang Y, Berger MS, Lin CH. Molecular targets of chromatin repressive mark H3K9me3 in primate progenitor cells within adult neurogenic niches. *Front Genet* 2014; **5**: 252 [PMID: 25126093 DOI: 10.3389/fgene.2014.00252]

90 **Lim DA**, Huang YC, Swigut T, Mirick AL, Garcia-Verdugo JM, Wysocka J, Ernst P, Alvarez-Buylla A. Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* 2009; **458**: 529-533 [PMID: 19212323 DOI: 10.1038/nature07726]

91 **Gonzales-Roybal G**, Lim DA. Chromatin-based epigenetics of adult subventricular zone neural stem cells. *Front Genet* 2013; **4**: 194 [PMID: 24115953 DOI: 10.3389/fgene.2013.00194]

92 **Ferguson-Smith AC**. Genomic imprinting: the emergence of an epigenetic paradigm. *Nat Rev Genet* 2011; **12**: 565-575 [PMID: 21765458 DOI: 10.1038/nrg3032]

93 **Edwards CA**, Ferguson-Smith AC. Mechanisms regulating imprinted genes in clusters. *Curr Opin Cell Biol* 2007; **19**: 281-289 [PMID: 17467259 DOI: 10.1016/j.ceb.2007.04.013]

94 **McEwen KR**, Ferguson-Smith AC. Distinguishing epigenetic marks of developmental and imprinting regulation. *Epigenetics Chromatin* 2010; **3**: 2 [PMID: 20180964 DOI: 10.1186/1756-8935-3-2]

95 **Hirasawa R**, Feil R. Genomic imprinting and human disease. *Essays Biochem* 2010; **48**: 187-200 [PMID: 20822494 DOI: 10.1042/bse0480187]

96 **Ferrón SR**, Charalambous M, Radford E, McEwen K, Wildner H, Hind E, Morante-Redolat JM, Laborda J, Guillemot F, Bauer SR, Fariñas I, Ferguson-Smith AC. Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature* 2011; **475**: 381-385 [PMID: 21776083 DOI: 10.1038/nature10229]

97 **DeChiara TM**, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 1991; **64**: 849-859 [PMID: 1997210]

98 **Giannoukakis N**, Deal C, Paquette J, Goodyer CG, Polychronakos C. Parental genomic imprinting of the human IGF2 gene. *Nat Genet* 1993; **4**: 98-101 [PMID: 8099843 DOI: 10.1038/ng0593-98]

99 **Lehtinen MK**, Zappaterra MW, Chen X, Yang YJ, Hill AD, Lun M, Maynard T, Gonzalez D, Kim S, Ye P, D'Ercole AJ, Wong ET, LaMantia AS, Walsh CA. The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron* 2011; **69**: 893-905 [PMID: 21382550 DOI: 10.1016/j.neuron.2011.01.023]

100 **Krishnakumar R**, Blelloch RH. Epigenetics of cellular reprogramming. *Curr Opin Genet Dev* 2013; **23**: 548-555 [PMID: 23948105 DOI: 10.1016/j.gde.2013.06.005]

101 **Mikkelsen TS**, Hanna J, Zhang X, Ku M, Wernig M, Schorderet P, Bernstein BE, Jaenisch R, Lander ES, Meissner A. Dissecting direct reprogramming through integrative genomic analysis. *Nature* 2008; **454**: 49-55 [PMID: 18509334 DOI: 10.1038/nature07056]

102 **Chin MH**, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, Ambartsumyan G, Aimiuwu O, Richter L, Zhang J, Khvorostov I, Ott V, Grunstein M, Lavon N, Benvenisty N, Croce CM, Clark AT, Baxter T, Pyle AD, Teitell MA, Pelegrini M, Plath K, Lowry WE. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 2009; **5**: 111-123 [PMID: 19570518 DOI: 10.1016/j.stem.2009.06.008]

103 **Papp B**, Plath K. Epigenetics of reprogramming to induced pluripotency. *Cell* 2013; **152**: 1324-1343 [PMID: 23498940 DOI: 10.1016/j.cell.2013.02.043]

104 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]

105 **Hester ME**, Song S, Miranda CJ, Eagle A, Schwartz PH, Kaspar BK. Two factor reprogramming of human neural stem cells into pluripotency. *PLoS One* 2009; **4**: e7044 [PMID: 19763260 DOI: 10.1371/journal.pone.0007044]

106 **Maherali N**, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007; **1**: 55-70 [PMID: 18371336 DOI: 10.1016/j.stem.2007.05.014]

107 **Park IH**, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008; **451**: 141-146 [PMID: 18157115 DOI: 10.1038/nature06534]

108 **Wernig M**, Meissner A, Cassady JP, Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2008; **2**: 10-12 [PMID: 18371415 DOI: 10.1016/j.stem.2007.12.001]

109 **Aoi T**, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T, Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 2008; **321**: 699-702 [PMID: 18276851 DOI: 10.1126/science.1154884]

110 **Hanna J**, Markoulaki S, Schorderet P, Carey BW, Beard C, Wernig M, Creyghton MP, Steine EJ, Cassady JP, Foreman R, Lengner CJ, Dausman JA, Jaenisch R. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* 2008; **133**: 250-264 [PMID: 18423197 DOI: 10.1016/j.cell.2008.03.028]

111 **Stadtfeld M**, Brennand K, Hochedlinger K. Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Curr Biol* 2008; **18**: 890-894 [PMID: 18501604 DOI: 10.1016/j.cub.2008.05.010]

112 **Eminli S**, Utikal J, Arnold K, Jaenisch R, Hochedlinger K. Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. *Stem Cells* 2008; **26**: 2467-2474 [PMID: 18635867 DOI: 10.1634/stemcells.2008-0317]

113 **Kim JB**, Sebastiano V, Wu G, Araúzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, Meyer J, Hübner K, Bernemann C, Ortmeier C, Zenke M, Fleischmann BK, Zaehres H, Schöler HR. Oct4-induced pluripotency in adult neural stem cells. *Cell* 2009; **136**: 411-419 [PMID: 19203577 DOI: 10.1016/j.cell.2009.01.023]

114 **Kim JB**, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Araúzo-Bravo MJ, Ruau D, Han DW, Zenke M, Schöler HR. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 2008; **454**: 646-650 [PMID: 18594515 DOI: 10.1038/nature07061]

115 **Lin SL**, Chang DC, Lin CH, Ying SY, Leu D, Wu DT. Regulation of somatic cell reprogramming through inducible mir-302 expression. *Nucleic Acids Res* 2011; **39**: 1054-1065 [PMID: 20870751 DOI: 10.1093/nar/gkq850]

116 **Lee HJ**, Hore TA, Reik W. Reprogramming the methylome: erasing memory and creating diversity. *Cell Stem Cell* 2014; **14**: 710-719 [PMID: 24905162 DOI: 10.1016/j.stem.2014.05.008]

117 **Hochedlinger K**, Plath K. Epigenetic reprogramming and induced pluripotency. *Development* 2009; **136**: 509-523 [PMID: 19168672 DOI: 10.1242/dev.020867]

118 **Singh RP**, Shiue K, Schomberg D, Zhou FC. Cellular epigenetic modifications of neural stem cell differentiation. *Cell Transplant* 2009; **18**: 1197-1211 [PMID: 19660178 DOI: 10.3727/096368909X12483162197204]

119 **Shi Y**, Desponts C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* 2008; **3**: 568-574 [PMID: 18983970 DOI: 10.1016/j.stem.2008.10.004]

120 **Apostolou E**, Hochedlinger K. Chromatin dynamics during cellular reprogramming. *Nature* 2013; **502**: 462-471 [PMID: 24153299 DOI: 10.1038/nature12749]

121 **Theunissen TW**, Jaenisch R. Molecular control of induced pluripotency. *Cell Stem Cell* 2014; **14**: 720-734 [PMID: 24905163 DOI: 10.1016/j.stem.2014.05.002]

122 **Wernig M**, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007; **448**: 318-324 [PMID: 17554336 DOI: 10.1038/nature05944]

123 **Kim K**, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, Huo H, Loh YH, Aryee MJ, Lensch MW, Li H, Collins JJ, Feinberg AP, Daley GQ. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat Biotechnol* 2011; **29**: 1117-1119 [PMID: 22119740 DOI: 10.1038/nbt.2052]

124 **Tobin SC**, Kim K. Generating pluripotent stem cells: differential epigenetic changes during cellular reprogramming. *FEBS Lett* 2012; **586**: 2874-2881 [PMID: 22819821 DOI: 10.1016/j.febslet.2012.07.024]

125 **Ying QL**, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A. The ground state of embryonic stem cell self-renewal. *Nature* 2008; **453**: 519-523 [PMID: 18497825 DOI: 10.1038/nature06968]

126 **Ficz G**, Hore TA, Santos F, Lee HJ, Dean W, Arand J, Krueger F, Oxley D, Paul YL, Walter J, Cook SJ, Andrews S, Branco MR, Reik W. FGF signaling inhibition in ESCs drives rapid genome-wide demethylation to the epigenetic ground state of pluripotency. *Cell Stem Cell* 2013; **13**: 351-359 [PMID: 23850245 DOI: 10.1016/j.stem.2013.06.004]

127 **Habibi E**, Brinkman AB, Arand J, Kroeze LI, Kerstens HH, Matarese F, Lepikhov K, Gut M, Brun-Heath I, Hubner NC, Benedetti R, Altucci L, Jansen JH, Walter J, Gut IG, Marks H, Stunnenberg HG. Whole-genome bisulfite sequencing of two distinct interconvertible DNA methylomes of mouse embryonic stem cells. *Cell Stem Cell* 2013; **13**: 360-369 [PMID: 23850244 DOI: 10.1016/j.stem.2013.06.002]

128 **Leitch HG**, Tang WW, Surani MA. Primordial germ-cell development and epigenetic reprogramming in mammals. *Curr Top Dev Biol* 2013; **104**: 149-187 [PMID: 23587241 DOI: 10.1016/B978-0-12-416027-9.00005-X]

**P- Reviewer:** Kan L, Leanza G, Perron M **S- Editor:** Song XX

**L- Editor:** **E- Editor:**

****

**Figure 1 The neurogenic niches in the adult murine mammalian brain.** (A)Sagittal view showing the adult mouse subventricular zone (SVZ) and the migrating neuroblasts (red) reaching the olfactory bulb (OB) through the rostral migratory stream (rms). Enlarged view of SVZ: type B1 stem cells (blue) contacting the ventricle with a thin process extended between the ependymal cells (e; gray); type B2 stem cells (blue) contacting the brain parenchyma; transit amplyfing progenitors (TAP) or type C cells (green) give rise to type A cells (red) that migrate through the rostral migratory stream (rms). Dividing stem cells and their TAP progeny are tightly opposed to blood vessels (bv); B: Schematic drawing showing the lineage progression in the SVZ; C: SVZ neural stem cell (NSC) cultures in self-renewal (neurosphere formation) and differentiation. The astrocyte marker GFAP in blue, the neuronal marker III-tubulin in green and the oligodendrocyte marker O4 in red; D: Coronal view showing the adult mouse subgranular zone (SGZ) and the newborn neurons (red) being integrated in the granular cell layer (gr). Enlarged view of the dentate gyrus (DG): Type I stem cells (blue) are GFAP+ and show a radial single prolongation through the granular layer; Type II precursors give rise to neuronal lineage-restricted progenitors type III cells (red) that differentiate into neurons in the granular layer; E: Schematic drawing showing the lineage progression in the SGZ; F: Confocal images showing immunostaining in the DG for the astrocyte marker GFAP in green, for the progenitor precursor TBR2 in red and for the neuronal precursor DCX in red. DAPI is used to stain DNA. Scale bar in C: Top left panel 100 µm, rest 10 µm; In f: 10 µm.

******

**Figure 2 Epigenetic regulation of gene expression.** A: Schematic of DNA methylation and histone modifications in NSCs. DNA is compressed through interactions with histones and methyl groups (M) are added to cytosine-guanine (CpGs) dinucleotides in regulatory regions. Histone methylation reactions are catalyzed by histone methyltransferases (HMTs) and the reverse process is mediated by histone demethylases (HDMs). Histone acetylation is mediated by histone acetyltransferases (HATs) that leads to chromatin decondensation (accessible chromatin) and transcription activation. Histone deacetylases (HDACs) catalyze the reverse process inducing inactivation of transcription (inaccessible chromatin); B: Schematic of DNA methylation at the cytosine-guanine dinucleotides in gene regulatory regions. Methylation reactions are mediated by DNA methyltransferases (DNMTs) that transfer methyl groups (M) to the fifth position of the pyrimidine ring. This is a reversible process mediated by TET1, TET2 and TET3 dioxygenases that catalyze the conversion of the modified genomic base 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) playing a key role in active DNA demethylation; C: Schematic of the histone tail showing multiple sites for epigenetic modifications as acetylation (Ac) or methylation (Me).