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REVIEW

Epigenetic regulation of stemness maintenance in the neurogenic niches

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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

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.

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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]. 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] (Figure 1A-C) and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus[3] (Figure 1D-F). Adult neurogenesis is supported by multipotent neural stem cells (NSCs) deriving from embryonic radial-glia and thus expressing astroglial characteristics[4,5]. 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[2,6,7]. Moreover, their slow division rate can be detected by the label retention of thymidine analogs incorporated during DNA replication[6,8,9]. 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[10,11] (Figure 1C).

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 and pigment epithelium derived factor[12-14]. 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[15,16]. 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[17,18] (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]. 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]. 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 polysialylated neural cell adhesion molecule (PSA-NCAM) 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]. 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], where they differentiate into myelinating and nonmyelinating oligodendrocytes[25].

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]. 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]. 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]. In this region, two types of neural progenitors can be identified according to different expression of molecular markers and their morphologies[23] (Figure 1D and E). Type Ⅰ progenitors exhibit a radial process spanning the granule cell layer and arborizing profusely in

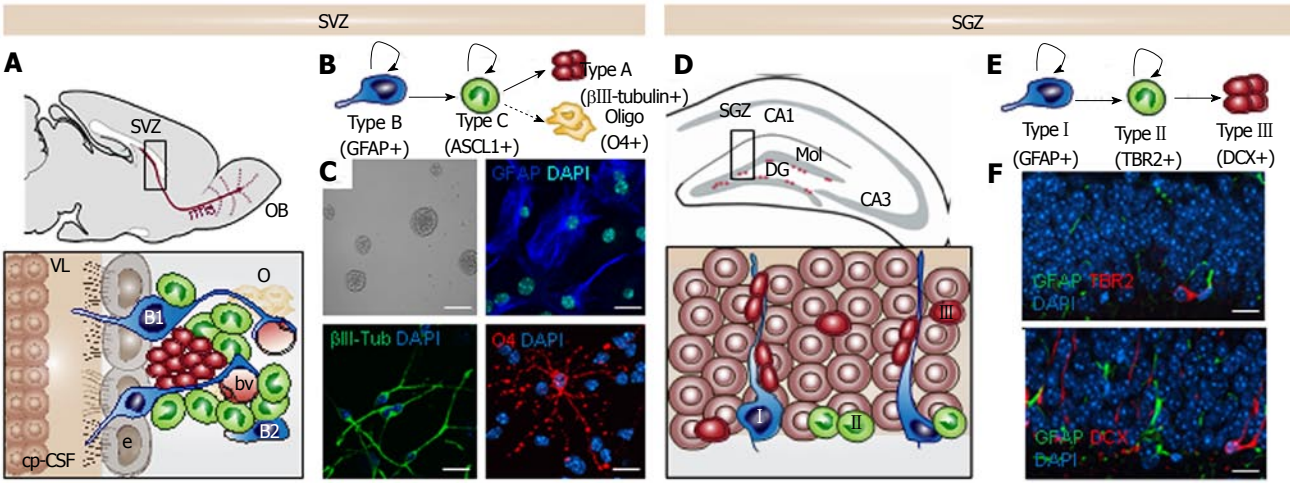

Figure 1 The neurogenic niches in the adult murine mammalian brain. A: Sagittal view showing the adult mouse subventricular zone (SVZ) and the migrating neuroblasts (red) reaching the olfactory bulb (OB) through the rostral migratory stream (rms). Enlarged view of SVZ: type B1 stem cells (blue) express the astrocyte marker glial fibrillary acidic protein (GFAP) and contact 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) express the achaete-scute homolog 1 (ASCL1) transcription factor and 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 βIII-tubulin in green and the oligodendrocyte marker O4 in red; The Choroid plexus-cerebrospinal fluid system (cp-CSF) is shown. 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 marker T-box brain protein 2 (TBR2) in red and for the neuronal precursor marker Doublecortin (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.

the molecular layer[27]. These cells express nestin, GFAP, and Sox2[31]. 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]. 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]. 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[32,33] 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[34,35]. 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[36,37] (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]. These different classes of epigenetic modifications are intimately related, resulting in multiple layers of control allowing cells to maintain their identity over time[34,38]. 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] where it promotes a closed chromatin structure and aids the prevention

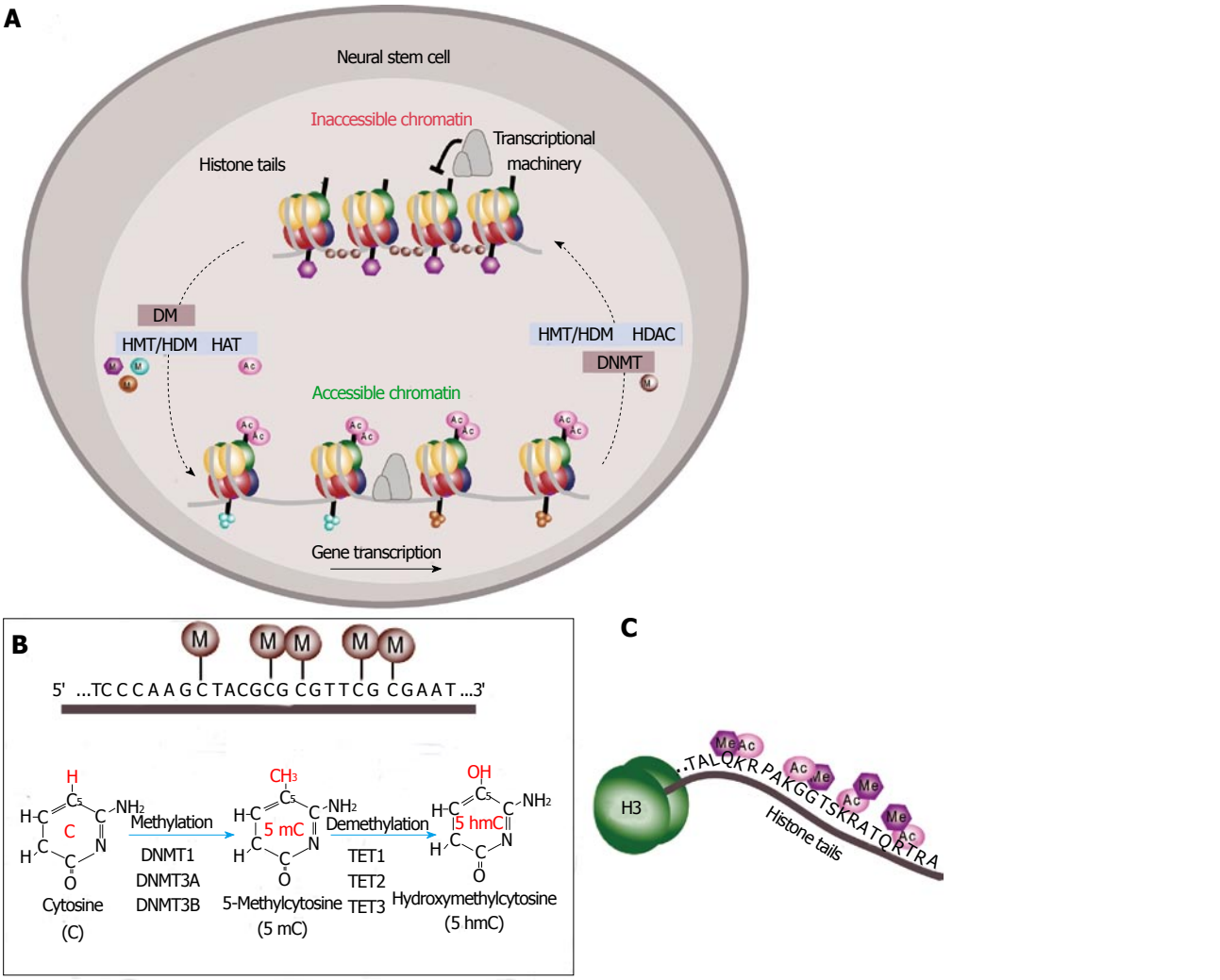

Figure 2 Epigenetic regulation of gene expression. A: Schematic of DNA methylation and histone modifications in neural stem cells (NSCs). DNA is compressed through interactions with histones and methyl groups (M) are added to cytosine-guanine (CpGs) dinucleotides in regulatory regions. Histone methylation 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 the ten-eleven translocation (TET) family of enzymes 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).

of expression[34,40]. 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]. 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].

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[45,46]. 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]. A lack of DNMT1 alters neuronal excitability and increases apoptosis in post-mitotic cortical neurons[50]. 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]. DNMT3a and DNMT3b are highly expressed in postnatal

NSCs and are required for neurogenesis and neuronal maturation[48,49,52,53]. Loss of DNMT3a results in gene silencing[53] and depletion of DNMT3b leads to deficient neuronal differentiation *in vitro*[54].

DNA is hypomethylated in neural progenitor cells and methylation is progressively increased during lineage commitment[55]. The suppression of astrogliogenesis during neuronal specification is also associated with changes in DNA methylation[56,57]. 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]. 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[60,61]. A similar alteration in methylation pattern occurs at another STAT3 binding site in the *S100β* promoter[58].

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] (Figure 2B). In mammals, three members of the ten-eleven translocation (TET) family of enzymes have been identified: TET1, TET2 and TET3[63,64]. TET hydroxylases may catalyze active DNA demethylation by oxidation of 5mC to 5hmC[65-67] (Figure 2B). 5hmC is relatively abundant in mouse embryonic stem cells (ESCs), the early embryo and in adult brain[68,69]. In the brain, 5hmC is enriched at active genes, associated with the strong depletion of 5mC from these regions[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[69,71]. Recent studies have also shown that TET1 is involved in the epigenetic regulation of neural progenitor cell proliferation in the adult hippocampus[72-74]. Mice lacking *Tet1* exhibit impaired hippocampal neurogenesis accompanied by poor learning and memory[72-74]. 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[75,76]. 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] (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]. 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 enhances the recruitment and activation of the transcriptional machinery and is generally associated with areas of active gene transcription[78]. However, histone deacetylases (HDACs) remove acetyl groups promoting the condensation of chromatin[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]. Deletion of HDAC2 in the SVZ results in a defective neurogenesis to the OB[81] and neurospheres treated with class Ⅰ and Ⅱ HDAC inhibitors promotes neuronal differentiation[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] and at neuronal genes such as *Sox2*[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 (H3K27me^3 and H3K9me^3) tends to associate with regions of inactive gene transcription, whereas H3K4, H3K36 and H3K79 methylations are associated with active transcription[85]. Histone demethylases (HDMs) also have a key role in regulating neural development[86]. 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]. 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]. Moreover, H3K9me^3 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]. 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 H3K27me^3 on the promoter indicating that *Mll1* is recruiting a H3K27 demethylase[90]. In summary, the above studies indicate that different chromatin modifiers have a critical role in adult neurogenesis[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]. 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]. 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]. 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 and Prader-Willi syndrome are all characterized by neurological and behavioral impairments and learning difficulties[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]. Notably, this selective absence of *Dlk1* imprinting is associated with acquisition of DNA methylation at the germline-derived imprinting control region[96]. *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[97,98]. Thus, CSF produced from the choroid plexus and blood vessels is a biallelic source of neurogenesis-promoting IGF2[99].

Epigenetic changes during NSCs reprogramming to induced pluripotent stem cells

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]. Reprogramming involves changes in the transcriptome and chromatin state of the reprogrammed cell type to that of a pluripotent cell[101-103]. This implicates different levels of changes in DNA factor binding, transcription and chromatin state[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], 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]. 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 synthesized 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 methy-

lation 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 show transcriptional 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 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.

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