·Minireview·

Epigenetics and neural stem cell commitment

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Abstract: Neural stem cell is presently the research hotspot in neuroscience. Recent progress indicates that epigenetic modulation is closely related to the self-renewal and differentiation of neural stem cell. Epigenetics refer to the study of mitotical/meiotical heritage changes in gene function that cannot be explained by changes in the DNA sequence. Major epigenetic mechanisms include DNA methylation, histone modification, chromatin remodeling, genomic imprinting, and non-coding RNA. In this review, we focus on the new insights into the epigenetic mechanism for neural stem cells fate.

Key words: stem cells; epigenesis; neuron restrictive silencer element; genomic imprinting; *H19*; non-coding RNA

Epigenetics, a newly arising subject in genetics, refers to the heritable changes of gene function that would ultimately result in cellular specification transformation without altering the genome sequence. In 1942, Waddington CH firstly raised the term "epigenetics", pointing out that it was related to genetics and mainly dealt with the links between genetic identity and epigenetic identity. Later, Holiday R drew more systemic conclusion that the objective of epigenetics was to study the heritable pattern of gene expression change without DNA sequence alteration[1]. The epigenetic mechanisms cover a wide range, containing DNA methylation, histone modification (such as acetylation), chromatin remodeling, genomic imprinting, and regulatory non-coding RNAs. These epigenetic factors are closely related to regulate gene expression (Fig. 1). Recently, more and more investigations have shown that epigenetic modifications play important roles in neural stem cell (NSC) commitment[2,3], which would be discussed in this review.

1 DNA methylation

One class of epigenetic modifications is DNA cytosine methylation, which consistently associated with diverse gene regulatory processes, for instance, genomic imprinting[4]. In vertebrates, DNA methylation mainly takes place at CpG dinucleotides. Clusters of CpG dimer known as CpG islands are located on the region of gene promoter. During the process of DNA methylation, cytosine is out of DNA double helix and enters the split that binds to the cytosine methyltransferase. The latter transfers the methyl of s-adenosylmethionine (SAM) to the 5' of cytosine, forming 5-methyl-cytosine. DNA methylation is carried out by the DNA methyl transferase (DNMT) protein family, which is split into three kinds in mammals: DNMT1, DNMT2 and DNMT3. DNMT3a and DNMT3b[5] was originally described as *de novo* methyltransferases; DNMT1 could maintain methylation; and DNMT2 was found as a homologue to the Schizosac-charomyces pombe DNA methyltransferase.

DNA methylation-mediated gene silencing is regulated through two mechanisms: 1, the methylation at CpG sites blocks transcription factor binding and leads to transcriptional inactivation; and 2, methyl-CpGs are bound by a family of methyl-CpG binding proteins (MBDs), including MBD1–4 and MeCP2. The MBDs binding and the further recruitment of histone deacetylase (HDAC) repressor complexes result in histone deacetylation and the inactivation of chromatin structures responsible for transcription repression. The mice deficient in MBD1 exhibit decreased neurogenesis and reduced long-term potentiation in the dentate gyrus of the hippocampus[6]; the mice lacking

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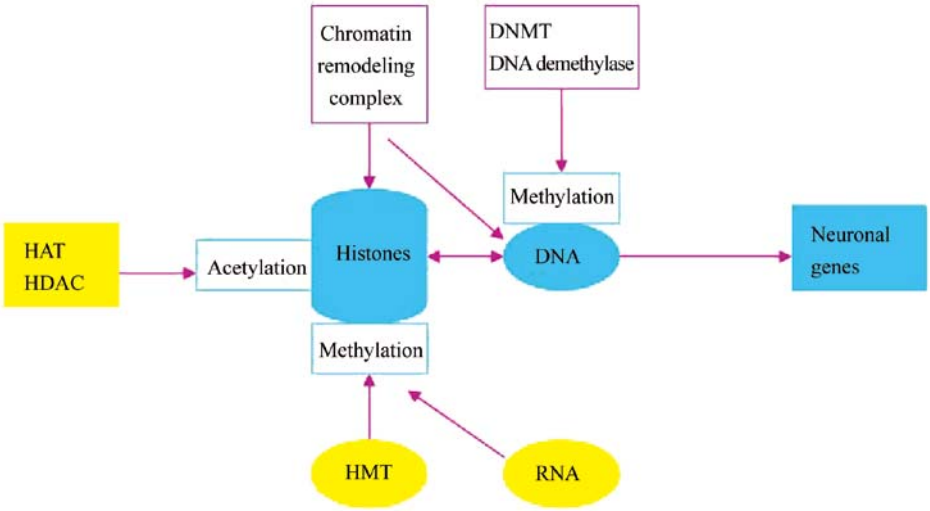

Fig.1 Schematic representation of epigenetic modifications in neural differentiation. DNA methylation [mediated by DNA methyl transferase (DNMT)], histone modification [associated with histone deacetylases (HDACs) and histone acetyltransferases (HATs)], chromatin remodeling and microRNAs (miRNAs) are all interlaced to promote neuronal genes expression during neural development and plasticity. HMT: histone methyltransferase.

MBD2 show mild maternal behavior deficits; the MBD3$^{-/-}$ mice die at an early embryonic stage[7]; and the mice deficient in MBD4 show deficits in DNA repair and increased tumor formation[8]. In human, mutations of MeCP2 would cause neurological deficits (Rett syndrome)[9] (Fig. 2A).

There are also increasing reports describing the relationships of DNA methylation-mediated transcription and neuronal plasticity[10,11]. Studies revealed that neurons could make use of epigenetic regulations, such as DNA methylation, to modulate gene expression in response to environmental or extracellular signals.

2 Histone modification

The role of chromatin structure modifications, particularly histone modification, in the inheritable changes of gene expression is the current research focus as well[12,13]. Histone covalent modifications involve methylation, acetylation, phosphorylation, ADP ribosylation, ubiquitination, and sumoylation[14,15]. These modifications are called "histone code", because they mark chromatin states and can be specifically recognized by other proteins.

Much has been presently known that the lysine acetylation is mediated by two enzyme groups: histone acetyltransferases (HATs) and histone deacetylases (HDACs). The process is reversible. HATs transfer the acetyl from acetyl coenzyme A to the ε-amino radical of the certain lysine residue within the N-terminal of core histones, by which decreases the interaction between the positively charged histone tails and the negatively charged phosphate backbone of DNA, resulting in nucleosomes relaxation and exposure of transcription factors to DNA, thus

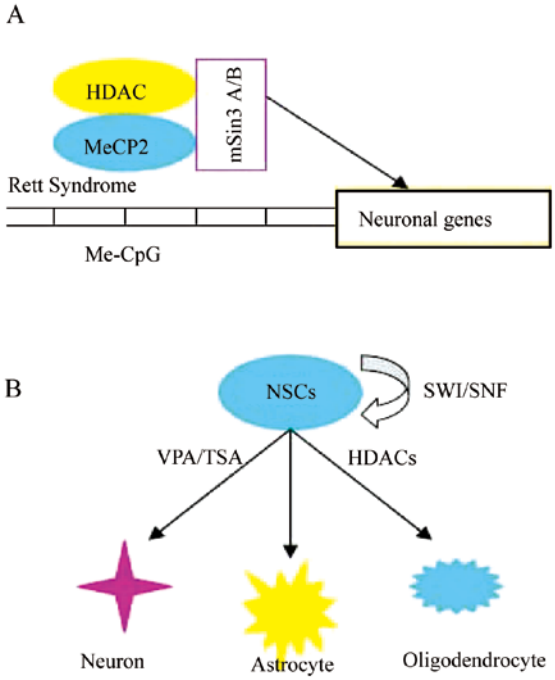

Fig.2 Epigenetic factors involved in neural stem cells (NSCs) differentiation. A: MeCP2 binds to methylated CpG sites and recruits the repressor complex to inactivate transcription. B: Switching/sucrose non-fermenting (SWI/SNF) complex promotes NSCs self-renewal and differentiation. Histone acetylation induces neuronal differentiation, but inhibits astrocyte and oligodendrocyte differentiation. VPA: valproic acid, TSA: Trichostatin A, HDAC: histone deacetylase.

activate the transcription of specific genes. On the contrary, HDACs catalyze the adverse reaction, i.e. prevent promoters from accessing to the transcription regulation element and repress gene transcription (Fig. 2B). Based on homology to the yeast proteins, HDACs are divided into 3 classes: class I[13,16,17], II[18-20] and III.

The functional relevance of histone H3 lysine methylations depends on their amino acid position: the methylation at lysine 4 (K4) leads to transcriptional activation, while the methylation at lysine 9 (K9) is associated with transcriptional silencing[16]. Researchers have shown that the process of cortical progenitor cells differentiating into astrocytes is accompanied by the induction of histone H3 K4 methylation and the suppression of histone H3 K9 methylation in GFAP promoter[21]. In addition, levels of histone H3 trimethyl K9 and histone H4 monomethyl K20 are elevated in the proliferating cells of neural tube, whereas histone H4 K20 trimethyl derivatives are enriched in differentiating neurons[13].

To investigate the effect of histone deacetylation, components that act as inhibitors have been studied in many researches, e.g. Trichostatin A (TSA) and valproic acid (VPA)[22]. Blocking the global HDAC activity inhibits the differentiation of embryonic stem (ES) cells[23] and the progression of oligodendrocyte progenitors into the mature oligodendrocytes[24], and promotes the differentiation of adult hippocampal progenitor into neurons[25].

3 Genomic imprinting

Genomic imprinting is an epigenetic mechanism by which certain genes are repressed at one of the two parental alleles, in other words, imprinted genes are expressed in a parent-of-origin-dependent manner. The *H19/Igf2* locus is a representative of genomic imprinting. *H19* and *Igf2* are closely located and share the same regulation mechanism. It has been shown that *H19/Igf2* plays important roles in mammalian development, tumorigenesis[26,27], and embryonic stem cells.

3.1 The structure and the expression of *H19/Igf2* *H19/Igf2* locus is conserved on mouse chromosome 7 and human chromosome 11. *H19* and *Igf2* are coordinately regulated, and harbor the common expression pattern and the reciprocal imprinting, i.e. *H19* is expressed from the maternally inherited allele (paternal imprinting), whereas *Igf2* is paternally transcribed (maternal imprinting).

The human *H19/IGF2* is located on chromosome 11p15.5. There is only 70 kb apart between the two genes, with *H19* on the downstream of *IGF2*. The 3-kb long *H19* gene is composed of five exons and four introns. It is suggested that *H19* produce RNA but not protein, to exert roles at the RNA level. Thus, *H19* is described as a constitutive element of ribose regulation factors. The length of insulin-like growth factor-2 gene (*IGF2*) is 8.8 kb, with 9 exons, 8 introns, and 4 promotors (P1-P4). *H19/IGF2* genes are expressed widely during embryonic development in identical tissues and are downregulated shortly after birth. However, in some brain regions, such as choroid plexus, *Igf2* is expressed but *H19* is not[26,27].

3.2 Mechanism of imprinting The regulation mechanism of imprinted expression of the *H19/Igf2* can be explained by the current postulated "boundary model" (Fig. 3). Differentially methylated regions (DMRs, of *Igf2*), also called imprinting control regions (ICRs), are essential for the process. DMD (differentially methylated domain, of *H19*) is located on the 5' of *H19* and rich in CpG dinucleotides.

DMR/DMD represents different methylation status of two parental alleles. On the maternal allele, the DMD is unmethylated and bound by the zinc finger protein CTCF, which acts as a boundary/insulator element[30,31]. This blocks the access of *Igf2* to the downstream enhancers, which are thus targeted to the *H19* promoter, resulting in *H19* expression from the maternal chromosome (Fig. 3). The unmethylated DMR1 also acts as the silencer to repress the maternal transcription. While on the paternally inher-

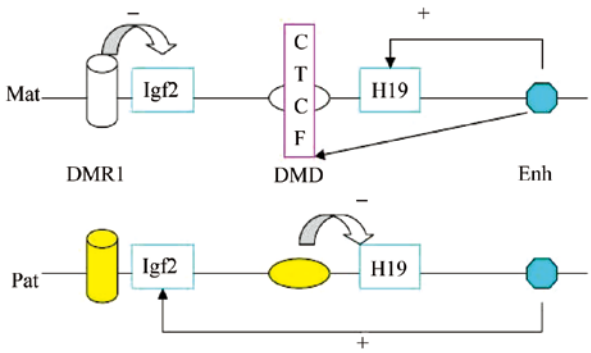

Fig.3 Boundary model of *H19/Igf2* imprinted gene regulation. On the maternal allele (Mat), CTCF binds to the unmethylated *H19* DMD to form a boundary/insulator and blocks the downstream enhancer Enh to act on *Igf2*, thus Enh can only interact with *H19* promoter and activate the maternal transcription (+). On the paternal allele (Pat), the DMD is methylated and prevent the CTCF binding and boundary formation. This permits Enh to interact with *Igf2* and activate paternal transcription (+). DMD: differentially methylated domain.

ited allele, the DMD is methylated, preventing the CTCF binding and the subsequent boundary formation. This permits the downstream enhancers freely interact with *Igf2*, resulting in *Igf2* expression from the paternal chromosome [32]. In addition, the methylated DMD also acts as the silencer to repress the transcription of the paernal *H19* allele.

Kono *et al*. strongly demonstrated that *H19* played a crucial role in development[33] and *cis*-regulation of *Igf2* expresstion. Additionally, the abnormality of *H19-IGF2* imprinting is linked to tumorigenesis. An extensive study of Wilms' cases showed that *IGF2* was biallelically expressed but *H19* was fully repressed, with the DMR of *H19* being hypermethylated on both alleles. Likewise, recent studies indicated that the microdeletions of the *H19* DMR in the Beckwith-Wiedemann syndrome (BWS) patients led to loss of imprinting, with the biallelic expression of *Igf2* and the silencing of *H19*[34,35]. Rugg-Gunn PJ's research on the epigenetic control of embryonic stem cell fate enlighten us on the impact of *H19/Igf2* on the NSCs proliferation and differentiation[28,29]. This issue will also be an important research topic in our lab in the future.

4 Chromatin remodeling

Modulations of histone-DNA interactions in nucleosomes are termed chromatin remodeling, accounting for another critical mechanism for the regulation of stem cell biology, cellular specification[36], and even neuronal plasticity[12]. Based on the ATPase unit and additional protein motifs, chromatin remodeling complexes are divided into several subgroups in mammals: switching (SWI; also known as sucrose non-fermenting, SNF), imitation switch (ISWI), Mi-2/NuRD, and other complexes[37]. Among them, the SWI/SNF complexes, which consist of one or more catalytic subunits: Brahma (Brm), Brahma-related gene 1 protein (Brg1) or Breastovarian cancer susceptibility protein 1 (Brca1), and polycomb repressors (Bmi-1), play roles in neural development. Muller *et al*. found that the SWI/SNF complexes were also associated with the cell differentiation and cell cycle arrest mediated by C/EBPα[38].

The SWI/SNF complexes interact with HATs or HDACs and/or sequence-specific transcription factors to activate or repress the target genes. Kondo *et al*. suggest that the conversion of oligodendrocyte precursor cells to neural-stem-like cells is associated with recruitment of the Brca1 and Brm (the catalytic subunit in a subset of SWI/SNF) to the promoter of a key transcription factor gene *Sox2*, which involves in maintaining the proliferation of NSCs[39] by methylation at K4 and acetylation at K9 in the histone H3 of the promoter.

Studies indicate that Brg1 may play important roles in neural development. Seo S *et al*. showed that loss of Brg1 was correlated with the augment of proliferating neural progenitors and the expansion of *Sox2*-positive cells in embryos at the later stage of neurogenesis[40]. Additionally, Brg1 was found to be associated with two proneural bHLH proteins—neurogenin-related-1 and NeuroD, and was required for these proneural genes to drive neurogenesis.

Furthermore, Bmi-1, the polycomb family transcriptional repressor, is required for post-natal maintenance of stem cells in multiple tissues including the central nervous system. Studies showed that Bmi-1 prevented the premature senescence of neural stem cells by repressing Ink4a and Arf, but additional pathways must also function downstream[41].

In addition to the effects on neural fate specification, chromatin remodeling is associated with neuronal plasticity. Recent studies reveal that different drugs induce the transient phosphorylation at serine 10 and acetylation at lysine 14 of histone H3, as well as up-regulation of *c-fos* transcription in the hippocampal neurons[42].

5 Non-coding RNAs

In recent years, the emerging roles of non-coding RNAs in gene expression have gained lots of attention[43]. Small non-coding RNAs, including small interfering RNAs (siRNAs) and micro RNAs (miRNAs), are involved in neuron restrictive silencer element or repressor element-1 (NRSE/RE-1), and neuronal restricted silencing factor or RE-1 silencing transcription factor (NRSF/REST) interaction. MiRNAs are novel epigenetic regulation factors[44] and play important roles in diverse biological processes, such as developmental timing and patterning in *Caenorhabditis elegans*[45] and apoptosis in *Drosophila melanogaster*[46]. Evidences also support the idea that miRNAs may be essential during late vertebrate development[47,48], particularly for lineage differentiation of various tissue types[49,50]. These highly conserved regulatory molecules have been isolated from animals, plants, yeasts and others[51,52], and they modulate gene expression posttranscriptionally by targeting mRNAs for translational repression or degradation.

5.1 The structure of miRNA Researches indicate that miRNAs are produced from larger primary transcripts origi-

nated from distinct genes[53]. The primary transcript undertakes two processing steps: generating stem-loop precursor transcript that is exported to the cytoplasm, and subsequently shaping it into the 21-23 nt miRNA. One strand of mature miRNA is preferentially incorporated into the RNA-induced silencing complex. This effector complex is then guided by miRNA to target the mRNAs bearing miRNA recognition motifs in their 3' untranslated region (3'UTR). It is suggested that most of the currently characterized small, non-coding RNAs play roles in transcriptional gene silencing. Kuwabara *et al*. reported that non-coding RNAs could also function in activating neuron-specific gene expression[54].

5.2 NRSE/NRSF interaction Many neuronal genes are repressed in the non-neuronal cells by a conserved neuron restrictive silencer element or repressor element-1 (NRSE/RE1), which interacts with a zinc-finger protein known as neuronal restricted silencing factor or RE-1 silencing transcription factor (NRSF/REST). NRSF mediates silencing of target genes through recruitment of HDACs by the corepressors associated with its repressor domains, hence negatively regulate gene transcription[55].

Discovered in 1995, the NRSE sequence is a 23-bp conserved motif[56,57] matching a large number of neuronal genes including axon-guiding molecules, ion channels, synaptic vesicle proteins, and neurotransmitter receptors[58,59]. NRSE mainly acts as a silencer mediating the transcriptional inhibition of neuron-specific genes. NRSF is a 116-kD zinc-finger protein, belonging to the Gli-Krüppel family. There is a cluster of eight zinc finger repeats near its N-terminus, followed by a region rich in basic amino acids, a cluster of six proline-rich repeats, and a single zinc-finger near the C terminus. Su *et al*. showed that the NSCs transfected with recombinant transcriptional factor NRSF-VP16 could directly activate the target genes inhibited by NRSF and efficiently induce NSCs to differentiate into neurons[60].

NRSE/NRSF can mediate transcriptional repression through associating with the mSin3A/B complex[61], N-CoR[62], and the novel co-repressor complex[55] CoREST/HDAC2[63]. CoREST can recruit other silencing machinery to NRSF target genes, including the methyl-DNA binding protein MeCP2, heterochromatin protein 1, and histone lysine methyltransferase (the suppressor of variegation 39H1)[59]. The transcriptional repression mediated by the N-terminus of NRSF is via recruiting mSin3A/B and a HDACs complex. HDACs deacetylate the lysine residue of nucleosomal core histones which consequently limit the accessibility of DNA to the transcription factors and RNA polymerase, resulting in transcriptional inhibition. The demethylating agents such as 5-aza-2'deoxycytidine (5-aza-CdR) and its homologues or valproic acid (VPA) are usually used for studying gene regulation[64]. Researches indicate that TSA can alleviate the transcription repression of the genes containing NRSE[61]. Hsieh *et al*.[25] found that adult multipotent neural progenitor cells predominantly differentiated into neurons but not glia since glial differentiation was actively suppressed in the presence of VPA (Fig.2B).

5.3 The regulative mechanism of non-coding RNAs Kuwabara and colleagues found that the sequence of a small, non-coding RNA matched *NRSE* gene and formed double-stranded (dsRNA) molecules (Fig. 4). The *NRSE* dsRNA may interact with NRSF protein to regulate the generation of neurons at the transcription level. This neuron fate commitment mechanism is different from RNA interference (RNAi)[54,65]. In RNAi, the miRNAs or siRNAs mainly prevent gene expression by inhibiting the transcription of target mRNA, or forming duplexes with mRNA, which are then degraded by RNA endonucleases.

Kuwabara *et al*. proposed a model wherein the *NRSE* dsRNA directly associated with NRSF and converted the NRSF from a repressor to an activator complex, perhaps by triggering a conformational change to prevent its association with co-repressor proteins, hence the activation of neuron-specific genes. Furthermore, Ballas and colleagues suggested two separated models for explaining the regulation of NRSF to the neuronal genes during embryonic and adult neurogenesis[66].

Studies revealed that non-coding miRNAs represent yet another set of target genes for NRSF. Cecilia and colleagues demonstrated the reciprocal actions of NRSF, and proved that miRNAs could promote neuronal identity[67]. All of these data suggest that small, non-coding RNAs have diverse modulation functions in development and stem cell biology, and they regulate cell behavior at both transcriptional and posttranscriptional levels[54].

6 Perspectives

Investigations on adult neurogenesis in past decades have paved the way for treatment of the neurological degeneration disorders and the functional reconstruction after traumatic brain injuries[68]. However, the number of tissue-

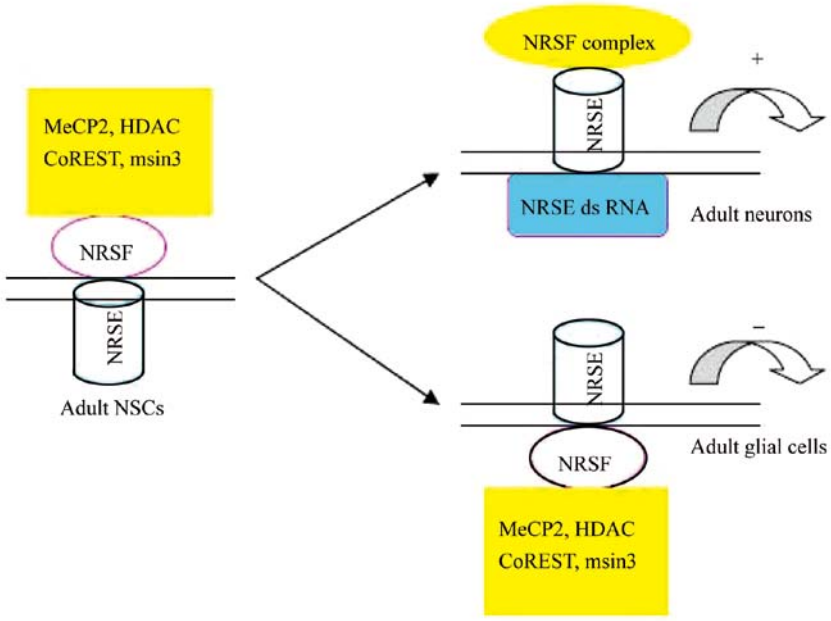

Fig.4 Illustration of the NSC differentiation regulated by NRSE dsRNA. The non-coding RNA forms NRSE dsRNA, directly acts on NRSF and converts it from a repressor to an activator, allowing adult NSCs differentiating. While, in glial cells, NRSE remains to be repressed by the NRSF complex.

specific stem cells in adult central nervous system is very limited for cell replacement therapy. Recent studies indicated that epigenetic modification might provide alternative sources of NSCs from other cell types[69].

Epigenetic mechanism could coordinately activate and inhibit the expression of related genes in certain developmental stage of stem cells[64]. The concept of epigenetics is opposite to genetics. Genes usually contain two kinds of inheritable information, one is traditional genetic message, which is delivered by the DNA sequence; the other is epigenetic message, and it instructs when, where and how the genetic messages should be applied. Epigenetic modulations have deepened and widened the study on NSC biological behaviors, e.g. proliferation and differentiation. Although many problems remain to be resolved, epigenetic regulation on NSC fate will definitely ensure a bright future for application of adult NSCs.

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表观调控与神经干细胞分化

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摘要：神经干细胞是当前神经科学领域的研究热点。最近的研究显示表观调控与神经干细胞的分化关系密切，而且为神经干细胞的移植治疗提供可能的细胞来源。表观调控是指在基因的DNA序列未改变的情况下，基因功能发生可遗传的变化而导致细胞表型发生改变，主要机制包括 DNA 甲基化、组蛋白修饰、基因印迹、染色体重组以及非编码小 RNA 等。本综述就表观调控对神经干细胞分化作用的最新进展作一回顾。

关键词：干细胞；表遗传；神经元限制性沉默元件；基因印记；*H19*；非编码 RNA