Published in final edited form as:

Prog Neurobiol. 2015 January ; 0: 28–48. doi:10.1016/j.pneurobio.2014.11.001.

Epigenetic Programming of Hypoxic-Ischemic Encephalopathy in Response to Fetal Hypoxia

Qingyi Ma and Lubo Zhang

Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA

Abstract

Hypoxia is a major stress to the fetal development and may result in irreversible injury in the developing brain, increased risk of central nervous system (CNS) malformations in the neonatal brain and long-term neurological complications in offspring. Current evidence indicates that epigenetic mechanisms may contribute to the development of hypoxic/ischemic-sensitive phenotype in the developing brain in response to fetal stress. However, the causative cellular and molecular mechanisms remain elusive. In the present review, we summarize the recent findings of epigenetic mechanisms in the development of the brain and their roles in fetal hypoxia-induced brain developmental malformations. Specifically, we focus on DNA methylation and active demethylation, histone modifications and microRNAs in the regulation of neuronal and vascular developmental plasticity, which may play a role in fetal stress-induced epigenetic programming of hypoxic/ischemic-sensitive phenotype in the developing brain.

Keywords

DNA methylation; DNA demethylation; histone modifications; micro RNAs

1. Introduction

Growing evidence from clinical and pre-clinical studies has clearly elucidated that intrauterine stress may have subtle or drastic impact on tissue/organ ontogeny, structure and function during the fetal development, leading to enhanced vulnerability or resiliency to challenges and diseases later in life (Dudley *et al.*, 2011; Xiong and Zhang, 2013). The developing brain in the gestational stage is highly plastic and vulnerable to various adverse environmental conditions. Among these, hypoxia is of critical importance and may occur in various conditions, such as pregnancy at high altitude, placental insufficiency, pregnancy anemia, and other maternal diseases. Exposure of the fetus to persistent hypoxia can cause

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Corresponding author: Lubo Zhang, PhD, Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University, School of Medicine, Loma Linda, CA 92350, Tel: 909-558-4325, Fax: 909-558-4029, lzhang@llu.edu.

Conflict of interest: None

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abnormal development of the brain *via* series of direct or indirect actions at cellular and molecular levels. As a major consequence, fetal hypoxia increases the risk of central nervous system (CNS) developmental malformations and may result in the development of neurological diseases in offspring (Gonzalez-Rodriguez *et al.*, 2014; Li *et al.*, 2012). However, the causative mechanisms of fetal hypoxia affecting the developing brain remain largely elusive.

Epigenetic modifications result in stable and heritable gene expression patterns without changes in the coding sequences and are the important mechanisms in developmental programming of health and disease (Chen and Zhang, 2011; Egger *et al.*, 2004; Gluckman *et al.*, 2008). Epigenetic mechanisms mainly include DNA methylation/demethylation, histone modifications and non-coding RNAs such as microRNAs, which regulate lineage-specific expression profiles of different cell types and the information for the transcriptional program of gene expression (Cantone and Fisher, 2013). Epigenetic modifications are very sensitive to various environmental stimuli, and are essential in controlling proper gene expression patterns in particular tissues at specific time points in response to endogenous or environmental signals (Cantone and Fisher, 2013; Gicquel *et al.*, 2008; John and Lefebvre, 2011). The brain is the central organ responsible for stress responses, and undergoes parallel alterations in its structure and function in response to stress events (McEwen, 2008). There is increasing evidence suggesting that epigenetic machinery orchestrates the development, plasticity, homeostasis and evolutionary innovations of the brain (Feng *et al.*, 2007; Mehler, 2008; Mehler and Mattick, 2007; Tsankova *et al.*, 2007). Recent studies suggest that epigenetic programming in response to fetal hypoxia is responsible for the development of hypoxic-ischemia sensitive phenotype in the brain (Gonzalez-Rodriguez *et al.*, 2014; Li *et al.*, 2012), implicating a key role of epigenetic modifications in fetal stress-mediated developmental programming of neuronal and vascular dysfunctions in the brain.

During the fetal development, the neuronal organization is remodeled and developed into the adult phenotype and functional properties, which is affected by intrauterine environment (Fagiolini *et al.*, 2009). In addition to neurons, vascular development is also a critical portion of the development and maturation of the CNS by providing metabolic nutrition, physical support and protection to the developing brain. The neurovascular cross-talk is initiated in the early stage of embryonic development and lasts throughout the life. For example, in the mouse the blood-brain barrier is functional at E15.5 (Ben-Zvi *et al.*, 2014) and protects the fragile CNS tissue from metabolic and cellular changes (Bautch and James, 2009). Neurovascular coupling is developed to undertake important physiological functions, such as the regulation of cerebral blood flow (CBF) in response to local neural activities, and its abnormality implicates various brain diseases, such as cerebral cavernous malformations and vascular dementia (Attwell *et al.*, 2010; Iadecola, 2004).

Recently, “active” DNA demethylation has been brought forward and is under extensive study in the developmental plasticity of the brain. Its role has been revealed in regulating a series of gene expression and cell fate determination in neural progenitor cell differentiation, which eventually determines the brain growth and function during the embryogenesis (Miller and Sweatt, 2007; Wheldon *et al.*, 2014). Moreover, the epigenetic mechanisms, i.e. DNA methylation/demethylation, histone modifications and miRNAs interact and determine

the development of the brain. Nonetheless, relatively little is known about the epigenetic mechanisms in fetal stress-mediated programming of the brain susceptibility to neurological dysfunction. Indeed, most current studies in stress-induced fetal programming of developmental abnormalities in the brain have focused on long-term functional outcomes. Few investigated molecular and cellular mechanisms underlying the effect of fetal hypoxia on the lineage specification of neural stem cells, neurogenesis, vasculogenesis, astrogliogenesis, and the interaction and communication of neuron, vascular and glial cells in the developing brain. In the present review, we summarize the current knowledge of epigenetic mechanisms in regulating the development of the brain, specifically neuronal and vascular development, and propose that the epigenetic programming of the developing brain at the cellular and molecular levels in response to fetal hypoxia may result in abnormal neuronal and vascular development, leading to the enhanced susceptibility of immature brain to postnatal hypoxic-ischemic encephalopathy (HIE). Of importance, findings in these studies may help provide insights in the design of effective diagnostic and therapeutic strategies in preventing and managing the adverse effects of fetal stress on the development of the brain.

2. Fetal hypoxia and hypoxic-ischemic encephalopathy

Hypoxia during gestation contributes to developmental malformations in the fetus. Exposure to severe hypoxia *in utero* results in acute neuronal and glial injury, an increase in apoptosis, and a reduction of brain growth and neural complexity, consequently contributing to chronic functional deficits in the brain. Fetal hypoxia can cause brain injury in both white matter and grey matter, and prenatal white matter damage is regarded as the main stream of developing brain injury. It has been classified into two subtypes: one is cystic periventricular leucomalacia (PVL) in which the necrotic lesions manifest in the periventricular white matter surrounded by astrogliosis and microgliosis, and the other type is non-cystic PVL in which the necrotic lesions are more diffused and are associated with activated glia. In addition to the white matter, the cortical and subcortical grey matter may also be significantly influenced by fetal hypoxia. One reason is that cerebral white matter damage may interrupt afferent and efferent cortical connections, therefore potentially result in cortical neuronal damage (Rees *et al.*, 2011). This notion has been confirmed by animal studies showing that severe acute hypoxia causes an identical pattern of subcortical white matter and gray matter injury in preterm fetal sheep at 0.6 and 0.7 of gestation with the term being 147-150 days (Bennet *et al.*, 2007; Dean *et al.*, 2006; George *et al.*, 2007).

Severe fetal hypoxia, whatever its cause, is widely considered to be a major factor leading to fetal brain injury (Volpe, 2009). Pontosubicular neuron necrosis is a form of brain circulation deficiency in perinatal period and often co-exists with other forms of cerebral hypoxic-ischemic injuries, such as PVL. Using histological methods, Takizawa and colleagues found pontosubicular neuron necrosis in six human premature infants who died 5.2 days after birth from perinatal hypoxia or respiration insufficiency (Takizawa *et al.*, 2006). Moreover, a large number of apoptotic cells and increased expression of activated caspase 3 were also observed (Takizawa *et al.*, 2006). Growing evidence indicates that fetal brain injury depends on the nature and severity of the insults and the gestational stage of the fetus during the insults. In sheep, the neurogenesis and neural migration reach the peak at

early-mid gestation, therefore, acute hypoxia at this time in fetal sheep slows neural migration in the hippocampus leading to neuronal cell death in pyramidal cells of the hippocampal CA1 region and in the cortical neurons (Rees *et al.*, 1999; Rees *et al.*, 1997). Similar to acute hypoxia, lipopolysaccharide (LPS), a bacterial endotoxin induces a transit hypoxemic effect in the brain and results in cerebral white matter injury ranging from diffused subcortical damage to PVL (Duncan *et al.*, 2002). In late gestation fetal sheep, acute hypoxemia caused by umbilical cord occlusion resulted in neuronal death, which mostly occurred in periventricular white matter. Excessive glutamate release may be involved in this damage process (Loeliger *et al.*, 2003). The consequences of exposure to long-term hypoxemia differ from those caused by acute insults in several aspects including a broad white matter injury and the reduction of brain weight. Using an umbilicoplacental embolization model in sheep, Dunca and colleagues found a marked reduction of the combined width of stratum oriens and pyramidal in the dorsal hippocampus besides structural abnormalities in the brain and retinae. Correspondingly, gliosis in either the cerebral white matter or the cerebellum and degeneration around blood vessels were also observed (Duncan *et al.*, 2004). Taken together, these studies confirm the conception that perinatal hypoxia contributes to fetal brain injury not only in periventricular white matter but also in gray matter, depending on the severity and duration of hypoxia and gestational stage as well.

Multiple mechanisms have been invoked to explain developing brain injury caused by hypoxia. Many of these envision a process that the reduced oxygen delivery to the developing brain causes an energy depletion and thereby leads to a loss of stem or progenitor cells and increased apoptotic cell death (Barrett *et al.*, 2007; Rees *et al.*, 2011). A sequence of events is associated with this process, including glutamate accumulation in the synaptic cleft and N-methyl-D-aspartate receptors (NMDA) over-activation, high concentrations of intracellular calcium, stimulation of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and reactive nitrogen species (RNS) such as nitric oxide (NO) and peroxynitrite ($ONOO^-$). These events will further affect mitochondrial function, and result in the release of cytotoxic enzymes and pro-apoptotic proteins. Considering that the immature oligodendrocytes predominantly distributed in the developing periventricular white matter are vulnerable to glutamate, cytokines and free radicals (Back *et al.*, 1998; Back *et al.*, 2002; Riddle *et al.*, 2006), all these events will heighten the susceptibility of the developing brain to PVL damage. Indeed, the presumed loss of oligodendrocytes is a hallmark of PVL.

Hypoxic-ischemia (HI) results in the aberrant development of the brain, and is an important cause of cerebral palsy and associated disabilities in children. If occurrence of HI is severe enough to damage the brain, it leads within 12 to 36 hours to HIE (Fatemi *et al.*, 2009; Volpe, 2001). HIE is not a single “event” but is rather an evolving process. The clinical signs of HIE reflect the progression of various delayed cellular or molecular responses triggered by initial insults probably occurred in gestational time (Fatemi *et al.*, 2009). Fetal hypoxia is one of these initial insults that may increase the susceptibility to HIE at perinatal stage. Many conditions may lead to fetal hypoxia, including pregnancy at high altitude, pregnancy with anemia, placental insufficiency, cord compression, preeclampsia or heart,

lung and kidney disease in the mother, and pregnancy with hemoglobinopathy (Chen and Zhang, 2011).

3. DNA methylation and active demethylation and the development of the brain

3.1. DNA methylation and active demethylation

In mammals, DNA methylation occurs almost exclusively on cytosine nucleotide in the symmetrical dinucleotide CpG sequence. Some of the genomic regions contain a G+C content of at least 50% and are defined as CpG islands. CpG islands are highly enriched at or near the gene promoter region. In mammalian genomes, about 60-70% of gene promoters, including housekeeping genes, tissue-specific and developmental genes, are associated with CpG islands (Blackledge and Klose, 2011; Deaton and Bird, 2011). Studies on genome-wide distribution of DNA methylation estimate that 60-90% of all the CpGs in mammals, including those present in intragenic regions, repetitive sequences and mobile elements, are methylated. In contrast, most CpG islands are unmethylated. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which transfer a methyl group from S-adenyl methionine to the fifth carbon of a cytosine residue to produce the 5-methylcytosine (5mC) (Goll and Bestor, 2005). There are four types of active DNMTs in mammals: DNMT3A, DNMT3B, DNMT1 and DNMT2. DNMT3A and DNMT3B are *de novo* methyltransferases that set up DNA methylation patterns by targeting unmethylated CpG sites. DNMT1 primarily acts to maintain the DNA methylation pattern during development and efficiently preserves epigenetic inheritance through cell division. DNMT1 localizes to replication foci during S-phase, and shows high catalytic activity on hemi-methylated CpGs through its interaction with protein UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) (Bostick *et al.*, 2007; Sharif *et al.*, 2007). Different from other active DNMTs, DNMT2 does not methylate DNA but instead methylates a small RNA (Goll *et al.*, 2006). Mutation of DNMT genes results in a reduction of DNA methylation leading to abnormal embryonic development and embryonic lethality (Li *et al.*, 1992; Okano *et al.*, 1999; Williams *et al.*, 2012). DNA methylation in promoter regions is usually associated with transcriptional repression, and this is believed to be mediated by a direct interference of transcription factor binding to the promoter or through two families of proteins called methyl-CpG-binding proteins (MBDs), including MeCP2 and MBD1, and structural-unrelated methyl-CpG binding zinc-finger proteins of the Kaiso family, including Kaiso/ZBTB33 and ZBTB4 (Bogdanovic and Veenstra, 2009; Williams *et al.*, 2012).

Loss of methylation or DNA demethylation has been observed at the genomic DNA in specific contexts, including both early development and somatic cells. DNA demethylation can occur through both passive and active mechanisms. Passive DNA demethylation is generally accompanied by the absence of functional DNA methylation maintenance machinery during DNA synthesis. The methylation at specific locus may be lost in daughter cells after cell division due to the “methylation dilution” by not replicating the methylated status to the newly synthesized strand (Wu and Zhang, 2010). Passive DNA demethylation is particularly relevant in rapidly dividing cells, such as embryonic stem cells. Unlike “passive” DNA demethylation, “active” DNA demethylation refers to the enzymatic

reaction process that removes or modifies the methyl group from 5mC. To date, no DNA demethylase has been found. The molecular mechanisms of active DNA demethylation are only beginning to be addressed and several pathways have been proposed. For instance, 5mC can be converted to thymine (T) through deamination by the activation-induced cytidine deaminase/apolipoprotein B mRNA-editing enzyme complex (AID/APOBEC); therefore, a G/T mismatch is created and induces the activation of base excision repair (BER) pathway to correct the mistake. The role of AID/APOBEC in demethylation has been shown in some animal cells. Overexpression of AID/APOBEC promoted DNA demethylation in zebrafish (Rai *et al.*, 2008), whereas downregulation of AID/APOBEC blocked the DNA demethylation of cellular reprogramming genes and the development related genes (Bhutani *et al.*, 2010; Popp *et al.*, 2010). Another mechanism in DNA demethylation is started with ten-eleven translocation (TET) proteins. The finding that TET1 protein catalyzes the conversion of 5mC into 5-hydroxymethylcytosine (5hmC), an intermediate product during loss of 5mC, suggests the potential mechanism of oxidative active demethylation in mammalian DNA (Tahiliani *et al.*, 2009). Other members of TET family proteins, the TET2 and TET3 are also 2-oxoglutarate and Fe (II)-dependent dioxygenases, and have the capacity to convert 5mC into 5hmC both *in vitro* and *in vivo* (Ito *et al.*, 2010; Tahiliani *et al.*, 2009). The TET family proteins are broadly expressed in different tissues. TET1, initially discovered as a fusion partner of the mixed lineage leukemia (MLL) gene (Lorsbach *et al.*, 2003), is mostly expressed in embryonic stem cells, whereas TET2 and TET3 are more ubiquitously expressed (Szwagierczak *et al.*, 2010; Tahiliani *et al.*, 2009; Williams *et al.*, 2012). TET proteins can add a hydroxyl group to 5mC to form 5hmC that is further converted back to a naked cytosine by other pathways. In one way, TET proteins continue to oxidize 5hmC to 5-formyl-cytosine and then to 5-carboxycytosine (Ito *et al.*, 2011). In another way, AID/APOBEC can be activated to convert 5hmC to 5-hydroxymethyluracil (5hmU) (Guo *et al.*, 2011). In all mechanisms of active demethylation, the BER pathway using thymine DNA glycosylase (TDG) to correct the mismatch with a naked cytosine is activated (Cortellino *et al.*, 2011; He *et al.*, 2011). TDG is required for normal development and knockout of TDG results in embryonic lethality in mice (Cortellino *et al.*, 2011).

3.2. DNA methylation/demethylation in the neuronal development

DNA methylation plays an essential role during the embryonic development. A switch between *de novo* DNA methylation and demethylation during the development alters the methylation pattern of the genomic DNA. As a consequence, a stable and unique DNA methylation pattern is developed in the differentiated cells to regulate tissue-specific gene transcription (Moore *et al.*, 2013). DNA methylation controls the switch of multipotent neural progenitor cells (NPCs) differentiation between neurogenesis and astrogliogenesis in embryonic period (Qian *et al.*, 2000; Sauvageot and Stiles, 2002). Astrocyte differentiation occurs late in the development of the brain, since the glial fibrillary acidic protein (*Gfap*) gene promoter is highly methylated and repressed till E11.5 (Takizawa *et al.*, 2001a; Teter *et al.*, 1996). The continuous expression of DNMT1 in NPCs plays a pivotal role to maintain the methylation pattern on the *Gfap* promoter during cell division (Fan *et al.*, 2005). At E14.5, the *Gfap* promoter undergoes DNA demethylation and astrogliogenesis is initiated (Teter *et al.*, 1996). Intriguingly, in line with the differentiation of the astrocytic lineage, the

DNMT3 level starts to decline. However, the DNMT3a level goes back to the peak at postnatal 3 weeks, which coincides with the postnatal remethylation of *Gfap* promoter (Fan *et al.*, 2005; Nguyen *et al.*, 2007). Knockout studies highlight the precise regulation of methylation and demethylation on the development of the CNS. In the neurogenesis period (E8.5-E11.5), *Dnmt1* deletion from NPCs results in demethylation of the *Gfap* promoter, therefore promotes astrocyte differentiation (Fan *et al.*, 2001; Fan *et al.*, 2005) (Figure 1). Accordingly, due to hypomethylation of neuron differentiation genes, multiple maturation defects have been observed, including dendritic arborization and impaired neuronal excitability (Fan *et al.*, 2001; Golshani *et al.*, 2005; Hutnick *et al.*, 2009). In addition, MBDs influence gene expression by cooperating with series of transcriptional repressors, and play an important role in the development of the brain (Kimura and Shiota, 2003; Sarraf and Stancheva, 2004). MBDs are expressed in embryonic stem cells and neural precursors and have little effect on neuronal or glia cell differentiation (Kishi and Macklis, 2004; Martin Caballero *et al.*, 2009), but are required for normal neuronal maturation. For example, MeCP2 can be phosphorylated by neural activity through which its ability of binding to gene promoters is affected (Tao *et al.*, 2009; Zhou *et al.*, 2006), whereas the disruption of MeCP2 phosphorylation leads to aberrant dendritic arborization and abnormal synapse development and plasticity (Cohen *et al.*, 2011; Li *et al.*, 2011; Nelson *et al.*, 2006). Thus, the precise regulation of the switch between *de novo* methylation and demethylation is essential for the differentiation and maturation of the mammalian CNS. In addition, active DNA demethylation pathway has been found to be involved in the NSC differentiation. Wheldon and colleagues reported that 5-carboxylcytosine (5caC), an oxidized product of 5mC by TET was transiently accumulated at the cell type-specific promoters during neuronal and glial differentiation of NSCs (Wheldon *et al.*, 2014). Moreover, knockdown of TDG, which cleaves 5caC to unmodified cytosine, also led to increased 5caC levels in the differentiation of NSCs to glial (Wheldon *et al.*, 2014). Furthermore, the results showed that 5caC was deposited at CpG-rich promoter regions of a number of key glial markers such as *Gfap* during glial differentiation, which corresponded to demethylation of specific CpGs in differentiated cells (Wheldon *et al.*, 2014). This study not only demonstrated the essential role of DNA demethylation in the cell fate determination but also revealed that TDG/BER-dependent active DNA demethylation pathway was active during the development of the embryo brain. Thus it is reasonable to speculate that the impairment of maintaining a normal DNA methylation pattern under intrauterine hypoxia may result in abnormal development of the brain and increase the susceptibility of the brain to neurological dysfunction later in life. Fan and colleagues reported that mutant embryos carrying hypomethylated neuron cells in the brain were viable in the postnatal animals (Fan *et al.*, 2001). However, DNA demethylation of *Gfap* in neurogenesis stage during embryogenesis resulted in the initiation of astrogliogenesis (Fan *et al.*, 2005), excess of which drove NSCs differentiation to astrocytes and led to inflammation and neuronal death (Vangeison and Rempe, 2009). In consideration of the fact that hypoxia induces a global demethylation, it is possible that fetal hypoxia results in the hypoxia/ischemic sensitive phenotype in the brain *via* disturbing programming of neural stem cell differentiation and enhancing neuronal death during embryogenesis.

3.3. DNA methylation/demethylation in the vascular development

Vascular endothelial growth factor (VEGF) is the essential regulator of vasculogenesis and angiogenesis during embryonic development and is highly expressed in many types of cells, including neuroblasts, neuroepithelial, radial glia, astrocytes, pericytes and endothelial cells (ECs). In humans, VEGF is strongly expressed in developing telencephalon from gestational week 9, later in neurons, glia and blood vessels throughout the brain, till 34 weeks (Sentilhes *et al.*, 2010; Virgintino *et al.*, 2003). Either deletion of one VEGF-A allele or mild overexpression of VEGF-A causes abnormal vascular network development and may lead to embryogenic lethality (Ferrara *et al.*, 1996; Miquerol *et al.*, 2000). In the fetal brain, VEGF is expressed in the embryonic neuroectoderm, the epithelium of the stem cell and progenitor regions of the brain (Baburamani *et al.*, 2012). Through binding to the high-affinity receptor (VEGFR2), VEGF guides the formation of new blood vessels and influences the vascular development and neuronal development in the embryonic brain (Hermann and Zechariah, 2009; Sentilhes *et al.*, 2010). Expression of VEGF is tightly regulated at multiple levels during embryogenesis. VEGF-A and its two types of tyrosine kinase receptors, VEGFR1 (encoded by *Flt-1*) and VEGFR2 (encoded by *Kdr*) have typical CpG islands in their promoter regions. It has been shown in cancer cells that expression of VEGF receptors was regulated, at least partly, by epigenetic mechanisms. For example, hypermethylation of *Flt-1* and *Kdr* promoters are correlated with the decreased expression of related genes (Kim *et al.*, 2012; Kim *et al.*, 2009b), however, no evidence of methylation associated regulation of VEGF-A has been reported. A further study in human umbilical cord endothelial cells provides evidence that the regulation of VEGF-A expression can be achieved by alteration of histone code. Inhibition of histone acetyltransferase co-activator reduced the glucose induced VEGF-A expression in human umbilical cord endothelial cells (Chen *et al.*, 2010a). Another VEGF family member, VEGF-C is present in the developing brain in embryonic stage and stimulates growth of non-vascular progenitor cells, such as oligodendrocyte progenitors through binding with VEGFR-3. In some gastric cancer cell lines, methylation of VEGF-C promoter was found along with the absence of VEGF-C expression whereas in other GC cell lines showing VEGF-C gene expression, the VEGF-C promoter was unmethylated. Moreover, expression of VEGF-C mRNA was retrieved by treatment with a demethylation agent, suggesting an important role of DNA demethylation in regulating VEGF-C gene expression (Matsumura *et al.*, 2007). Little has been reported about the demethylation regulation of VEGF mediated angiogenesis in the development of the brain or its role in programming of hypoxic/ischemic-sensitive phenotype in response to hypoxia, although it is well known that the VEGF family is essential for the embryogenesis, especially the development of the vascular network. Dysregulation of VEGF signals results in the abnormalities of vasculature (Carmeliet, 2003; Coultas *et al.*, 2005). Under hypoxic conditions, VEGF was up-regulated and functioned as a double edged sword (Vangeison and Rempe, 2009). On one hand, it protected the neuron and promoted long-term brain functional recovery by angiogenesis (Zhao *et al.*, 2006); on the other hand, it increased the blood-brain barrier permeability and resulted in brain edema (Zhang *et al.*, 2000). Based on current evidence that VEGF can be regulated by DNA demethylation and its prominent roles in hypoxia-induced angiogenesis, it is reasonable to postulate that the epigenetic regulation of VEGF may be involved in fetal hypoxia-induced CNS malformations.

In addition to VEGF, the expression of other vascular-related genes may also be regulated by DNA methylation/demethylation mechanism. Recently, Shirodkar and colleagues analyzed the differential DNA methylation pattern in the proximal promoter regions of the EC-enriched genes, including CD31/PECAM1, Endoglin/ENG, ICAM-2, P-selectin/SELP, Tie-2/TEK, VE-Cadherin/CDH5 and vWF, in terminally differentiated ECs and non-EC type of cells. They found that most of the EC-enriched genes were differentially methylated in EC and non-EC types (Shirodkar *et al.*, 2013). To further achieve functionalities of DNA methylation in EC-enriched gene repression in non-EC types, they treated non-EC cells with DNA methyltransferase inhibitor and/or histone deacetylase inhibitor, and found that most of EC-expression genes were reactivated except VEGFR1 and VEGFR2, which were also hypomethylated in non-EC cells. These results provide evidence of DNA methylation in the regulation of EC-enriched gene expression, and also confirm previous studies demonstrating that DNA methylation did not play a role in transcriptional regulation of VEGFR1 and VEGFR2 (Shirodkar *et al.*, 2013). To date, direct evidence of DNA demethylation, especially active demethylation in regulating vascular-related genes in the developing brain is limited, and thus future studies focusing on the genomic programs in the brain vascular development may help enhance our understanding of this area.

4. Histone modifications and the development of the brain

4.1. Histone modifications

Chromatin is the combination of DNA along with histone and non-histone proteins. The smallest functional unit of chromatin, nucleosomes are made of 147 bp of DNA wrapped around an octamer of core pairs of histone proteins called H2A, H2B, H3, and H4. Basically, nucleosomes contribute to fine-tuning and precise regulation of DNA folding, binding of transcription factors to DNA and the following gene expression. Histones are key players in epigenetic regulation. Histone modifications work together with the DNA methylation to ensure an efficient remodeling of chromatin and silencing of specific gene expression (Kouzarides, 2007b). All histones are subject to several post-transcriptional modifications, including acetylation, methylation, phosphorylation, and ubiquitination, which mainly positioned within the N-terminal tails of core histones. As a result, actively transcribed genes are always characterized by high levels of histone H3 lysine 4 trimethylation (H3K4me3), histone H3 lysine 27 acetylation (H3K27ac), histone H2B lysine 5 acetylation (H2BK5ac) and histone H4 lysine 20 methylation (H4K20me1) in the promoter region and histone H3 lysine 79 methylation (H3K79me1) and histone H4 lysine 20 methylation (H4K20me1) along the gene body (Karlic *et al.*, 2010).

Histone modifications are processed by a series of enzymes. Histone acetyltransferases (HATs) catalyze the direct transfer of an acetyl group from acetyl-CoA to the ϵ -NH⁺ group of the lysine residues (K). This process is reversed by the histone deacetylases (HDACs), which remove the acetyl from lysine residues. Generally, HDACs are divided into two types: Class I including HDAC 1, 2, 3, and 8; Class II encompassing HDAC 4, 5, 6, 7, 9, 10, and 11. A new subtype of HDACs family, Class III was discovered in 2004, called the SIR2 family of HDACs. Most of the HATs and HDACs can modify more than one residue. Interestingly, a large number of transcriptional co-activators have been described to have

HAT activity, whereas many transcriptional co-repressor complexes contain subunits possessing HDAC activity (Wang *et al.*, 2008b). As a result, most HDACs in the human genome function to reset chromatin by removing acetylation at active genes, whereas HATs, by contrast, are mainly linked to transcriptional activation (Wang *et al.*, 2009).

Histones methylation is catalyzed by histone methyltransferases (HMTs). Similar to acetylation modification, histone methylation happens on ϵ -NH⁺ groups of lysine residues (K). Lysine can be methylated up to three times; therefore, three different states of lysine methylation, mono-(me1), di-(me2) or trimethylated (me3) exist to undertake reversed functions. A number of human genes encode proteins with histone methyltransferases activities, such as MLL (Ziemin-van der Poel *et al.*, 1991) and EZH2 (Cao *et al.*, 2002; Fiskus *et al.*, 2006). In general, trimethylation of H3K4, H3K36 and H3K79 are usually enriched at active genes, while H3K9me3/me2, H4K20me3 and H3K27me3 are associated with gene silencing (Pedersen and Helin, 2010) (Figure 2). In addition to lysine residues, arginine residues (R) within histones can also form mono- or dimethylated states, which are catalyzed by protein arginine methyltransferases. Histone methylation is reversed by histone demethylases (HDMs), which was first discovered in 2004 (Shi *et al.*, 2004). Since then, two classes of HDMs have been identified, including the KDM1 (Lysine demethylase family) and the Jumonji C (JmjC) domain (Kooistra and Helin, 2012; Pedersen and Helin, 2010).

4.2. Histone modification in the neuronal development

During the development of the brain, time and space specific expression of the neural cell specific genes is critical for the generation of numerous cell types constituting the CNS. Mounting evidence has shown that transcription factors were in control of neurogenesis and neural cell-fate specification (Ross *et al.*, 2003; Wegner and Stolt, 2005) and chromatin modifying factors may be involved in these processes. Some of the lysine residues in histone tails can be acetylated and consequently led to the relaxation of the higher order of chromatin (Kouzarides, 2007a). Subsequently, acetylation of the histone tails will allow transcription factors and the RNA pol II complex to bind with DNA transiently for transcription. On the contrary, when the histone tails are deacetylated by HDACs, DNA will be rewrapped up into condensed chromatin, leading to transcriptional inactivation. Therefore, HATs and HDACs that catalyze acetylation or deacetylation of histones may play an essential role in the embryonic NPC development.

CREB binding protein (CBP) and p300 are co-activators with HAT activity, and both are involved in regulation of neural cell differentiation during development (Lee and Lee, 2010). Deletion of CBP and p300 results in severe neural tube closure defects (Bu *et al.*, 2007; Lee *et al.*, 2009; Tanaka *et al.*, 2000; Yao *et al.*, 1998). Whereas, little is known about the molecular mechanisms by which extrinsic and intrinsic cues collaboratively trigger the chromatin modifications in the development of the CNS. Lee and colleagues reported that the coupling of retinoic acid and intrinsic transcription factor Neurogenin2 (Ngn2) can trigger chromatin remodeling and transcriptionally activation of spinal motor neuron genes during development. The retinoic acid receptor (RAR) binds with Ngn2 to form a RAR-Ngn2 complex, which can move to Ngn2 targeting genes in motor neurons. Upon binding of retinoic acid to the RAR, CBP was recruited to the Ngn2/RAR complex, which induced

chromatin H3/H4-acetylation, subsequent motor neuron gene expression and neuron differentiation (Lee *et al.*, 2009) (Figure 3). In addition to the Ngn2 signaling pathway, CBP is also involved in the neurogenin (Ngn1) mediated neural stem cell differentiation. Sun and colleagues reported that Ngn1 inhibited neural stem cell differentiation to astrocyte by dissociating the transcription complex CBP-Smad1 from the *Gfap* gene and by inhibiting the STAT1/STAT3 activation, which were essential for gliogenesis. In contrast, Ngn1 recruited the CBP/P300-Smad1 complex to neural-specific gene promoters, such as *NeuroD*, thereby promoting the expression of neuronal differentiation genes (Sun *et al.*, 2001). These findings indicate that HATs regulate cell-type specific gene expression and subsequent cell differentiation of NPCs by integrating extrinsic signals and transcription factors.

Histone deacetylase HDAC1 and HDAC2 also exhibit critical functions in the switch of neuronal and oligodendrocyte differentiation by controlling the neurogenic and gliogenic gene expression during neural development. It has been reported that HDAC inhibitors, such as valproic acid and trichostatin A influence the proliferation and differentiation of NPCs in the embryonic and adult brain. Shen and colleagues administered valproic acid to rodents during the first 10 postnatal days, and found that the HDAC inhibition in perinatal rodent brain resulted in delayed differentiation of oligodendrocytes and significant hypomyelination (Shen *et al.*, 2005). To specify the critical role of individual HDAC in neuronal development, a genetic deletion technique was employed in the embryonic stage. Montgomery and colleagues found that the deprivation of both HDAC1 and HDAC2 in developing neurons resulted in severe hippocampal abnormalities, absence of cerebellar foliation, disorganization of cortical neurons, and lethality by postnatal day 7, which is potentially due to the failure of differentiation from neuronal precursors to mature neurons, or excessive cell death (Montgomery *et al.*, 2009). Valproic acid stimulated the overexpression of neuron-specific gene *NeuroD* and induced the differentiation of NPCs to neuron but not glial (Hsieh *et al.*, 2004). This finding was confirmed by another study showing that removal of *Hdac1* and *Hdac2* genes in oligodendrocyte lineage cells abolished oligodendrocyte differentiation in the brain and spinal cord (Ye *et al.*, 2009). Moreover, valproic acid treatment also induced expression of *Ngn1*, *Math1* and *p15* genes during neuronal differentiation. Notably, *in vitro* study using cultured hippocampal neural progenitor cells showed that the promoters of these neuronal genes were all associated with acetylated histone H4 (Ac-H4) (Yu *et al.*, 2009). Such evidence demonstrates that HATs and HDACs are associated with the regulation of neurogenesis and neural cell-fate specification by harmonizing multiple extrinsic signals and intrinsic transcription factors.

Mounting evidence indicates that histone methylation/demethylation complexes also play essential roles during the development of the CNS. Multiple HMTs and HDMs are involved in neural development by regulating chromatin structure modification and neurogenic-related gene regulation. Generally, high levels of trimethylation of H3K4 are associated with active gene transcription, while methylation of H3K9 and H3K27 results in a condensed/transcriptionally inactive chromatin state (Kouzarides, 2007a). For example, the *Gfap* promoter of NPCs has high level of H3K9me3, when NPCs differentiated into astrocytes, H3K9me3 levels decreased whereas H3K4me3 levels increased (Song and Ghosh, 2004). Similar to the role of HATs and HDACs in the cell-type specific differentiation of NPCs, a

complex relationship between HMTs and HDMs were observed to coordinate chromatin modifications at specific genes to promote neurogenesis. For example, H3K4 specific methyltransferases, MLL1 deletion caused a severe impairment of the neuronal differentiation in the subventricular zone (SVZ) of the mouse postnatal brain *via* reduction of *Dlx2* expression, a key downstream regulator of SVZ neurogenesis. The *Dlx2* encodes a homeodomain-containing transcription factor, the promoter of which can recruit MLL1 specifically. Further experiment showed that *Dlx2* was the direct downstream target of MLL1 since *Dlx2* was not expressed in *Mll1*-deficient cells and overexpression of *Dlx2* rescued neurogenesis impaired by *Mll1*-deficient. Intriguingly, the authors found that in differentiating wild-type SVZ cells, *Dlx2* promoter was marked with high levels of H3K4me3, whereas in *Mll1*-deficient cells, *Dlx2* was bivalently marked by both H3K4me3 and H3K27me3, which silenced *Dlx2* gene expression. These results suggest that MLL1 regulates *Dlx2* gene expression by recruitment of H3K27-specific demethylases (K27DM) (Lim *et al.*, 2009). Another specific H3 trimethyl K27 demethylase encoded by jumonji-domain containing gene *JMJD3*, is capable of activating specific components of the neurogenic program in NPCs, including *Dlx5*, *Gad1/2* and *Dcx* markers of differentiated interneurons (Jepsen *et al.*, 2007). It also plays role in neural lineage commitment of embryonic stem cells (ES cells) by resolving the bivalent domain at the *Nestin* promoter (Burgold *et al.*, 2008). Moreover, it has been discovered that the *JMJD3* gene was repressed by nuclear receptor co-repressor 2 (NCoR2) during neurogenesis, consequently inhibiting its functions in neuronal differentiations of embryonic NPCs and in maintenance of the neural stem cell state (Jepsen *et al.*, 2007). In addition to specific H3K27 demethylase, the X-linked mental retardation (XLMR) gene *SMCX* encoding a JmjC-domain protein, which possesses H3K4me3 demethylase activity and converts H3K4me3 to di- and mono- but not unmethylated products, has been reported to play important roles in neuronal survival and dendritic morphogenesis of cerebellar granular neurons (Iwase *et al.*, 2007). Furthermore, the X-linked intellectual disability caused by the mutations in the Jumonji AT-rich interactive domain 1C (*JARID1C/SMCX/KDM5C*) gene is probably due to the loss of demethylase activities (Jensen *et al.*, 2010; Santos-Reboucas *et al.*, 2011). Tsukada and colleagues reported that inhibition of KDM7 orthologs, which is a JmjC domain-containing protein and catalyzes demethylation of both mono- and dimethylated H3K9 and H3K27, resulted in developmental brain defects (Tsukada *et al.*, 2010). All these evidence suggest a critical role of histone modifications in the development of CNS by strictly controlling chromatin architecture and genes transcription patterns. And multiple chromatin remodeling factors cooperatively regulates the gene expression and silencing. Thus, the dysfunction of chromatin remodeling factors may contribute to various neurodevelopment diseases.

4.3. Histone modification in the vascular development

The notion that the histone modification links to endothelial differentiation first came from Rossig's study in 2005, in which they found that the endothelial differentiation of adult endothelial progenitor cells (EPCs) was blocked by the HDAC inhibitor trichostatin A (Rossig *et al.*, 2005). HoxA9, one of the homeobox transcription factors that are essential for migration and tube-forming capacity of mature endothelial cells, is regarded as the potential downstream effector of HDAC. Overexpression of HoxA9 rescued HDAC inhibition-mediated endothelial differentiation (Bruhl *et al.*, 2004). Further experiments revealed that

HoxA9 regulated the expression of a sequence of the prototypical endothelial-committed genes in progenitor cells, including endothelial nitric oxide synthase (eNOS), VEGF-R2, and VE-cadherin. Moreover, HoxA9 mediated the shear stress-induced maturation of endothelial cells. A lower number of endothelial progenitor cells and an impaired postnatal neovascularization capacity after the induction of ischemia was observed in HoxA9-deficient mice (Rossig *et al.*, 2005), which further suggest an essential role of HDAC in endothelial cell differentiation and neovascularization.

Compared to global HDACs inhibition, the individual HDAC may have specific and different biological effects. It is well known that laminar flow enhances endothelial differentiation of ESCs (Illi *et al.*, 2005; Zeng *et al.*, 2006) and peripheral blood- or bone marrow-derived progenitor cells (Rossig *et al.*, 2005; Yamamoto *et al.*, 2003; Zeng *et al.*, 2006), whereas little is known about the underlying mechanisms. Zeng and colleagues investigated the role of HDAC3 in ESC differentiation stimulated by shear stress and VEGF delivery, and found that HDAC3 played a crucial role in this process *via* regulation of p53-p21 pathway (Zeng *et al.*, 2006). Silencing of HDAC3 specifically inhibited the expression of endothelial specific genes stimulated by laminar flow. Moreover, the authors found that laminar flow and VEGF activated the Flk-1-PI3K-Akt pathway and upregulated the expression of HDAC3, which in turn deacetylated p53 and led to p21 activation during embryogenesis (Zeng *et al.*, 2006). In addition to HDAC3, laminar flow also induced and/or activates HDAC1 and HDAC5 in retinal endothelial cells in the development of diabetic retinopathy (Zhong and Kowluru, 2010). HDAC7, a member of class II HDACs, is dedicated to the control of tissue growth and development. Chang and colleagues reported that HDAC7 was specifically expressed in vascular endothelial cells during early embryogenesis and acted as a vascular integrity maintainer by suppression of matrix metalloproteinase 10 (MMP-10). In the embryonic vascular endothelial cells deleting HDAC7, MMP10 level was upregulated while its endogenous antagonist, tissue inhibitor of metalloproteinase 1 (TIMP1) was downregulated (Chang *et al.*, 2006). Considering the effect of MMP10 on extracellular matrix degradation, these results explain the lethality of HDAC embryonic deletion in mice, which is caused by vascular dilatation and rupture due to loss of adhesion between endothelial cells (Chang *et al.*, 2006). Thus, this finding indicates that HDAC7 plays a critical role in the process of angiogenesis and vascular remodeling during development. SIRT1, a member of class III HDACs, is a nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase in mammalian (Frye, 2000). Study from Potente and colleagues showed that loss of SIRT1 function blocked sprout formation and branching morphogenesis of endothelial cells during angiogenesis along with downregulation of endothelial specific genes involved in blood vessel development and vascular remodeling. Further study revealed that the effect of SIRT1 on angiogenesis was associates with its deacetylase activity and its cofactor, forkhead transcription factor Foxo1. These results implicated an important role of SIRT1 in postnatal vascular growth and angiogenesis by modulating endothelial gene expression (Potente *et al.*, 2007) (Figure 4).

In addition to histone acetylation, histone methylation also regulates vascular differentiation. HMT Ezh2, part of the polycomb repressor complex 2 (PRC2), suppresses gene expression related to endothelial cell differentiation. Silencing of Ezh2 led to the re-expression of

endothelial specific genes and consequently promoted the endothelial tube formation (Richter *et al.*, 2009). This finding suggests that Ezh2 is a negative regulator of angiogenesis. In another study, Ezh2 was shown to be upregulated by VEGF and stimulated tumor angiogenesis through blockage of the angiogenesis inhibitor vasohibin 1 (Lu *et al.*, 2010). These controversial findings suggest that Ezh2 regulates gene expression through different manners in normal endothelial cells and tumor cells. Other histone methyltransferase, such as MLL, was shown to regulate endothelial cell functions by regulation of HoxA9 and HoxD3, and thereby control endothelial gene expression (Diehl *et al.*, 2007; Rossig *et al.*, 2005). In summary, increasing evidence suggests that histone modification enzymes play essential role in vascular development during embryonic development, and the disruption of their biological effects may lead to abnormal vascular development and vascular diseases.

5. MicroRNA and the development of the brain

5.1. Biogenesis of miRNA

MiRNAs are highly conserved, short ~21-22 nucleotides (nt) non-coding RNAs that bind to 3' untranslated region (3'UTR) of target mRNAs and suppress the expression of target proteins, either by inhibiting translation or causing degradation of target mRNAs. The human genome encodes over one thousand miRNAs that potentially target several thousand of genes and participate in the control of many fundamental processes (Jackson and Standart, 2007). Most miRNAs are expressed in a developmental or tissue-specific manner, and many of them are specifically enriched in the brain. This is consistent with the growing evidence showing the important role of miRNAs in the brain development and function (Jackson and Standart, 2007; Kosik, 2006; Krol *et al.*, 2010; Schratt, 2009).

Primary miRNA (pri-miRNA) transcription begins with a 7-methylguanosine cap (m⁷Gppp) and ends with a 3' poly (A) tail by RNA polymerase II (Pol II) in the nucleus. Pri-miRNA is processed into hairpin-shaped precursors in length of ~70-100 nucleotide by Drosha, an endonuclease that forms complexes with a double-stranded RNA (dsRNA)-binding domain protein, DGCR8 (in mammals) or Pasha (in flies) (Han *et al.*, 2004; Lee *et al.*, 2003). The resultant precursor miRNA (pre-miRNA) is then transported into the cytoplasm *via* an Exportin-5- and Ran-GTP-dependent pathway (Lund *et al.*, 2004). In the cytoplasm, pre-miRNA is further processed to produce ~20-22 base pair mature miRNA duplexes by Dicer, the double-stranded ribonuclease, together with its dsRNA-binding partner TRBP (transactivation-response RNA-binding protein) in mammals or Loquacious (Loqs) in flies (Hutvagner *et al.*, 2001). The mature miRNA duplexes are first recruited into an Argonaute (AGO) protein as a dsRNA, and then cleaved by the effector ribonucleic acid-induced silencing complex (RISC), in which one strand of the duplex is degraded to form a mature single stranded miRNA incorporating with the RISC. MiRNAs specifically bind to the 3'UTR of the target mRNAs through a perfect or near-perfect complementary match to repress the expression of mRNA (Guo *et al.*, 2010; Huntzinger and Izaurralde, 2011; Kane *et al.*, 2012).

5.2. MiRNA in the neuronal development

Central nervous system is the prominent site for miRNA expression. It is estimated that approximate 70% of current detectable miRNAs are expressed in the brain, and half of them are either brain specific or brain enriched (Cao *et al.*, 2006). Bioinformatics data focusing on the spatial and temporal region of zebrafish brain manifest that miRNAs are present in different expression patterns during neural cells differentiation and/or proliferation. For example, miR-92b is primarily expressed in neuronal precursors and stem cells; miR-124 is constitutively expressed in mature neurons and is associated with transition from proliferation to differentiation; and miR-9 is expressed in both proliferative cells and their differentiated progeny. Some miRNAs show regionally restricted expression, such as miR-222 that is mainly expressed in telencephalon. Some miRNAs show cell-specific expression, such as miR-218a that is mainly expressed in motor neurons (Kapsimali *et al.*, 2007). These findings are further confirmed by a large-scale expression profiling assay of miRNAs performed in a human embryonal carcinoma cell line during retinoic acid-induced transition from progenitors to fully differentiated neural phenotypes (Smith *et al.*, 2010). The results suggest that dynamic changes of miRNA patterns can be linked to specific neurodevelopmental stages, such as cell fate commitment, progenitor expansion and differentiation into neuron and glia (Smith *et al.*, 2010). Based on these findings, the pivotal modes of miRNA functionalities in neurogenesis, neuronal maturation, neuronal differentiation and maintenance, and neuroplasticity are likely predicted.

Neuronal maturation is a critical step of the development of the brain. Some miRNAs participate in the neuronal maturation process and play essential roles in several steps, including dendritic and axonal growth, spine development and synapse formation. For example, overexpression of the neuronal enriched miR-137 abolished dendritic morphogenesis, phenotypic maturation and spine development in the brain by targeting mind bomb-1 (Mib1) protein, which is an ubiquitin ligase known to be involved in neurodevelopment (Smrt *et al.*, 2010). Many miRNAs are expressed in the neuronal dendritic spines and regulate synaptic plasticity (Lugli *et al.*, 2008; Smalheiser and Lugli, 2009). MiR-134 regulated dendritic spine morphology by inhibition of the translation of Limk1 mRNA at the synapse (Schratt *et al.*, 2006), and silence of miR-134 expression *in vivo* reduced the hippocampal CA3 pyramidal neuron dendrite spine density (Jimenez-Mateos *et al.*, 2012). RNA-binding protein Pumilio2 (Pum2) is another target of miR-134 during the regulation of dendritogenesis (Fiore *et al.*, 2009). Similar to miR-134, the brain-enriched miRNA, miR-138, was expressed in dendrites and negatively regulates the growth of dendritic spines in rat hippocampal neurons by targeting acyl protein thioesterase 1 (APT1) (Siegel *et al.*, 2009). MiR-132 was involved in dendritic structural and functional plasticity development by targeting a Rho family GTPase-activating protein, the p250GAP (Wayman *et al.*, 2008).

In addition to neuronal maturation, miRNAs are also associated with neuronal differentiation, thus influence the development of the brain. MiR-124, one of the most abundant and well-conserved miRNAs in adult and embryonic brains, is exclusively present at presynaptic terminal of Aplysia (Rajasethupathy *et al.*, 2009). During neural differentiation, miR-124 regulates the splicing pattern in neurons *via* repression of

polypyrimidine tract-binding protein 1 (PTB; neural variant, nPTB), which encodes a global repressor of alternative pre-mRNA splicing in non-neuronal cells, and subsequently promotes neuronal differentiation (Makeyev *et al.*, 2007). Intriguingly, recent study showed that PTB can reversely block miRNAs action on multiple components of the REST complex during the neural progenitor cell differentiation, thereby suppressing a large number of neuronal genes, and multiple neuronal-specific transcription factors in non-neuronal cells (Xue *et al.*, 2013). MiR-124 is also implicated in neurogenesis in adult mammalian brain by repression of SRY-box transcription factor SOX9. Overexpression of miR-124 promoted neuronal differentiation of SVZ stem cells, whereas genetic knockdown or blocking of miR124 maintained SVZ stem cells followed by a delayed neuronal formation (Cheng *et al.*, 2009). It is known that the REST-miR-124 signaling axis functions in the control of neuronal differentiation and phenotype maintenance. Repressor element 1-silencing transcription factor (REST) is an endogenous inhibitor of neuronal gene expression in non-neuronal and neural progenitor cells, and is downregulated during the transition from pluripotent cell to post-mitotic neuron to allow transcription of fundamental neuronal genes (Ballas *et al.*, 2005). Effect of REST on neuronal gene expression is mediated by miR-124. In non-neuronal cells, REST blocking miR-124 allows non-neuronal genes transcription, whereas in differentiated neurons, REST dismissed from binding site of miR-124 gene loci leads to selective degradation of non-neuronal gene transcripts (Conaco *et al.*, 2006). On the contrary, miR-124 also antagonizes REST functions during embryonic central nervous system development through inhibition of Small C-terminal domain phosphatase 1 (SCP1), which is an anti-neural factor in non-neuronal tissues cooperating with REST (Visvanathan *et al.*, 2007) (Figure 5). MiR-9 is another prominent miRNA in balancing embryonic neural progenitor cell proliferation (Coolen *et al.*, 2013). Its expression starts at mid-embryogenesis stage and is dynamically regulated during the development of the brain *in vivo* and induced neurogenesis *in vitro* (Coolen *et al.*, 2013; Krichevsky *et al.*, 2006; Miska *et al.*, 2004). Mounting evidence shows that miR-9 is associated with neurogenic progenitor cells predominantly located in ventricular zone (Bonev *et al.*, 2011; Coolen *et al.*, 2012). MiR-9 also exhibits anti-progenitor proliferative effect by targeting multiple transcription factors, including the forkhead transcription factor FoxG1, the homeobox factor Gsx2, the orphan nuclear receptor Tlx/Nr2e1, REST and the zinc finger transcription factor Zic5 (Bonev *et al.*, 2011; Laneve *et al.*, 2010; Shibata *et al.*, 2011). Accordingly, these transcription factors also negatively regulate miR-9 expression and their upregulation leads to a miR-9 deficient-like phenotype. In miR-9-2/3 double mutant mice, upregulation of FoxG1 and Gsx2 protein levels leads to an increased proliferation of embryonic pallial and subpallial progenitor cells (Shibata *et al.*, 2011). Numlike (Numbl), a known regulator of the development of the brain, suppresses proliferation but enhances differentiation. MiR-184 binds to 3'UTR of Numbl mRNA and regulates its translation, thereby orchestrators the balance of proliferation and differentiation of neural stem cells. More intriguingly, the axis of miR-184 and Numbl is governed by MBD1, which represses miR-184 and modulates the neural stem cell proliferation and differentiation (Liu *et al.*, 2010).

MiR-132 is another subset of brain-enriched miRNAs that can be activated in response to neuron stimulation in the rodent brain or culture neurons (Nudelman *et al.*, 2010; Wayman *et al.*, 2008), and may play a role in synaptic plasticity (Krol *et al.*, 2010). Ngan Vo and

colleagues reported that activity-dependent regulators of neuroplasticity, such as the cAMP response binding protein (CREB) and brain-derived neurotrophic factor (BDNF) upregulated miR-132 transcription and induced neurite outgrowth in cultured cortical and hippocampal neurons (Vo *et al.*, 2005). The p250GAP that is highly enriched in the CNS (Nakazawa *et al.*, 2003), is the target of miR-132 (Vo *et al.*, 2005). Later on, another study further discovered that miR132/p250GAP circuit regulated Rac1 activity and spine formation by modulating synapse-specific Kalirin7-Rac1 signaling, thereby activated a Rac1-Pak actin remodeling pathway (Impey *et al.*, 2010). In addition to its function in dendritic spine growth, miR-132 also plays a role in synaptic excitability by increasing α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and NMDA glutamate receptor-mediated currents in postsynaptic cells (Edbauer *et al.*, 2010; Lambert *et al.*, 2010). Another established target for miR-132 is the deacetylase (SIRT1) (Strum *et al.*, 2009), which enhances synaptic plasticity in mouse hippocampus through a mechanism involving repression of miR-134 and the resultant upregulation of BDNF and CREB (Gao *et al.*, 2010).

Thus, these findings demonstrate that miRNAs regulate neuronal morphogenesis, plasticity and maintenance during the development of the brain. It is believed that multiple miRNAs cooperate to control the gene expression profiles, thereby affecting these cellular events. Therefore, abnormal expression of miRNAs in response to adverse environmental conditions may disturb neurodevelopment and contribute to neurological disorders.

5.3. MiRNA in the vascular development

Classic theories have documented the formation of the vascular plexus surrounding the embryonic brain and spinal cord, and the subsequent ingression of vascular into the neural tissue (Bautch and James, 2009; Nakao *et al.*, 1988). To date, a growing body of evidence indicates that the genetic programs previously described to control the growth of nerve cells also guide the vascular development (Vogel, 2005). Accordingly, miRNAs play critical roles in the vascular development. Dicer is a key enzyme involved in the biogenesis of mature miRNA. In *Dicer* mutant mice, Bernstein and colleagues observed embryo morphological abnormality at E7.5 and early embryonic lethality as well (Bernstein *et al.*, 2003). In another study with *Dicer-ex1/2* homozygous mice, embryos had a severe defect in blood vessel formation and maintenance, and died at mid-gestation stage. Correspondingly, the aberrant vascular development is correlated with the altered expression of a sequence of early endothelial markers in the *Dicer1* mutant embryos, including *Vegf*, *Flt1*, *Kdr*, and *Tie1* (Yang *et al.*, 2005). This finding suggests a role of miRNA in embryonic angiogenesis, probably through regulating expression of angiogenesis-related genes, and provides initial evidence for the contribution of miRNA in EC differentiation. The notion that Dicer is a prerequisite for EC function was first demonstrated *in vitro* (Kuehbacher *et al.*, 2007). In *Dicer* siRNA transfected ECs, capillary sprouting, tube forming and EC migration were abolished, which was consistent with let-7 family (let-7f) and mir-27b inhibition in these ECs. Further experiments indicated that Dicer and Drosha siRNAs reduced the expression both *let-7f* and *mir-27b* (Kuehbacher *et al.*, 2007; Suarez *et al.*, 2007). In order to address the direct effect of Dicer on EC development *in vivo*, two approaches were utilized to generate EC-Dicer-specific-knockout animals: one is the homozygous for the floxed Dicer

allele and Cre-recombinase under the regulation of Tie2 promoter, and the other is Tamoxifen (TMX)-inducible Cre-recombinase (Cre-ERT2) under the regulation of vascular endothelial cadherin (*VEcad*) promoter (Suarez *et al.*, 2008). Results from these knockout animals showed that postnatal vascular growth in Dicer conditional KO models was reduced when confronting a variety of stimuli, such as exogenous VEGF and ischemia (Suarez *et al.*, 2008).

MiRNAs have tissue-specific expression manners, therefore, one miRNA may be highly enriched in one tissue but have no or low expression in other tissues (Lagos-Quintana *et al.*, 2002). To identify the vascular-specific miRNAs, Kuehbachner and colleagues analyzed the profiles of 168 human miRNAs using real-time PCR and found that members of the let-7f, mir-21, mir-126, mir-221, and mir-222 were highly expressed in endothelial cells (Kuehbachner *et al.*, 2007). Later studies with microarray and realtime PCR revealed that miR-126 was the most frequently expressed vascular miRNA in primary human ECs from veins, arteries, skin, and brain (Bonauer *et al.*, 2009; Harris *et al.*, 2008; Liu *et al.*, 2011; Poliseno *et al.*, 2006). In mice, miR-126 was located within the intron 7 of the *Egf7* gene, deletion of which led to a delayed vascular development in retina and brain, and impairment of adult VEGF-dependent corneal angiogenesis (Kuhnert *et al.*, 2008). Mechanistically, the miR-126 deletion inhibited VEGF-dependent Akt and Erk signaling by targeting p85 β subunit of PI3 kinase and Spred1, respectively (Kuhnert *et al.*, 2008). Another study confirmed the role of miR-126 in vascular development by showing that the deletion of miR-126 in mice caused defects in EC proliferation, migration, and angiogenesis, thereby leading to vascular integrity impairment, hemorrhaging, and partial embryonic lethality (Wang *et al.*, 2008a). Moreover, the abnormal vascular development in miR-126 deletion mice resembled consequences similar to that observed in blocking of VEGF signaling (Lee *et al.*, 2007). These studies indicate an essential role of miR-126 in the vascular development, and suggest that miR-126 may have a direct association with the VEGF signaling pathway.

MiR-132 is another widely studied miRNA associated with the vascular development. Mounting evidence exhibits that miR-132 is highly upregulated in a human embryonic stem cell model of vasculogenesis (Anand *et al.*, 2010). Overexpression of miR-132 in ECs *in vitro* increased the growth and tube-forming ability, whereas intraocular injection of miR-132 antagomir reduced postnatal retinal vascular development in mice. The Ras pathway functions as an active downstream signaling of VEGF during vascular growth and the p120RasGAP is the endogenous inhibitor of Ras pathway. Study by Anand and colleagues revealed that miR-132 increased Ras activity by directly suppressing p120RasGAP expression, and activation of p120RasGAP reversed the effect of miR-132 on vascular development (Anand *et al.*, 2010). This finding suggests that miR-132 may act as an angiogenic switch by suppression of endothelial p120RasGAP expression, and activation of miR-132 induces neovascularization process. A recent study confirmed in multiple rodent models that miR-132 affected angiogenic sprouting and neovascularization by negatively regulating p120RasGAP expression, and indicated that anti-miR-132 had more potent therapeutic benefits than the widely used VEGF antagonist (Westenskow *et al.*, 2013) (Figure 6).

Both MiR-221/222 and miR-155 are associated with angiogenesis. MiR-221/222 is located in close proximity on Xp11.3 chromosome (Altuvia *et al.*, 2005) and regulates Ang II-induced endothelial migration (Zhu *et al.*, 2011). MiR-221/222 can inhibit angiogenesis by targeting key regulators in angiogenesis, including c-Kit, STAT5A and ZEB2 (Chen *et al.*, 2010b; Dentelli *et al.*, 2010; Poliseno *et al.*, 2006). Moreover, miR-221/222 is required for the endothelial tip cell behavior during vascular development in zebrafish (Nicoli *et al.*, 2012). MiR-221 knockdown phenocopied defects associated with loss of the tip cell-expressed Flt4 receptor and prevented “hyper-angiogenesis” defects associated with Notch deficiency. Two targets of miR-221 were involved in this process, one is the cyclin dependent kinase inhibitor 1b (cdkn1b), and the other is phosphoinositide-3-kinase regulatory subunit 1 (pik3r1) (Nicoli *et al.*, 2012). MiR-155 has been shown to co-express in ECs and vascular smooth muscle cells (VSMCs), and represses the translation of AT1R *in vivo* (Martin *et al.*, 2006). Ets-1, a key endothelial transcription factor for angiogenesis, vascular remodeling and inflammation, has been identified as a target of both miR-221/222 and miR-155 (Zhu *et al.*, 2011).

Other miRNAs, such as miR-34 family (miR-34a, b and c) may also be potentially involved in the vascular development, although their roles still remain to be elucidated. In a recent study, Iqbal N and colleagues demonstrated the effect of miR-34a on the expression of *Pdgfrβ* (Iqbal *et al.*, 2014), which was specifically expressed in smooth muscle cells and pericytes and played an important role in cell proliferation. They found that ectopic miR-34a could directly target *Pdgfrβ* gene, moreover, both the *Arf* gene driven repression of *Pdgfrβ* and the PDGF-B stimulated DNA synthesis were completely interrupted by anti-miR-34a. Further analysis indicated that the expression of miR-34a, and the related miR-34b and c were controlled by *Arf* during normal mouse development (Iqbal *et al.*, 2014).

6. Cross-linking of epigenetic modifications

6.1. Cross-linking between DNA methylation and histone modifications

During embryonic development, both DNA methylation and histone modifications are associated with establishing patterns of gene repression. As a consequence, the gene expression profile is tightly influenced by chromatin architecture alterations, which is primarily orchestrated by modifications to DNA or chromatin-associated proteins, especially histones. Moreover, it appears that there is a close interaction between these two systems that work cooperatively to modulate gene expression. Some histone modifications need a template served by DNA methylation, and in turn histone methylation may directly guarantee the methylation modification process of DNA. The most obvious example is that MBPs bind to methylated DNA, and proteins with MBP are capable of recruiting other chromatin modifiers. Consequently, these actions may reposition nucleosomes or induce changes in post-translational histone modification states leading to chromatin condensation (Nan *et al.*, 1998). Indeed, it has been reported by Klose and colleagues that MeCP2 can bind tightly to chromosomes and mediate transcriptional repression through recruitment of a chromatin-remodeling complex containing the transcriptional repressor mSin3A and HDACs, suggesting that DNA methylation-related chromatin remodeling is critical for gene regulation (Klose and Bird, 2004). Moreover, it has been reported that several histone

methyltransferases were able to direct DNA methylation through recruiting DNMTs to specific genomic targets (Tachibana *et al.*, 2008; Zhao *et al.*, 2009), helping in this way to set the silenced state established by the repressive histone marks. Moreover, histone methyltransferases and demethylases can also regulate DNA methylation levels, thereby affecting the stability of DNMT proteins (Esteve *et al.*, 2009; Wang *et al.*, 2009) (Figure 2).

In the mammalian genome, CpGs islands are protected from methylation reprogramming during early development. Although the precise mechanisms are not clear, studies suggest that common *cis*-acting sequences and active demethylation may direct this process (Brandeis *et al.*, 1994; Frank *et al.*, 1991). Therefore, this evidence suggests that although DNA and histone methylation are catalyzed by different sets of enzymes, these are connected through interactions between the enzymes. Moreover, another study suggests that the histone modification may contribute to the establishment of the basic DNA methylation profile (Ooi *et al.*, 2007). Based on this model, the authors found that the RNA polymerase II, which is mostly bound to CpG islands in the early embryo, was involved in the setup of H3K4 methylation pattern that might be formed throughout the genome before occurrence of the *de novo* demethylation. As a result, only CpG islands were marked with H3K4me, while the rest of the genome still contained unmethylated H3K4 (Ooi *et al.*, 2007). It is well known that DNA methyltransferase DNMT3A and 3B with the assistance of DNMT3L modulated the *de novo* demethylation process. These recruit the methyltransferase to DNA through binding to histone H3 in the nucleosome. The methylated histone H3 inhibits the interaction between DNMT3 L and the nucleosome, however. Thus, these prevent DNA sequences within the CpG islands from *de novo* methylation (Cedar and Bergman, 2009).

6.2. Cross-linking between miRNA and DNA methylation/demethylation

DNA methylation/demethylation and miRNAs regulate gene expression through transcriptional and post-transcriptional levels, respectively, but they interact tightly during the development. DNA methylation may regulate transcription of both protein-coding genes and miRNAs by affecting the binding of transcriptional factors to the target DNA regions. On the other hand, miRNAs may influence DNA methylation/demethylation process *via* regulating related enzymes and the expression of methylation binding proteins.

As mentioned above, pri-miRNA is transcribed by RNA polymerase II and gene expression can be repressed by DNA methylation on its promoter. As a result, the transcription of pri-miRNAs may be affected by DNA methylation. In the human colon cancer cell line HCT116, by comparing the miRNAs profiles between wildtype and *Dnmt1* and *Dnmt3b* double KO cells, it was found that about 10% miRNAs was regulated by DNA methylation. Moreover, pharmacological inhibition or genetic deletion of DNMT1 alone cannot recapitulate miRNA profiles observed in the double KO cells. This result suggests that miRNAs expression is tightly controlled by DNA methylation and partial demethylation process is not sufficient to induce miRNA re-expression (Han *et al.*, 2007). The identification of transcriptional start site (TSS) is the priority concern to study miRNA genes. Bhadra and colleagues built a classifier model to predict miRNA TSS sites by analyzing available brain specific methylation data, and found that this model is highly sensitivity and specificity for miRNA prediction (Bhadra *et al.*, 2013). This study also

provides evidence that the DNA methylation is directly related to the miRNA expression. In addition, MBDs may affect miRNA expression. MBD1 plays an important role in cellular growth and its deletion in mice results in brain-associated phenotypes, including impaired adult neurogenesis, defective hippocampus-dependent learning and susceptibility to depression (Allan *et al.*, 2008; Zhao *et al.*, 2003). Liu and colleagues found that MBD1 deficiency damaged adult neural stem/progenitor cell (aNSC) differentiation and the neurogenesis process, potentially targeting miR-184 that promotes NSC proliferation and inhibits differentiation. Numbl, a known regulator of the development of the brain and embryonic NSC proliferation and differentiation (Johnson, 2003; Li *et al.*, 2003), is the downstream target of miR-184. Through binding to the 3'UTR of Numbl mRNA, miR184 affects its translation. Further study showed that the expression of exogenous Numbl rescued the aNSC defects resulted from either miR-184 overexpression or MBD1 deficiency (Liu *et al.*, 2010). Such evidence suggests that MBD1 may control the balance between proliferation and differentiation through repression of miR-184 in aNSCs.

On the other hand, miRNAs can regulate DNA methylation process by regulating the expression of methylation related enzymes and MBDs. MeCP2 is critical for normal brain function and mutation or overexpression of MeCP2 leads to various neurological diseases. In humans, MeCP2 is expressed in fetal brains and upregulated in the postnatal stage, which may be associated with timely regulation of development-related gene expression (Chahrouh *et al.*, 2008). MeCP2 is a putative target of miRNAs owing to the unusually long 3'UTR of predominant MeCP2 transcripts. MiR-483-5p, an intragenic miRNA of the imprinted *IGF2*, regulates MeCP2 levels through a human-specific binding site in the MeCP2 long 3'UTR (Coy *et al.*, 1999). In fibroblasts, overexpression of miR-483-5p leads to a reduction of the MeCP2 level (Han *et al.*, 2013). In human developing brain and fibroblasts from Beckwith-Wiedemann Syndrome (RWS) patients, an inverse correlation between miR-483-5p and the MeCP2 level was found. Moreover, in mouse hippocampal neurons, miR-483-5p expression rescued the abnormal dendritic spine phenotype caused by overexpression of human MeCP2. Additionally, miR-483-5p also modulates the expression of other members of MeCP2-interacting co-repressor complexes, such as HDAC4 and TBL1X (Han *et al.*, 2013). These findings suggest that miR-483-5p tightly controls the expression of MeCP2 and interacting proteins during human fetal development, thus affects DNA methylation pattern.

MiRNAs are involved in the establishment and/or maintenance of DNA methylation (Rajewsky, 2006). The miR-29 family members have been reported to directly bind to 3'UTRs of DNMT3A and DNMT3B, and suppress their expression. As a result, methylation silenced tumor suppressor genes is reactivated and thereby restore normal patterns of DNA methylation in non-small cell lung cancer cells (Fabbri *et al.*, 2007). MiR-29 family not only inhibits expression of DNA methyltransferases but also targets active demethylation related enzymes, such as TET1 and TDG. In A549 or PC9 cells, co-transfection of miR-29 with luciferase-labelled TET1, TDG, DNMT3A and DNMT3B constructs results in the significantly reduced luciferase activity after 48 hours of transfection (Morita *et al.*, 2013). MiR-148 also can regulate expression of DNMT3B, and repression of miR-148 leads to an increase of DNMT3B expression. Moreover, the specific binding site of miR-148 is located at the protein coding sequence of DNMT3B mRNA, and mutation of this putative miR-148

targeting site abolishes the suppression effect of miR-148 on DNMT3B expression (Duursma *et al.*, 2008).

6.3. Cross-linking between miRNA and histone modification

Histone modifications alter the histone's interaction with DNA and transcription factors, thereby influencing DNA transcription, including microRNA. Suzuki and colleagues performed genome-wide assay for the chromatin signatures including H3K4me3, H3K27me3, and H3K79me2 in colorectal cancer cell lines (Suzuki *et al.*, 2011). They found that the H3K4me3 marks was enriched around the proximal upstream CpG islands (CGIs) regions of two abundantly expressed miRNA clusters, miR-200b and miR-17 in both wild-type HCT116 and DKO cells while the CGIs of miR-34b/c, miR-124-1, and miR-9-3 were completely devoid of H3K4me3 and H3K79me2 marks in HCT116 cells. On the contrary, a moderate enrichment of H3K27me3 was observed in miR-124-1 and miR-9-3 clusters (Suzuki *et al.*, 2011). The regulation of histone modification on miRNAs expression is mediated by histone acetylation-related enzymes. It has been reported that a non-competitive inhibitor of HDAC, butyrate-induced p21 protein expression was dampened by treatment with a miR-106b mimic. Moreover, HDAC inhibition is also associated to the aberrant expression of 44 miRNAs in HCT-116 cells, including miRs-17, -20a, -20b, -93, -106a and -106b, etc. (Hu *et al.*, 2011). Moreover, another HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA) also strongly influenced miRNAs expression in colorectal cancer cell line (Shin *et al.*, 2009). All these studies provide evidence regarding the histone modification regulating miRNAs expression although the precise mechanism of HDAC on miRNAs expression still need to be further determined.

Conversely, expression of HDACs is also modulated by a subset of miRNAs. For example, miR-1 promotes myogenesis by targeting HDAC4, a repressor of muscle differentiation and skeletal muscle gene expression, whereas overexpression of HDAC4 counteracted the effects of miR-1 on myogenesis. Luciferase reporter gene assay showed that miR-1 bound to 3'UTRs of HDAC4 and repressed its expression (Chen *et al.*, 2006). HDAC4 is also one of the important targets of miR-140 that induces expression of p53 and p21 in colon cancer cells through suppression of HDAC4 (Song *et al.*, 2009). Recently, Bruneteau and colleagues reported that HDAC4 targeted by miR-206 in human patients with amyotrophic lateral sclerosis may be associated with muscle reinnervation and disease progression (Bruneteau *et al.*, 2013). In addition, miR-449a results in cell-cycle arrest, apoptosis and a senescent-like phenotype *via* directly repressing the expression of HDAC1 in prostate cancer cells (Noonan *et al.*, 2009).

A growing body of evidence indicates that miRNAs may also influence chromatin structure by directly affect effectors of epigenetic machinery, such as the post-transcriptional regulatory polycomb repressive complex (PRC) genes. The polycomb group proteins are transcriptional repressors that regulate lineage choices occurring during development and differentiation, are crucial in embryonic development. It silences chromatin domains via (di- or tri-) methylation of Lys 27 of histone H3 (H3K27me2/3) (Covic *et al.*, 2010). In humans, there are two main families of PRC, called PRC1 and PRC2, are both needed to sustain gene repression (Margueron and Reinberg, 2011). EZH2 is a histone methyltransferase belonging

to PRC2 family, which is crucial for timing in neuronal differentiation (Pereira *et al.*, 2010). MiR-137 was found to repress EZH2 feeds back to chromatin, leading to a global decrease in H3K27me3 in NSC cell cultures. However, with coexpression of *Ezh2* the effect of miR-137 overexpression-related phenotypes was restored, suggesting that interaction between miR-137 and *Ezh2* play a critical role in neurogenesis (Szulwach *et al.*, 2010). MiR-214 has been reported to regulate skeletal muscle and embryonic stem cells differentiation by regulating *Ezh2* protein levels. The developmental regulators, MyoD and myogenin are recruited at the miR-214 region and enhance its transcriptional activation. Moreover, in undifferentiated skeletal muscle cells, miR-214 transcriptional unit is occupied and repressed by PcG proteins (Juan *et al.*, 2009). Additionally, other miRNAs including miR-128 (Peruzzi *et al.*, 2013), miR-323-3p (Zhang *et al.*, 2013), miR-200 (Iliopoulos *et al.*, 2010) also modulate the expression and effect of PRC component.

Thus, miRNAs and classical epigenetic mechanisms cooperate with each other to modulate the gene expression profile, thereby maintaining physiological functions during the development. When this cooperation machinery is disrupted, the normal physiological functions may be interfered with, leading to various diseases.

7. Hypoxia, epigenetic mechanisms and the abnormal development of the CNS

7.1. Epigenetic mechanisms in fetal hypoxia-induced brain injury

The developing brain is vulnerable to various adverse environmental conditions, including fetal hypoxia, which may lead to a hypoxic/ischemia-sensitive phenotype in the developing brain (Li *et al.*, 2012). The molecular mechanisms underlying fetal hypoxia-induced injury in the developing brain have been investigated. Similar early gene expression pattern was observed in the developing brain and the placenta in response to late-gestational intrauterine hypoxia, including the upregulation of hypoxia-inducible factors (HIFs)-dependent and immediate early genes (e.g. *Fos*, *Jun*, *Egr1*, *Bhlhb2*), apoptosis-promoting factors (e.g. *Bnip3*, *Dusp1*, *Ier3*), and the down-regulation of genes modulating RNA binding and translation (e.g. *Rbm3*, *Thap2*, *Lig4*, *Rbm12b*) (Trollmann *et al.*, 2010). The finding that many of these genes such as *Bnip3* and *Fos* have been demonstrated to be the targets of epigenetic modifications (Dyrvig *et al.*, 2012; Ha *et al.*, 2014) implicates a role of epigenetic mechanisms induced by intrauterine hypoxia. A recent study by Gonzalez-Rodriguez and colleagues showed that the epigenetic modification of glucocorticoid receptor (GR) in response to fetal hypoxia resulted in enhanced brain vulnerability to HI injury in neonatal rats (Gonzalez-Rodriguez *et al.*, 2014). This finding of fetal hypoxia-induced epigenetic repression of GR in the developing brain provides a novel mechanism in the understanding of HIE at the molecular level. Another study from Wang and colleagues demonstrated that gestational intermittent hypoxia (GIH) increased anxiety in a sex-dependent manner in 90-day-old (P90) male rat offspring, which was linked to DNA demethylation at several specific CpG sites of corticotropin-releasing hormone type-1 receptor promoter in hypothalamus and paraventricular nucleus (Wang *et al.*, 2013b). These findings suggest that epigenetic mechanisms play a role in fetal hypoxia-induced adverse effects in the development of the brain.

7.2. Hypoxia impacts epigenetic machineries

Hypoxia-induced transcription factors (HIF), especially HIF-1 α is a key mediator of the transcriptional responses induced by hypoxia. HIF-1 α binds to hypoxia response elements (HREs) in promoter and enhancer regions of the target genes, contributing to the formation of hypoxic-inducible phenotype (Watson *et al.*, 2010). Accumulating evidence suggests that the expression of HIF-1 α may be regulated by epigenetic mechanisms. HIF-1 α promoter has CpG rich regions. DNA methylation suppresses the expression of HIF-1 α , and demethylation by an inhibition of DNMT up-regulates its expression (Koslowski *et al.*, 2011; Walczak-Drzewiecka *et al.*, 2010). Kenneth and colleagues reported that the chromatin-remodeling complex SWI/SNF was required to mediate hypoxia-induced cellular responses, including HIF-1 α (Kenneth *et al.*, 2009). Recently, a large number of miRNAs have been discovered in multiple cell types to be involved in the HIF pathway under hypoxic conditions, such as miR-23, miR-24, miR-26, miR-107, miR-210, miR-373, etc. (Crosby *et al.*, 2009; Huang *et al.*, 2009). Many of them participate in the regulation of HIF-1 expression. For example, during hypoxia miR-200 overexpression increased HIF-1 α stability by targeting PHD2, a hydroxylase for HIF-1 α proteosomal degradation under normoxic conditions (Garcia-Bonilla *et al.*, 2014). MiR-199a and miR-20b directly target HIF-1 α mRNA during hypoxia in cardiac myocytes (Lei *et al.*, 2009; Rane *et al.*, 2009). Some of them are the HIF-1 downstream targets, such as miR-210. On one hand, the expression of miR-210 is regulated by HIF (Chan *et al.*, 2012; McCormick *et al.*, 2013; Nakada *et al.*, 2011). On the other hand, miR-210 also regulates HIF's stability (Wang *et al.*, 2014).

The stability of HIF-1 α is posttranslationally controlled by prolyl hydroxylase domains (PHDs) and von-Hippel-Lindau-tumour-suppressor (VHL)-mediated ubiquitination process. Recent studies suggest that the expression and activity of PHDs and VHL are regulated by the methylation status of their promoters (Hatzimichael *et al.*, 2010; Herman *et al.*, 1994; Nguyen *et al.*, 2013). Moreover, the finding that HDAC regulates VHL expression and activity suggests a role of histone modification in HIF-1 α stability under hypoxia (Kim *et al.*, 2001). In addition, several epigenetic factors have been found to be involved in the HIF-1 α coactivation complex and have direct contact with HIF-1 α during the initial transcriptional response to hypoxia. For instance, the histone acetyltransferase enzyme, CBP/p300 is recruited into nuclear and acts as the coactivator of HIF-1 α in hypoxic cells (Kallio *et al.*, 1998).

Many studies have investigated the regulation of HIF-1 α transactivation activity by focusing on the epigenetic modification of HREs, thereby the expression of HIF-1 downstream genes (Shahrzad *et al.*, 2007; Watson *et al.*, 2009). For example, the increase of HRE methylation levels of erythropoietin 3' enhancer inhibits hypoxia-induced erythropoietin gene expression (Wenger *et al.*, 1998). BNIP3, an apoptosis regulator, is also regulated by HIF-1 α binding to its promoter region. Several studies demonstrated that DNMT inhibitors (Ishida *et al.*, 2007; Okami *et al.*, 2004) or HDAC inhibitors (Bacon *et al.*, 2007) reversed hypoxia-induced BNIP3 expression. In addition, Mahon and colleagues reported that a transcriptional corepressor that consists of factor inhibiting HIF-1 (FIH-1) and VHL inhibits HIF-1 α transactivation by recruiting HDAC (Mahon *et al.*, 2001). This finding was further

supported by another study showing that histone acetylation regulated HIF function by targeting HIF-1 α /p300 complex, which was blocked by HDAC inhibitors (Fath *et al.*, 2006).

Hypoxia also directly affects the epigenetic factors. For example, TET1 expression was found to be increased through a HIF-1-dependent mechanism in neuroblastoma, resulting in an increase in global 5-hmC in hypoxia (Mariani *et al.*, 2014). Similar to TET1, Jumonji family histone demethylases, JMJD1A and JMJD2B that regulate the methylation level of H3K9 residues, were also transcriptionally up-regulated by HIF-1 binding to HREs under hypoxic conditions (Beyer *et al.*, 2008; Krieg *et al.*, 2010; Wellmann *et al.*, 2008). These findings were confirmed by ChIP-seq assays mapping the HIF-binding sites across the genome (Schodel *et al.*, 2011).

Consistent with the changes of epigenetic factors in response to hypoxia, hypoxia-induced global changes in many histone modifications, such as H3K4me3 that is associated with transcriptional activation, and H3K27me3 that is a marker of transcriptional silencing, have been reported (Johnson *et al.*, 2008). Meantime, hypoxia-mediated site-specific changes in histone modifications also have been investigated (Luo *et al.*, 2012; van den Beucken *et al.*, 2009). Luo and colleagues found that JMJD2C enhanced HIF-1 binding to HRE by a decrease in H3K9me3 levels, resulting in the transcription of metabolic reprogramming genes (Luo *et al.*, 2012). Hypoxia also regulates noncoding transcriptome through release of pre-bound promoter-paused RNA polymerase 2, and HIF is a major direct regulator of this process (Choudhry *et al.*, 2014). Furthermore, hypoxia also affects miRNA expression by regulating phosphorylation level of AGO2, an upstream regulator of miRNA biogenesis (Shen *et al.*, 2013). Taken together, these findings suggest that epigenetic modifications play a crucial role in the cellular response to hypoxia.

7.3. Hypoxia, epigenetic and neuronal development

Mammalian embryonic development is under a physiologically hypoxic environment, and thus hypoxia has been shown to promote cell reprogramming (Yoshida *et al.*, 2009). Mutoh and colleagues showed that oxygen levels in the embryonic brain are essential for the determination of the fate of NPC switching during the development of the brain (Mutoh *et al.*, 2012). It has been reported that DNA methylation is a critical determinant for the differentiation of NPCs to astrocytes or neurons (Namiyama *et al.*, 2009), especially in the mid-gestation stage, in which the genes encoding typical astrocyte markers are hypermethylated (Fan *et al.*, 2005; Takizawa *et al.*, 2001b). However, the mechanisms in the effect of hypoxia on the cell fate determination in the developing brain remain unclear. Mutoh *et al.* found that the Notch pathway was activated during hypoxia, and cooperated with HIF-1 α to induce the expression of the transcription factor nuclear factor IA (NFIA) (Mutoh *et al.*, 2012; Namiyama *et al.*, 2009), which binds to the promoters of astrocyte-specific gene promoters, such as *Gfap*, and induces dissociation of DNMT1, resulting in demethylation and gene expression (Mutoh *et al.*, 2012). These findings implicate a crucial role of hypoxic environment in the regulation of cell fate determination in the developing brain. To gain a broad understanding of epigenetic changes of neuronal genes during hypoxia, Hartley and colleagues investigated the changes in DNA methylation following short-term hypoxic exposure in primary hippocampal neuronal cultures (Hartley *et al.*,

2013). Using RNA-Seq, these investigators analyzed transcriptome profiling and showed that 369 differentially expressed genes with 225 being up-regulated in response to hypoxia probably due to the hypoxic-induced demethylation. Many of these genes are correlated to the DNA methylation status at their promoter regions, and are associated with the CNS development and function. Interestingly, these CpG islands maintained a long-lasting hypomethylation levels after hypoxic stress was removed, suggesting that epigenetic modifications may contribute to the development of post-hypoxia phenotype (Hartley *et al.*, 2013). In addition to the genomic assay *in vitro*, Zeng injected a lentiviral vector carrying miR-210 (LV-miR-210) into the mouse brain and found that the overexpression of miR-210 in the brain hemisphere greatly increased neural progenitor cells in the SVZ, as compared with the controls. This finding provides evidence that miR-210 may be a potential therapeutic target for ischemia stroke (Zeng *et al.*, 2014). Furthermore, the expression of miR-210 in neuronal development is also regulated by epigenetic modification under hypoxic conditions. Xiong and colleagues reported that DNA demethylation regulated the expression of miR-210 in neural progenitor cells subjected to hypoxia (Xiong *et al.*, 2012). Other studies also showed that miR-210 under hypoxic conditions were related to the stem cell survival and differentiation (Chio *et al.*, 2013; Kim *et al.*, 2009a; Wang *et al.*, 2013a).

7.4. Hypoxia, epigenetic and vascular development

HIF-1 regulates the expression of various downstream target genes to adapt to low oxygen environment, many of which have been identified to be related to tumor growth (Benizri *et al.*, 2008), angiogenesis (Manalo *et al.*, 2005), etc.. Indeed, it has been reported that HIF-1 is an essential regulator of cephalic vascularization. Using a null mutation at the HIF-1 α locus *via* homologous recombination in mouse embryonic stem (ES) cells, Ryan and colleagues found abnormal neural development by E8.0 and a lack of vascularization in many tissues by E8.5 in HIF-1 α null mutant embryos, and the expression of HIF-1 target genes, such as the glucose transporter-1 (GLUT1), VEGF, *etc.* were significantly reduced (Ryan *et al.*, 1998). This finding indicates that HIF-1 is essential in the embryonic development. VEGF, the major angiogenic factor for new vessel growth is the target of HIF-1. Recently, a study by Tudisco and colleagues reported that placental growth factor (PlGF), a member of the VEGF family was induced in cultured human and mouse endothelial cells exposed to hypoxia (Tudisco *et al.*, 2014). Further molecular studies revealed that hyperacetylation of histones H3 and H4 occurred in the HREs located in the second intron of *Plgf*; whereas the DNA methylation level at the *Plgf* CpG-island was not changed. This finding suggests that hypoxia induced an angiogenesis-related gene, *Plgf* expression through chromatin remodeling of HREs sites (Tudisco *et al.*, 2014).

In addition to chromatin modifications, micro RNAs also play an important role in hypoxia-mediated angiogenesis. MiR-210 is a downstream target of HIF-1 and is the master of hypoxia-induced miRNAs because of its magnitude and consistent up-regulation under hypoxic conditions (Chan *et al.*, 2012). Fasanaro and colleagues found that miR-210 overexpression in endothelial cells stimulated the formation of capillary-like structures and cell migration, and hypoxia-mediated angiogenesis was inhibited by anti-sense miR-210 inhibitor (Fasanaro *et al.*, 2008). Moreover, luciferase reporter assays showed that 3'UTR of Ephrin-A3, a tyrosine kinase receptor Eph ligand that plays a crucial role in the development

of the vascular system and vascular remodeling (Kuijper *et al.*, 2007) contains a miR-210 binding sequence, and therefore, was a direct target of miR-210 (Fasanaro *et al.*, 2008). Another study from Zeng and colleagues confirmed the role of miR-210 in angiogenesis *in vivo* (Zeng *et al.*, 2014). After the LV-miR-210 injection into the mouse brain, they found that microvessel density and downstream angiogenic factors, such as VEGF were up-regulated 4 weeks later (Zeng *et al.*, 2014). Further studies showed that miR-210 is up-regulated in patients with pre-eclampsia caused by insufficient uteroplacental oxygenation, which inhibited the migration and invasion capability of trophoblast cells through suppression of HoxA9 and Ephrin-A3 (EFNA3) (Zhang *et al.*, 2012). Taken together, these findings suggest a possible involvement of epigenetic mechanisms in the adverse effect of hypoxia on the neurovascular development in the brain.

8. Conclusions and perspectives

As we have discussed above, epigenetic regulations, including DNA methylation/demethylation, histone modifications and miRNAs, interact and regulate the neuronal and vascular development of the brain. During the fetal development the environmental conditions, such as hypoxia may influence the development of the brain at the molecular level by stimulating various epigenetic modifications, and therefore increasing the susceptibility of neurological disorders in offspring (Figure 7). Indeed, it is likely that epigenetic modifications are the major mechanism by which the early environmental factors during the fetal development regulate the output of genomic information and impact the phenotypic development later in life. Therefore, the understanding of epigenetic mechanisms in the development of the brain will improve our knowledge of brain susceptibility to neurological dysfunction.

At present, although epigenetic mechanisms have been broadly studied in neuronal and vascular development, relatively little is known about their involvement and interaction in the effect of fetal stress on programming of hypoxic/ischemic-sensitive phenotype in the developing brain. Because hypoxia is one of the most important and clinically relevant stresses to the fetal development, and clinical and animal studies indicate a link between fetal stress and an increased risk of HIE in the developing brain, it is important to understand the molecular mechanisms underlying fetal stress-mediated programming of the developmental plasticity of the brain. Particularly, future studies should focus on the epigenetic mechanisms in fetal stress-mediated programming of neuronal differentiation, maturation and synaptic plasticity, astrocyte differentiation, vascular network development and neurovascular coupling (Chang *et al.*, 2006; Girouard and Iadecola, 2006). The outcome of these studies should also help identify “epigenetic biomarkers” for the brain at risk. Recently, transcriptomic analysis of brain samples from newborn rats has demonstrated that prenatal stress induces the epigenetic signatures such as miRNAs related to neurological disorders in offspring (Zucchi *et al.*, 2013). Similarly, in a model of prenatal undernutrition-induced metabolic disease methylation of insulin like growth factor (*IGF*) has been found lower, whereas methylation of non-imprinted interleukin 10 promoters, imprinted loci of signaling G-protein alpha subunit gene *GNAS*-antisense 1 and maternally expressed protein *MEG3* genes is higher (Dominguez-Salas *et al.*, 2012; Tobi *et al.*, 2009). In the brain, it has been reported that translational dysregulation of mRNAs may be associated with the

expression of fragile X mental retardation protein (FMRP) in fragile X syndrome, a brain disorder caused by the absence of the RNA binding FMR protein (Brown *et al.*, 2001). Obviously, the role of epigenetic mechanisms in fetal stress-induced developmental programming of hypoxic/ischemic-sensitive phenotype and the discovery of epigenetic biomarkers in the developing brain are far from being solved, and more endeavors are required.

Acknowledgments

This work was supported in part by National Institutes of Health Grants HL083966 (LZ), HL110125 (LZ), HL118861 (LZ) and HD031226 (LZ). We apologize to those authors whose excellent studies covered by the scope of this review were unable to be cited due to space restriction.

References

- Allan AM, Liang X, Luo Y, Pak C, Li X, Szulwach KE, Chen D, Jin P, Zhao X. The loss of methyl-CpG binding protein 1 leads to autism-like behavioral deficits. *Hum Mol Genet.* 2008; 17:2047–2057. [PubMed: 18385101]
- Altuvia Y, Landgraf P, Lithwick G, Elefant N, Pfeffer S, Aravin A, Brownstein MJ, Tuschl T, Margalit H. Clustering and conservation patterns of human microRNAs. *Nucleic Acids Res.* 2005; 33:2697–2706. [PubMed: 15891114]
- Anand S, Majeti BK, Acevedo LM, Murphy EA, Mukthavaram R, Schepke L, Huang M, Shields DJ, Lindquist JN, Lapinski PE, King PD, Weis SM, Cheres DA. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. *Nat Med.* 2010; 16:909–914. [PubMed: 20676106]
- Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA. Glial and neuronal control of brain blood flow. *Nature.* 2010; 468:232–243. [PubMed: 21068832]
- Baburamani AA, Ek CJ, Walker DW, Castillo-Melendez M. Vulnerability of the developing brain to hypoxic-ischemic damage: contribution of the cerebral vasculature to injury and repair? *Frontiers in physiology.* 2012; 3:424. [PubMed: 23162470]
- Back SA, Gan X, Li Y, Rosenberg PA, Volpe JJ. Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. *J Neurosci.* 1998; 18:6241–6253. [PubMed: 9698317]
- Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM. Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. *J Neurosci.* 2002; 22:455–463. [PubMed: 11784790]
- Bacon AL, Fox S, Turley H, Harris AL. Selective silencing of the hypoxia-inducible factor 1 target gene BNIP3 by histone deacetylation and methylation in colorectal cancer. *Oncogene.* 2007; 26:132–141. [PubMed: 16799636]
- 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. [PubMed: 15907476]
- Barrett RD, Bennet L, Davidson J, Dean JM, George S, Emerald BS, Gunn AJ. Destruction and reconstruction: hypoxia and the developing brain. *Birth Defects Res C Embryo Today.* 2007; 81:163–176. [PubMed: 17963273]
- Bautch VL, James JM. Neurovascular development: The beginning of a beautiful friendship. *Cell adhesion & migration.* 2009; 3:199–204. [PubMed: 19363295]
- Ben-Zvi A, Lacoste B, Kur E, Andreone BJ, Mayshar Y, Yan H, Gu C. Mfsd2a is critical for the formation and function of the blood-brain barrier. *Nature.* 2014; 509:507–511. [PubMed: 24828040]
- Benizri E, Ginouves A, Berra E. The magic of the hypoxia-signaling cascade. *Cellular and molecular life sciences : CMLS.* 2008; 65:1133–1149. [PubMed: 18202826]

- Bennet L, Roelfsema V, George S, Dean JM, Emerald BS, Gunn AJ. The effect of cerebral hypothermia on white and grey matter injury induced by severe hypoxia in preterm fetal sheep. *J Physiol.* 2007; 578:491–506. [PubMed: 17095565]
- Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet.* 2003; 35:215–217. [PubMed: 14528307]
- Beyer S, Kristensen MM, Jensen KS, Johansen JV, Staller P. The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. *The Journal of biological chemistry.* 2008; 283:36542–36552. [PubMed: 18984585]
- Bhadra T, Bhattacharyya M, Feuerbach L, Lengauer T, Bandyopadhyay S. Correction: DNA Methylation Patterns Facilitate the Identification of MicroRNA Transcription Start Sites: A Brain-Specific Study. *PLoS One.* 2013; 8
- Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature.* 2010; 463:1042–1047. [PubMed: 20027182]
- Blackledge NP, Klose R. CpG island chromatin: a platform for gene regulation. *Epigenetics.* 2011; 6:147–152. [PubMed: 20935486]
- Bogdanovic O, Veenstra GJ. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. *Chromosoma.* 2009; 118:549–565. [PubMed: 19506892]
- Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, Burchfield J, Fox H, Doebele C, Ohtani K, Chavakis E, Potente M, Tjwa M, Urbich C, Zeiher AM, Dimmeler S. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science.* 2009; 324:1710–1713. [PubMed: 19460962]
- Bonev B, Pisco A, Papalopulu N. MicroRNA-9 reveals regional diversity of neural progenitors along the anterior-posterior axis. *Developmental Cell.* 2011; 20:19–32. [PubMed: 21238922]
- Bostick M, Kim JK, Esteve PO, Clark A, Pradhan S, Jacobsen SE. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science.* 2007; 317:1760–1764. [PubMed: 17673620]
- Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H. Sp1 elements protect a CpG island from de novo methylation. *Nature.* 1994; 371:435–438. [PubMed: 8090226]
- Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, Darnell RB, Warren ST. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell.* 2001; 107:477–487. [PubMed: 11719188]
- Bruhl T, Urbich C, Aicher D, Acker-Palmer A, Zeiher AM, Dimmeler S. Homeobox A9 transcriptionally regulates the EphB4 receptor to modulate endothelial cell migration and tube formation. *Circulation research.* 2004; 94:743–751. [PubMed: 14764452]
- Bruneteau G, Simonet T, Bauche S, Mandjee N, Malfatti E, Girard E, Tanguy ML, Behin A, Khiami F, Sariali E, Hell-Remy C, Salachas F, Pradat PF, Fournier E, Lacomblez L, Koenig J, Romero NB, Fontaine B, Meininger V, Schaeffer L, Hantai D. Muscle histone deacetylase 4 upregulation in amyotrophic lateral sclerosis: potential role in reinnervation ability and disease progression. *Brain : a journal of neurology.* 2013; 136:2359–2368. [PubMed: 23824486]
- Bu P, Evrard YA, Lozano G, Dent SY. Loss of Gcn5 acetyltransferase activity leads to neural tube closure defects and exencephaly in mouse embryos. *Mol Cell Biol.* 2007; 27:3405–3416. [PubMed: 17325035]
- Burgold T, Spreafico F, De Santa F, Totaro MG, Prosperini E, Natoli G, Testa G. The histone H3 lysine 27-specific demethylase Jmjd3 is required for neural commitment. *PLoS One.* 2008; 3:e3034. [PubMed: 18716661]
- Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. *Nature structural & molecular biology.* 2013; 20:282–289.
- Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science.* 2002; 298:1039–1043. [PubMed: 12351676]

- Cao X, Yeo G, Muotri AR, Kuwabara T, Gage FH. Noncoding RNAs in the mammalian central nervous system. *Annu Rev Neurosci*. 2006; 29:77–103. [PubMed: 16776580]
- Carmeliet P. Angiogenesis in health and disease. *Nature medicine*. 2003; 9:653–660.
- Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nature reviews Genetics*. 2009; 10:295–304.
- Chahrouh M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science*. 2008; 320:1224–1229. [PubMed: 18511691]
- Chan YC, Banerjee J, Choi SY, Sen CK. miR-210: the master hypoxamir. *Microcirculation*. 2012; 19:215–223. [PubMed: 22171547]
- Chang S, Young BD, Li S, Qi X, Richardson JA, Olson EN. Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. *Cell*. 2006; 126:321–334. [PubMed: 16873063]
- Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nature genetics*. 2006; 38:228–233. [PubMed: 16380711]
- Chen M, Zhang L. Epigenetic mechanisms in developmental programming of adult disease. *Drug Discov Today*. 2011; 16:1007–1018. [PubMed: 21945859]
- Chen S, Feng B, George B, Chakrabarti R, Chen M, Chakrabarti S. Transcriptional coactivator p300 regulates glucose-induced gene expression in endothelial cells. *American journal of physiology Endocrinology and metabolism*. 2010a; 298:E127–137. [PubMed: 19903865]
- Chen Y, Banda M, Speyer CL, Smith JS, Rabson AB, Gorski DH. Regulation of the expression and activity of the antiangiogenic homeobox gene GAX/MEOX2 by ZEB2 and microRNA-221. *Mol Cell Biol*. 2010b; 30:3902–3913. [PubMed: 20516212]
- Cheng LC, Pastrana E, Tavazoie M, Doetsch F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat Neurosci*. 2009; 12:399–408. [PubMed: 19287386]
- Chio CC, Lin JW, Cheng HA, Chiu WT, Wang YH, Wang JJ, Hsing CH, Chen RM. MicroRNA-210 targets antiapoptotic Bcl-2 expression and mediates hypoxia-induced apoptosis of neuroblastoma cells. *Arch Toxicol*. 2013; 87:459–468. [PubMed: 23108914]
- Choudhry H, Schodel J, Oikonomopoulos S, Camps C, Grampp S, Harris AL, Ratcliffe PJ, Ragoussis J, Mole DR. Extensive regulation of the non-coding transcriptome by hypoxia: role of HIF in releasing paused RNAPol2. *EMBO reports*. 2014; 15:70–76. [PubMed: 24363272]
- Cohen S, Gabel HW, Hemberg M, Hutchinson AN, Sadacca LA, Ebert DH, Harmin DA, Greenberg RS, Verdine VK, Zhou Z, Wetsel WC, West AE, Greenberg ME. Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron*. 2011; 72:72–85. [PubMed: 21982370]
- Conaco C, Otto S, Han JJ, Mandel G. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci U S A*. 2006; 103:2422–2427. [PubMed: 16461918]
- Coolen M, Katz S, Bally-Cuif L. miR-9: a versatile regulator of neurogenesis. *Front Cell Neurosci*. 2013; 7:220. [PubMed: 24312010]
- Coolen M, Thieffry D, Drivenes O, Becker TS, Bally-Cuif L. miR-9 controls the timing of neurogenesis through the direct inhibition of antagonistic factors. *Developmental Cell*. 2012; 22:1052–1064. [PubMed: 22595676]
- 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. [PubMed: 21722948]
- Coultas L, Chawengsaksophak K, Rossant J. Endothelial cells and VEGF in vascular development. *Nature*. 2005; 438:937–945. [PubMed: 16355211]
- Covic M, Karaca E, Lie DC. Epigenetic regulation of neurogenesis in the adult hippocampus. *Heredity*. 2010; 105:122–134. [PubMed: 20332807]
- Coy JF, Sedlacek Z, Bachner D, Delius H, Poustka A. A complex pattern of evolutionary conservation and alternative polyadenylation within the long 3′-untranslated region of the methyl-CpG-binding

- protein 2 gene (MeCP2) suggests a regulatory role in gene expression. *Hum Mol Genet.* 1999; 8:1253–1262. [PubMed: 10369871]
- Crosby ME, Kulshreshtha R, Ivan M, Glazer PM. MicroRNA regulation of DNA repair gene expression in hypoxic stress. *Cancer research.* 2009; 69:1221–1229. [PubMed: 19141645]
- Dean JM, Gunn AJ, Wassink G, George S, Bennet L. Endogenous alpha2-adrenergic receptor-mediated neuroprotection after severe hypoxia in preterm fetal sheep. *Neuroscience.* 2006; 142:615–628. [PubMed: 16952424]
- Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev.* 2011; 25:1010–1022. [PubMed: 21576262]
- Dentelli P, Rosso A, Orso F, Olgasi C, Taverna D, Brizzi MF. microRNA-222 controls neovascularization by regulating signal transducer and activator of transcription 5A expression. *Arterioscler Thromb Vasc Biol.* 2010; 30:1562–1568. [PubMed: 20489169]
- Diehl F, Rossig L, Zeiher AM, Dimmeler S, Urbich C. The histone methyltransferase MLL is an upstream regulator of endothelial-cell sprout formation. *Blood.* 2007; 109:1472–1478. [PubMed: 17047146]
- Dominguez-Salas P, Cox SE, Prentice AM, Hennig BJ, Moore SE. Maternal nutritional status, C(1) metabolism and offspring DNA methylation: a review of current evidence in human subjects. *Proc Nutr Soc.* 2012; 71:154–165. [PubMed: 22124338]
- Dudley KJ, Li X, Kobor MS, Kippin TE, Bredy TW. Epigenetic mechanisms mediating vulnerability and resilience to psychiatric disorders. *Neurosci Biobehav Rev.* 2011; 35:1544–1551. [PubMed: 21251925]
- Duncan JR, Cock ML, Loeliger M, Louey S, Harding R, Rees SM. Effects of exposure to chronic placental insufficiency on the postnatal brain and retina in sheep. *J Neuropathol Exp Neurol.* 2004; 63:1131–1143. [PubMed: 15581181]
- Duncan JR, Cock ML, Scheerlinck JP, Westcott KT, McLean C, Harding R, Rees SM. White matter injury after repeated endotoxin exposure in the preterm ovine fetus. *Pediatr Res.* 2002; 52:941–949. [PubMed: 12438674]
- Duursma AM, Kedde M, Schrier M, le Sage C, Agami R. miR-148 targets human DNMT3b protein coding region. *RNA.* 2008; 14:872–877. [PubMed: 18367714]
- Dyrvig M, Hansen HH, Christiansen SH, Woldbye DP, Mikkelsen JD, Lichota J. Epigenetic regulation of Arc and c-Fos in the hippocampus after acute electroconvulsive stimulation in the rat. *Brain research bulletin.* 2012; 88:507–513. [PubMed: 22613772]
- Edbauer D, Neilson JR, Foster KA, Wang CF, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp PA, Sheng M. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron.* 2010; 65:373–384. [PubMed: 20159450]
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature.* 2004; 429:457–463. [PubMed: 15164071]
- Esteve PO, Chin HG, Benner J, Feehery GR, Samaranyake M, Horwitz GA, Jacobsen SE, Pradhan S. Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. *Proc Natl Acad Sci U S A.* 2009; 106:5076–5081. [PubMed: 19282482]
- Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A.* 2007; 104:15805–15810. [PubMed: 17890317]
- Fagioli M, Jensen CL, Champagne FA. Epigenetic influences on brain development and plasticity. *Current opinion in neurobiology.* 2009; 19:207–212. [PubMed: 19545993]
- Fan G, Beard C, Chen RZ, Csankovszki G, Sun Y, Siniaia M, Biniszkievicz 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. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2001; 21:788–797. [PubMed: 11157065]
- 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 astroglialogenesis through regulation of JAK-STAT signaling. *Development.* 2005; 132:3345–3356. [PubMed: 16014513]

- Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, Capogrossi MC, Martelli F. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *The Journal of biological chemistry*. 2008; 283:15878–15883. [PubMed: 18417479]
- Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. *Clin Perinatol*. 2009; 36:835–858, vii. [PubMed: 19944838]
- Fath DM, Kong X, Liang D, Lin Z, Chou A, Jiang Y, Fang J, Caro J, Sang N. Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF- α . *The Journal of biological chemistry*. 2006; 281:13612–13619. [PubMed: 16543236]
- Feng J, Fouse S, Fan G. Epigenetic regulation of neural gene expression and neuronal function. *Pediatric research*. 2007; 61:58R–63R.
- Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature*. 1996; 380:439–442. [PubMed: 8602242]
- Fiore R, Khudayberdiev S, Christensen M, Siegel G, Flavell SW, Kim TK, Greenberg ME, Schratt G. Mef2-mediated transcription of the miR379-410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. *EMBO J*. 2009; 28:697–710. [PubMed: 19197241]
- Fiskus W, Pranpat M, Balasis M, Herger B, Rao R, Chinnaiyan A, Atadja P, Bhalla K. Histone deacetylase inhibitors deplete enhancer of zeste 2 and associated polycomb repressive complex 2 proteins in human acute leukemia cells. *Molecular cancer therapeutics*. 2006; 5:3096–3104.
- Frank D, Keshet I, Shani M, Levine A, Razin A, Cedar H. Demethylation of CpG islands in embryonic cells. *Nature*. 1991; 351:239–241. [PubMed: 2041571]
- Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochemical and biophysical research communications*. 2000; 273:793–798. [PubMed: 10873683]
- Gao J, Wang WY, Mao YW, Graff J, Guan JS, Pan L, Mak G, Kim D, Su SC, Tsai LH. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature*. 2010; 466:1105–1109. [PubMed: 20622856]
- Garcia-Bonilla L, Benakis C, Moore J, Iadecola C, Anrather J. Immune mechanisms in cerebral ischemic tolerance. *Frontiers in neuroscience*. 2014; 8:44. [PubMed: 24624056]
- George S, Scotter J, Dean JM, Bennet L, Waldvogel HJ, Guan J, Faull RL, Gunn AJ. Induced cerebral hypothermia reduces post-hypoxic loss of phenotypic striatal neurons in preterm fetal sheep. *Exp Neurol*. 2007; 203:137–147. [PubMed: 16962098]
- Gicquel C, El-Osta A, Le Bouc Y. Epigenetic regulation and fetal programming. *Best practice & research Clinical endocrinology & metabolism*. 2008; 22:1–16. [PubMed: 18279777]
- Girouard H, Iadecola C. Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *Journal of applied physiology*. 2006; 100:328–335. [PubMed: 16357086]
- Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *The New England journal of medicine*. 2008; 359:61–73. [PubMed: 18596274]
- Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. *Annu Rev Biochem*. 2005; 74:481–514. [PubMed: 15952895]
- Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH. Methylation of tRNA^{Asp} by the DNA methyltransferase homolog Dnmt2. *Science*. 2006; 311:395–398. [PubMed: 16424344]
- Golshani P, Hutnick L, Schweizer F, Fan G. Conditional Dnmt1 deletion in dorsal forebrain disrupts development of somatosensory barrel cortex and thalamocortical long-term potentiation. *Thalamus Relat Syst*. 2005; 3:227–233. [PubMed: 17710197]
- Gonzalez-Rodriguez PJ, Xiong F, Li Y, Zhou J, Zhang L. Fetal hypoxia increases vulnerability of hypoxic-ischemic brain injury in neonatal rats: Role of glucocorticoid receptors. *Neurobiology of disease*. 2014; 65:172–179. [PubMed: 24513088]
- Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*. 2010; 466:835–840. [PubMed: 20703300]

- Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell*. 2011; 145:423–434. [PubMed: 21496894]
- Ha SD, Han CY, Reid C, Kim SO. HDAC8-Mediated Epigenetic Reprogramming Plays a Key Role in Resistance to Anthrax Lethal Toxin-Induced Pyroptosis in Macrophages. *Journal of immunology*. 2014; 193:1333–1343.
- Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev*. 2004; 18:3016–3027. [PubMed: 15574589]
- Han K, Gennarino VA, Lee Y, Pang K, Hashimoto-Torii K, Choufani S, Raju CS, Oldham MC, Weksberg R, Rakic P, Liu Z, Zoghbi HY. Human-specific regulation of MeCP2 levels in fetal brains by microRNA miR-483-5p. *Genes & development*. 2013; 27:485–490. [PubMed: 23431031]
- Han L, Witmer PD, Casey E, Valle D, Sukumar S. DNA methylation regulates MicroRNA expression. *Cancer Biol Ther*. 2007; 6:1284–1288. [PubMed: 17660710]
- Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. *Proc Natl Acad Sci U S A*. 2008; 105:1516–1521. [PubMed: 18227515]
- Hartley I, Elkhoury FF, Heon Shin J, Xie B, Gu X, Gao Y, Zhou D, Haddad GG. Long-lasting changes in DNA methylation following short-term hypoxic exposure in primary hippocampal neuronal cultures. *PloS one*. 2013; 8:e77859. [PubMed: 24205000]
- Hatzimichael E, Dasoula A, Shah R, Syed N, Papoudou-Bai A, Coley HM, Dranitsaris G, Bourantas KL, Stebbing J, Crook T. The prolyl-hydroxylase EGLN3 and not EGLN1 is inactivated by methylation in plasma cell neoplasia. *Eur J Haematol*. 2010; 84:47–51. [PubMed: 19737309]
- 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. [PubMed: 21817016]
- Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarr JR, Linehan WM, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci U S A*. 1994; 91:9700–9704. [PubMed: 7937876]
- Hermann DM, Zechariah A. Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2009; 29:1620–1643.
- Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH. Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc Natl Acad Sci U S A*. 2004; 101:16659–16664. [PubMed: 15537713]
- Hu S, Dong TS, Dalal SR, Wu F, Bissonnette M, Kwon JH, Chang EB. The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer. *PloS one*. 2011; 6:e16221. [PubMed: 21283757]
- Huang X, Ding L, Bennenwith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT, Giaccia AJ. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. *Mol Cell*. 2009; 35:856–867. [PubMed: 19782034]
- Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet*. 2011; 12:99–110. [PubMed: 21245828]
- 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. [PubMed: 19433415]
- Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science*. 2001; 293:834–838. [PubMed: 11452083]
- Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nature reviews Neuroscience*. 2004; 5:347–360.
- Iliopoulos D, Lindahl-Allen M, Polytharchou C, Hirsch HA, Tschlis PN, Struhl K. Loss of miR-200 inhibition of Suz12 leads to polycomb-mediated repression required for the formation and maintenance of cancer stem cells. *Molecular cell*. 2010; 39:761–772. [PubMed: 20832727]

- Illi B, Scopece A, Nanni S, Farsetti A, Morgante L, Biglioli P, Capogrossi MC, Gaetano C. Epigenetic histone modification and cardiovascular lineage programming in mouse embryonic stem cells exposed to laminar shear stress. *Circ Res*. 2005; 96:501–508. [PubMed: 15705964]
- Impey S, Davare M, Lesiak A, Fortin D, Ando H, Varlamova O, Obrietan K, Soderling TR, Goodman RH, Wayman GA. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Mol Cell Neurosci*. 2010; 43:146–156. [PubMed: 19850129]
- Iqbal N, Mei J, Liu J, Skapek SX. miR-34a is essential for p19-driven cell cycle arrest. *Cell Cycle*. 2014; 13
- Ishida M, Sunamura M, Furukawa T, Akada M, Fujimura H, Shibuya E, Egawa S, Unno M, Horii A. Elucidation of the relationship of BNIP3 expression to gemcitabine chemosensitivity and prognosis. *World journal of gastroenterology : WJG*. 2007; 13:4593–4597. [PubMed: 17729412]
- 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. [PubMed: 20639862]
- Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*. 2011; 333:1300–1303. [PubMed: 21778364]
- Iwase S, Lan F, Bayliss P, de la Torre-Ubieta L, Huarte M, Qi HH, Whetstone JR, Bonni A, Roberts TM, Shi Y. The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell*. 2007; 128:1077–1088. [PubMed: 17320160]
- Jackson RJ, Standart N. How do microRNAs regulate gene expression? *Sci STKE*. 2007; 2007:re1. [PubMed: 17200520]
- Jensen LR, Bartenschlager H, Rujirabanjerd S, Tzschach A, Numann A, Janecke AR, Sporle R, Stricker S, Raynaud M, Nelson J, Hackett A, Fryns JP, Chelly J, de Brouwer AP, Hamel B, Gecz J, Ropers HH, Kuss AW. A distinctive gene expression fingerprint in mentally retarded male patients reflects disease-causing defects in the histone demethylase KDM5C. *PathoGenetics*. 2010; 3:2. [PubMed: 20181063]
- Jepsen K, Solum D, Zhou T, McEvilly RJ, Kim HJ, Glass CK, Hermanson O, Rosenfeld MG. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. *Nature*. 2007; 450:415–419. [PubMed: 17928865]
- Jimenez-Mateos E, Engel T, Merino-Serrais P, McKiernan R, Tanaka K, Mouri G, Sano T, O'Tuathaigh C, Waddington J, Prenter S, Delanty N, Farrell M, O'Brien D, Conroy R, Stallings R, DeFelipe J, Henshall D. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. *Nature medicine*. 2012; 18:1087–1094.
- John RM, Lefebvre L. Developmental regulation of somatic imprints. *Differentiation; research in biological diversity*. 2011; 81:270–280.
- Johnson AB, Denko N, Barton MC. Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. *Mutation research*. 2008; 640:174–179. [PubMed: 18294659]
- Johnson JE. Numb and Numlike control cell number during vertebrate neurogenesis. *Trends Neurosci*. 2003; 26:395–396. [PubMed: 12900165]
- Juan AH, Kumar RM, Marx JG, Young RA, Sartorelli V. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. *Molecular cell*. 2009; 36:61–74. [PubMed: 19818710]
- Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, Poellinger L. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. *The EMBO journal*. 1998; 17:6573–6586. [PubMed: 9822602]
- Kane NM, Howard L, Descamps B, Meloni M, McClure J, Lu R, McCahill A, Breen C, Mackenzie RM, Delles C, Mountford JC, Milligan G, Emanueli C, Baker AH. Role of microRNAs 99b, 181a, and 181b in the differentiation of human embryonic stem cells to vascular endothelial cells. *Stem Cells*. 2012; 30:643–654. [PubMed: 22232059]
- Kapsimali M, Kloosterman WP, de Bruijn E, Rosa F, Plasterk RH, Wilson SW. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol*. 2007; 8:R173. [PubMed: 17711588]

- Karlic R, Chung HR, Lasserre J, Vlahovicek K, Vingron M. Histone modification levels are predictive for gene expression. *Proc Natl Acad Sci U S A*. 2010; 107:2926–2931. [PubMed: 20133639]
- Kenneth NS, Mudie S, van Uden P, Rocha S. SWI/SNF regulates the cellular response to hypoxia. *The Journal of biological chemistry*. 2009; 284:4123–4131. [PubMed: 19097995]
- Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic preconditioning augments survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. *The Journal of biological chemistry*. 2009a; 284:33161–33168. [PubMed: 19721136]
- Kim J, Hwang J, Jeong H, Song HJ, Shin J, Hur G, Park YW, Lee SH, Kim J. Promoter methylation status of VEGF receptor genes: a possible epigenetic biomarker to anticipate the efficacy of intracellular-acting VEGF-targeted drugs in cancer cells. *Epigenetics : official journal of the DNA Methylation Society*. 2012; 7:191–200. [PubMed: 22395469]
- Kim JY, Hwang JH, Zhou W, Shin J, Noh SM, Song IS, Kim JY, Lee SH, Kim J. The expression of VEGF receptor genes is concurrently influenced by epigenetic gene silencing of the genes and VEGF activation. *Epigenetics : official journal of the DNA Methylation Society*. 2009b; 4:313–321. [PubMed: 19633424]
- Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nature medicine*. 2001; 7:437–443.
- Kimura H, Shiota K. Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. *J Biol Chem*. 2003; 278:4806–4812. [PubMed: 12473678]
- Kishi N, Macklis JD. MECP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. *Mol Cell Neurosci*. 2004; 27:306–321. [PubMed: 15519245]
- Klose RJ, Bird AP. MeCP2 behaves as an elongated monomer that does not stably associate with the Sin3a chromatin remodeling complex. *J Biol Chem*. 2004; 279:46490–46496. [PubMed: 15322089]
- Kooistra SM, Helin K. Molecular mechanisms and potential functions of histone demethylases. *Nature reviews Molecular cell biology*. 2012; 13:297–311.
- Kosik KS. The neuronal microRNA system. *Nat Rev Neurosci*. 2006; 7:911–920. [PubMed: 17115073]
- Koslowski M, Luxemburger U, Tureci O, Sahin U. Tumor-associated CpG demethylation augments hypoxia-induced effects by positive autoregulation of HIF-1alpha. *Oncogene*. 2011; 30:876–882. [PubMed: 21042279]
- Kouzarides T. Chromatin modifications and their function. *Cell*. 2007a; 128:693–705. [PubMed: 17320507]
- Kouzarides T. SnapShot: Histone-modifying enzymes. *Cell*. 2007b; 131:822. [PubMed: 18022374]
- Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells*. 2006; 24:857–864. [PubMed: 16357340]
- Krieg AJ, Rankin EB, Chan D, Razorenova O, Fernandez S, Giaccia AJ. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. *Molecular and cellular biology*. 2010; 30:344–353. [PubMed: 19858293]
- Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Richter J, Duebel J, Bicker S, Fehling HJ, Schubeler D, Oertner TG, Schratt G, Bibel M, Roska B, Filipowicz W. Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. *Cell*. 2010; 141:618–631. [PubMed: 20478254]
- Kuehbach A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circulation research*. 2007; 101:59–68. [PubMed: 17540974]
- Kuhnert F, Mancuso MR, Hampton J, Stankunas K, Asano T, Chen CZ, Kuo CJ. Attribution of vascular phenotypes of the murine *Egfl7* locus to the microRNA miR-126. *Development*. 2008; 135:3989–3993. [PubMed: 18987025]
- Kuijper S, Turner CJ, Adams RH. Regulation of angiogenesis by Eph-ephrin interactions. *Trends in cardiovascular medicine*. 2007; 17:145–151. [PubMed: 17574121]

- Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol*. 2002; 12:735–739. [PubMed: 12007417]
- Lambert TJ, Storm DR, Sullivan JM. MicroRNA132 modulates short-term synaptic plasticity but not basal release probability in hippocampal neurons. *PLoS One*. 2010; 5:e15182. [PubMed: 21206919]
- Laneve P, Gioia U, Andriotto A, Moretti F, Bozzoni I, Caffarelli E. A minicircuitry involving REST and CREB controls miR-9-2 expression during human neuronal differentiation. *Nucleic Acids Res*. 2010; 38:6895–6905. [PubMed: 20624818]
- Lee S, Chen TT, Barber CL, Jordan MC, Murdock J, Desai S, Ferrara N, Nagy A, Roos KP, Iruela-Arispe ML. Autocrine VEGF signaling is required for vascular homeostasis. *Cell*. 2007; 130:691–703. [PubMed: 17719546]
- Lee S, Lee B, Lee JW, Lee SK. Retinoid signaling and neurogenin2 function are coupled for the specification of spinal motor neurons through a chromatin modifier CBP. *Neuron*. 2009; 62:641–654. [PubMed: 19524524]
- Lee S, Lee SK. Crucial roles of histone-modifying enzymes in mediating neural cell-type specification. *Curr Opin Neurobiol*. 2010; 20:29–36. [PubMed: 20137907]
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003; 425:415–419. [PubMed: 14508493]
- Lei Z, Li B, Yang Z, Fang H, Zhang GM, Feng ZH, Huang B. Regulation of HIF-1 α and VEGF by miR-20b tunes tumor cells to adapt to the alteration of oxygen concentration. *PloS one*. 2009; 4:e7629. [PubMed: 19893619]
- Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*. 1992; 69:915–926. [PubMed: 1606615]
- Li H, Zhong X, Chau KF, Williams EC, Chang Q. Loss of activity-induced phosphorylation of MeCP2 enhances synaptogenesis, LTP and spatial memory. *Nat Neurosci*. 2011; 14:1001–1008. [PubMed: 21765426]
- Li HS, Wang D, Shen Q, Schonemann MD, Gorski JA, Jones KR, Temple S, Jan LY, Jan YN. Inactivation of Numb and Numlike in embryonic dorsal forebrain impairs neurogenesis and disrupts cortical morphogenesis. *Neuron*. 2003; 40:1105–1118. [PubMed: 14687546]
- Li Y, Gonzalez P, Zhang L. Fetal stress and programming of hypoxic/ischemic-sensitive phenotype in the neonatal brain: mechanisms and possible interventions. *Progress in neurobiology*. 2012; 98:145–165. [PubMed: 22627492]
- Lim DA, Huang YC, Swigut T, Mirick AL, Garcia-Verdugo JM, Wsocka J, Ernst P, Alvarez-Buylla A. Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature*. 2009; 458:529–533. [PubMed: 19212323]
- Liu C, Teng ZQ, Santistevan NJ, Szulwach KE, Guo W, Jin P, Zhao X. Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. *Cell Stem Cell*. 2010; 6:433–444. [PubMed: 20452318]
- Liu D, Krueger J, Le Noble F. The role of blood flow and microRNAs in blood vessel development. *Int J Dev Biol*. 2011; 55:419–429. [PubMed: 21858767]
- Loeliger M, Watson CS, Reynolds JD, Penning DH, Harding R, Bocking AD, Rees SM. Extracellular glutamate levels and neuropathology in cerebral white matter following repeated umbilical cord occlusion in the near term fetal sheep. *Neuroscience*. 2003; 116:705–714. [PubMed: 12573713]
- Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). *Leukemia*. 2003; 17:637–641. [PubMed: 12646957]
- Lu C, Han HD, Mangala LS, Ali-Fehmi R, Newton CS, Ozburn L, Armaiz-Pena GN, Hu W, Stone RL, Munkarah A, Ravoori MK, Shahzad MM, Lee JW, Mora E, Langley RR, Carroll AR, Matsuo K, Spannuth WA, Schmandt R, Jennings NB, Goodman BW, Jaffe RB, Nick AM, Kim HS, Guven EO, Chen YH, Li LY, Hsu MC, Coleman RL, Calin GA, Denkbass EB, Lim JY, Lee JS, Kundra V, Birrer MJ, Hung MC, Lopez-Berestein G, Sood AK. Regulation of tumor angiogenesis by EZH2. *Cancer Cell*. 2010; 18:185–197. [PubMed: 20708159]

- Lugli G, Torvik VI, Larson J, Smalheiser NR. Expression of microRNAs and their precursors in synaptic fractions of adult mouse forebrain. *J Neurochem*. 2008; 106:650–661. [PubMed: 18410515]
- Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science*. 2004; 303:95–98. [PubMed: 14631048]
- Luo W, Chang R, Zhong J, Pandey A, Semenza GL. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:E3367–3376. [PubMed: 23129632]
- Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes & development*. 2001; 15:2675–2686. [PubMed: 11641274]
- Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Molecular cell*. 2007; 27:435–448. [PubMed: 17679093]
- Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005; 105:659–669. [PubMed: 15374877]
- Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011; 469:343–349. [PubMed: 21248841]
- Mariani CJ, Vasanthakumar A, Madzo J, Yesilkamal A, Bhagat T, Yu Y, Bhattacharyya S, Wenger RH, Cohn SL, Nanduri J, Verma A, Prabhakar NR, Godley LA. TET1-Mediated Hydroxymethylation Facilitates Hypoxic Gene Induction in Neuroblastoma. *Cell reports*. 2014; 7:1343–1352. [PubMed: 24835990]
- Martin Caballero I, Hansen J, Leaford D, Pollard S, Hendrich BD. The methyl-CpG binding proteins MeCP2, Mbd2 and Kaiso are dispensable for mouse embryogenesis, but play a redundant function in neural differentiation. *PLoS One*. 2009; 4:e4315. [PubMed: 19177165]
- Martin MM, Lee EJ, Buckenberger JA, Schmittgen TD, Elton TS. MicroRNA-155 regulates human angiotensin II type 1 receptor expression in fibroblasts. *The Journal of biological chemistry*. 2006; 281:18277–18284. [PubMed: 16675453]
- Matsumura S, Oue N, Mitani Y, Kitadai Y, Yasui W. DNA demethylation of vascular endothelial growth factor-C is associated with gene expression and its possible involvement of lymphangiogenesis in gastric cancer. *International journal of cancer Journal international du cancer*. 2007; 120:1689–1695. [PubMed: 17230534]
- McCormick RI, Blick C, Ragoussis J, Schoedel J, Mole DR, Young AC, Selby PJ, Banks RE, Harris AL. miR-210 is a target of hypoxia-inducible factors 1 and 2 in renal cancer, regulates ISCU and correlates with good prognosis. *British journal of cancer*. 2013; 108:1133–1142. [PubMed: 23449350]
- McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European journal of pharmacology*. 2008; 583:174–185. [PubMed: 18282566]
- Mehler MF. Epigenetics and the nervous system. *Annals of neurology*. 2008; 64:602–617. [PubMed: 19107999]
- Mehler MF, Mattick JS. Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. *Physiological reviews*. 2007; 87:799–823. [PubMed: 17615389]
- Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron*. 2007; 53:857–869. [PubMed: 17359920]
- Miquerol L, Langille BL, Nagy A. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. *Development*. 2000; 127:3941–3946. [PubMed: 10952892]
- Miska EA, Alvarez-Saavedra E, Townsend M, Yoshii A, Sestan N, Rakic P, Constantine-Paton M, Horvitz HR. Microarray analysis of microRNA expression in the developing mammalian brain. *Genome Biol*. 2004; 5:R68. [PubMed: 15345052]

- Montgomery RL, Hsieh J, Barbosa AC, Richardson JA, Olson EN. Histone deacetylases 1 and 2 control the progression of neural precursors to neurons during brain development. *Proc Natl Acad Sci U S A*. 2009; 106:7876–7881. [PubMed: 19380719]
- Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology*. 2013; 38:23–38. [PubMed: 22781841]
- Morita S, Horii T, Kimura M, Ochiya T, Tajima S, Hatada I. miR-29 represses the activities of DNA methyltransferases and DNA demethylases. *Int J Mol Sci*. 2013; 14:14647–14658. [PubMed: 23857059]
- Mutoh T, Sanosaka T, Ito K, Nakashima K. Oxygen levels epigenetically regulate fate switching of neural precursor cells via hypoxia-inducible factor 1alpha-notch signal interaction in the developing brain. *Stem cells*. 2012; 30:561–569. [PubMed: 22213097]
- Nakada C, Tsukamoto Y, Matsuura K, Nguyen TL, Hijiya N, Uchida T, Sato F, Mimata H, Seto M, Moriyama M. Overexpression of miR-210, a downstream target of HIF1alpha, causes centrosome amplification in renal carcinoma cells. *The Journal of pathology*. 2011; 224:280–288. [PubMed: 21465485]
- Nakao T, Ishizawa A, Ogawa R. Observations of vascularization in the spinal cord of mouse embryos, with special reference to development of boundary membranes and perivascular spaces. *The Anatomical record*. 1988; 221:663–677. [PubMed: 3414988]
- Nakazawa T, Watabe AM, Tezuka T, Yoshida Y, Yokoyama K, Umemori H, Inoue A, Okabe S, Manabe T, Yamamoto T. p250GAP, a novel brain-enriched GTPase-activating protein for Rho family GTPases, is involved in the N-methyl-D-aspartate receptor signaling. *Mol Biol Cell*. 2003; 14:2921–2934. [PubMed: 12857875]
- Namihira M, Kohyama J, Semi K, Sanosaka T, Deneen B, Taga T, Nakashima K. Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. *Developmental cell*. 2009; 16:245–255. [PubMed: 19217426]
- Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*. 1998; 393:386–389. [PubMed: 9620804]
- Nelson ED, Kavalali ET, Monteggia LM. MeCP2-dependent transcriptional repression regulates excitatory neurotransmission. *Curr Biol*. 2006; 16:710–716. [PubMed: 16581518]
- Nguyen MP, Lee S, Lee YM. Epigenetic regulation of hypoxia inducible factor in diseases and therapeutics. *Arch Pharm Res*. 2013; 36:252–263. [PubMed: 23440580]
- Nguyen S, Meletis K, Fu D, Jhaveri S, Jaenisch R. Ablation of de novo DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev Dyn*. 2007; 236:1663–1676. [PubMed: 17477386]
- Nicoli S, Knyphausen CP, Zhu LHJ, Lakshmanan A, Lawson ND. miR-221 Is Required for Endothelial Tip Cell Behaviors during Vascular Development. *Developmental Cell*. 2012; 22:418–429. [PubMed: 22340502]
- Noonan EJ, Place RF, Pookot D, Basak S, Whitson JM, Hirata H, Giardina C, Dahiya R. miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene*. 2009; 28:1714–1724. [PubMed: 19252524]
- Nudelman AS, DiRocco DP, Lambert TJ, Garelick MG, Le J, Nathanson NM, Storm DR. Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. *Hippocampus*. 2010; 20:492–498. [PubMed: 19557767]
- Okami J, Simeone DM, Logsdon CD. Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. *Cancer research*. 2004; 64:5338–5346. [PubMed: 15289340]
- 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. [PubMed: 10555141]
- Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, Erdjument-Bromage H, Tempst P, Lin SP, Allis CD, Cheng X, Bestor TH. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*. 2007; 448:714–717. [PubMed: 17687327]
- Pedersen MT, Helin K. Histone demethylases in development and disease. *Trends in cell biology*. 2010; 20:662–671. [PubMed: 20863703]

- Pereira JD, Sansom SN, Smith J, Dobenecker MW, Tarakhovsky A, Livesey FJ. Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:15957–15962. [PubMed: 20798045]
- Peruzzi P, Bronisz A, Nowicki MO, Wang Y, Ogawa D, Price R, Nakano I, Kwon CH, Hayes J, Lawler SE, Ostrowski MC, Chiocca EA, Godlewski J. MicroRNA-128 coordinately targets Polycomb Repressor Complexes in glioma stem cells. *Neuro-oncology*. 2013; 15:1212–1224. [PubMed: 23733246]
- Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, Mercatanti A, Hammond S, Rainaldi G. MicroRNAs modulate the angiogenic properties of HUVECs. *Blood*. 2006; 108:3068–3071. [PubMed: 16849646]
- Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, Jacobsen SE, Reik W. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature*. 2010; 463:1101–1105. [PubMed: 20098412]
- Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E, Alt FW, Zeiher AM, Dimmeler S. SIRT1 controls endothelial angiogenic functions during vascular growth. *Genes Dev*. 2007; 21:2644–2658. [PubMed: 17938244]
- Qian CN, Zhang CQ, Guo X, Hong MH, Cao SM, Mai WY, Min HQ, Zeng YX. Elevation of serum vascular endothelial growth factor in male patients with metastatic nasopharyngeal carcinoma. *Cancer*. 2000; 88:255–261. [PubMed: 10640954]
- Rai K, Huggins JJ, James SR, Karpf AR, Jones DA, Cairns BR. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. *Cell*. 2008; 135:1201–1212. [PubMed: 19109892]
- Rajasethupathy P, Fiumara F, Sheridan R, Betel D, Puthanveetil SV, Russo JJ, Sander C, Tuschl T, Kandel E. Characterization of small RNAs in *Aplysia* reveals a role for miR-124 in constraining synaptic plasticity through CREB. *Neuron*. 2009; 63:803–817. [PubMed: 19778509]
- Rajewsky N. microRNA target predictions in animals. *Nat Genet*. 2006; 38(Suppl):S8–13. [PubMed: 16736023]
- Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M. Downregulation of miR-199a derepresses hypoxia-inducible factor-1 α and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. *Circulation research*. 2009; 104:879–886. [PubMed: 19265035]
- Rees S, Breen S, Loeliger M, McCrabb G, Harding R. Hypoxemia near mid-gestation has long-term effects on fetal brain development. *J Neuropathol Exp Neurol*. 1999; 58:932–945. [PubMed: 10499436]
- Rees S, Harding R, Walker D. The biological basis of injury and neuroprotection in the fetal and neonatal brain. *Int J Dev Neurosci*. 2011; 29:551–563. [PubMed: 21527338]
- Rees S, Stringer M, Just Y, Hooper SB, Harding R. The vulnerability of the fetal sheep brain to hypoxemia at mid-gestation. *Brain Res Dev Brain Res*. 1997; 103:103–118.
- Richter GH, Plehm S, Fasan A, Rossler S, Unland R, Bennani-Baiti IM, Hotfilder M, Lowel D, von Luetichau I, Mossbrugger I, Quintanilla-Martinez L, Kovar H, Staeger MS, Muller-Tidow C, Burdach S. EZH2 is a mediator of EWS/FLI1 driven tumor growth and metastasis blocking endothelial and neuro-ectodermal differentiation. *Proc Natl Acad Sci U S A*. 2009; 106:5324–5329. [PubMed: 19289832]
- Riddle A, Luo NL, Manese M, Beardsley DJ, Green L, Rorvik DA, Kelly KA, Barlow CH, Kelly JJ, Hohimer AR, Back SA. Spatial heterogeneity in oligodendrocyte lineage maturation and not cerebral blood flow predicts fetal ovine periventricular white matter injury. *J Neurosci*. 2006; 26:3045–3055. [PubMed: 16540583]
- Ross SE, Greenberg ME, Stiles CD. Basic helix-loop-helix factors in cortical development. *Neuron*. 2003; 39:13–25. [PubMed: 12848929]
- Rossig L, Urbich C, Bruhl T, Dembach E, Heeschen C, Chavakis E, Sasaki K, Aicher D, Diehl F, Seeger F, Potente M, Aicher A, Zanetta L, Dejana E, Zeiher AM, Dimmeler S. Histone deacetylase activity is essential for the expression of HoxA9 and for endothelial commitment of progenitor cells. *J Exp Med*. 2005; 201:1825–1835. [PubMed: 15928198]

- Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *The EMBO journal*. 1998; 17:3005–3015. [PubMed: 9606183]
- Santos-Reboucas CB, Fintelman-Rodrigues N, Jensen LR, Kuss AW, Ribeiro MG, Campos M Jr, Santos JM, Pimentel MM. A novel nonsense mutation in KDM5C/JARID1C gene causing intellectual disability, short stature and speech delay. *Neuroscience letters*. 2011; 498:67–71. [PubMed: 21575681]
- Sarraf SA, Stancheva I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol Cell*. 2004; 15:595–605. [PubMed: 15327775]
- Sauvageot CM, Stiles CD. Molecular mechanisms controlling cortical gliogenesis. *Curr Opin Neurobiol*. 2002; 12:244–249. [PubMed: 12049929]
- Schodel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood*. 2011; 117:e207–217. [PubMed: 21447827]
- Schratt G. microRNAs at the synapse. *Nat Rev Neurosci*. 2009; 10:842–849. [PubMed: 19888283]
- Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME. A brain-specific microRNA regulates dendritic spine development. *Nature*. 2006; 439:283–289. [PubMed: 16421561]
- Sentilhes L, Michel C, Lecourtois M, Catteau J, Bourgeois P, Laudenbach V, Marret S, Laquerriere A. Vascular endothelial growth factor and its high-affinity receptor (VEGFR-2) are highly expressed in the human forebrain and cerebellum during development. *Journal of neuropathology and experimental neurology*. 2010; 69:111–128. [PubMed: 20084021]
- Shahrzad S, Bertrand K, Minhas K, Coomber BL. Induction of DNA hypomethylation by tumor hypoxia. *Epigenetics*. 2007; 2:119–125. [PubMed: 17965619]
- Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K, Tajima S, Mitsuya K, Okano M, Koseki H. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature*. 2007; 450:908–912. [PubMed: 17994007]
- Shen J, Xia W, Khotskaya YB, Huo L, Nakanishi K, Lim SO, Du Y, Wang Y, Chang WC, Chen CH, Hsu JL, Wu Y, Lam YC, James BP, Liu X, Liu CG, Patel DJ, Hung MC. EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. *Nature*. 2013; 497:383–387. [PubMed: 23636329]
- Shen S, Li J, Casaccia-Bonnel P. Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. *J Cell Biol*. 2005; 169:577–589. [PubMed: 15897262]
- Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA, Shi Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*. 2004; 119:941–953. [PubMed: 15620353]
- Shibata M, Nakao H, Kiyonari H, Abe T, Aizawa S. MicroRNA-9 regulates neurogenesis in mouse telencephalon by targeting multiple transcription factors. *J Neurosci*. 2011; 31:3407–3422. [PubMed: 21368052]
- Shin S, Lee EM, Cha HJ, Bae S, Jung JH, Lee SM, Yoon Y, Lee H, Kim S, Kim H, Lee SJ, Park IC, Jin YW, An S. MicroRNAs that respond to histone deacetylase inhibitor SAHA and p53 in HCT116 human colon carcinoma cells. *International journal of oncology*. 2009; 35:1343–1352. [PubMed: 19885557]
- Shirodkar AV, St Bernard R, Gavryushova A, Kop A, Knight BJ, Yan MS, Man HS, Sud M, Hebbel RP, Oettgen P, Aird WC, Marsden PA. A mechanistic role for DNA methylation in endothelial cell (EC)-enriched gene expression: relationship with DNA replication timing. *Blood*. 2013; 121:3531–3540. [PubMed: 23449636]
- Siegel G, Obernosterer G, Fiore R, Oehmen M, Bicker S, Christensen M, Khudayberdiev S, Leuschner PF, Busch CJ, Kane C, Hubel K, Dekker F, Hedberg C, Rengarajan B, Drepper C, Waldmann H, Kauppinen S, Greenberg ME, Draguhn A, Rehmsmeier M, Martinez J, Schratt GM. A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. *Nat Cell Biol*. 2009; 11:705–716. [PubMed: 19465924]

- Smalheiser NR, Lugli G. microRNA regulation of synaptic plasticity. *Neuromolecular Med.* 2009; 11:133–140. [PubMed: 19458942]
- Smith B, Treadwell J, Zhang D, Ly D, McKinnell I, Walker PR, Sikorska M. Large-scale expression analysis reveals distinct microRNA profiles at different stages of human neurodevelopment. *PLoS One.* 2010; 5:e11109. [PubMed: 20559549]
- Smrt RD, Szulwach KE, Pfeiffer RL, Li X, Guo W, Pathania M, Teng ZQ, Luo Y, Peng J, Bordey A, Jin P, Zhao X. MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem Cells.* 2010; 28:1060–1070. [PubMed: 20506192]
- Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, Gavin E, Wan Y, Formentini A, Kornmann M, Fodstad O, Ju J. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. *Oncogene.* 2009; 28:4065–4074. [PubMed: 19734943]
- Song MR, Ghosh A. FGF2-induced chromatin remodeling regulates CNTF-mediated gene expression and astrocyte differentiation. *Nat Neurosci.* 2004; 7:229–235. [PubMed: 14770186]
- Strum JC, Johnson JH, Ward J, Xie H, Feild J, Hester A, Alford A, Waters KM. MicroRNA 132 regulates nutritional stress-induced chemokine production through repression of SirT1. *Mol Endocrinol.* 2009; 23:1876–1884. [PubMed: 19819989]
- Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res.* 2007; 100:1164–1173. [PubMed: 17379831]
- Suarez Y, Fernandez-Hernando C, Yu J, Gerber SA, Harrison KD, Pober JS, Iruela-Arispe ML, Merckenschlager M, Sessa WC. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. *Proc Natl Acad Sci U S A.* 2008; 105:14082–14087. [PubMed: 18779589]
- 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. [PubMed: 11239394]
- Suzuki H, Takatsuka S, Akashi H, Yamamoto E, Nojima M, Maruyama R, Kai M, Yamano HO, Sasaki Y, Tokino T, Shinomura Y, Imai K, Toyota M. Genome-wide profiling of chromatin signatures reveals epigenetic regulation of MicroRNA genes in colorectal cancer. *Cancer research.* 2011; 71:5646–5658. [PubMed: 21734013]
- Szulwach KE, Li X, Smrt RD, Li Y, Luo Y, Lin L, Santistevan NJ, Li W, Zhao X, Jin P. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *The Journal of cell biology.* 2010; 189:127–141. [PubMed: 20368621]
- Szwagierczak A, Bultmann S, Schmidt CS, Spada F, Leonhardt H. Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA. *Nucleic Acids Res.* 2010; 38:e181. [PubMed: 20685817]
- Tachibana M, Matsumura Y, Fukuda M, Kimura H, Shinkai Y. G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. *EMBO J.* 2008; 27:2681–2690. [PubMed: 18818694]
- 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. [PubMed: 19372391]
- 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. *Developmental Cell.* 2001a; 1:749–758. [PubMed: 11740937]
- 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. *Developmental cell.* 2001b; 1:749–758. [PubMed: 11740937]
- Takizawa Y, Takashima S, Itoh M. A histopathological study of premature and mature infants with pontosubicular neuron necrosis: neuronal cell death in perinatal brain damage. *Brain Res.* 2006; 1095:200–206. [PubMed: 16712812]
- Tanaka Y, Naruse I, Hongo T, Xu M, Nakahata T, Maekawa T, Ishii S. Extensive brain hemorrhage and embryonic lethality in a mouse null mutant of CREB-binding protein. *Mech Dev.* 2000; 95:133–145. [PubMed: 10906457]

- Tao J, Hu K, Chang Q, Wu H, Sherman NE, Martinowich K, Klose RJ, Schanen C, Jaenisch R, Wang W, Sun YE. Phosphorylation of MeCP2 at Serine 80 regulates its chromatin association and neurological function. *Proc Natl Acad Sci U S A*. 2009; 106:4882–4887. [PubMed: 19225110]
- Teter B, Rozovsky I, Krohn K, Anderson C, Osterburg H, Finch C. Methylation of the glial fibrillary acidic protein gene shows novel biphasic changes during brain development. *Glia*. 1996; 17:195–205. [PubMed: 8840161]
- Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Human molecular genetics*. 2009; 18:4046–4053. [PubMed: 19656776]
- Trollmann R, Rehrauer H, Schneider C, Krischke G, Huemmler N, Keller S, Rascher W, Gassmann M. Late-gestational systemic hypoxia leads to a similar early gene response in mouse placenta and developing brain. *Am J Physiol Regul Integr Comp Physiol*. 2010; 299:R1489–1499. [PubMed: 20926767]
- Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nature reviews Neuroscience*. 2007; 8:355–367.
- 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 & development*. 2010; 24:432–437. [PubMed: 20194436]
- Tudisco L, Della Ragione F, Tarallo V, Apicella I, D'Esposito M, Matarazzo MR, De Falco S. Epigenetic control of hypoxia inducible factor-1alpha-dependent expression of placental growth factor in hypoxic conditions. *Epigenetics : official journal of the DNA Methylation Society*. 2014; 9:600–610. [PubMed: 24504136]
- van den Beucken T, Koritzinsky M, Niessen H, Dubois L, Savelkoul K, Mujcic H, Jutten B, Kopacek J, Pastorekova S, van der Kogel AJ, Lambin P, Voncken W, Rouschop KM, Wouters BG. Hypoxia-induced expression of carbonic anhydrase 9 is dependent on the unfolded protein response. *The Journal of biological chemistry*. 2009; 284:24204–24212. [PubMed: 19564335]
- Vangeison G, Rempé DA. The Janus-faced effects of hypoxia on astrocyte function. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*. 2009; 15:579–588.
- Virgintino D, Errede M, Robertson D, Girolamo F, Masciandaro A, Bertossi M. VEGF expression is developmentally regulated during human brain angiogenesis. *Histochemistry and cell biology*. 2003; 119:227–232. [PubMed: 12649737]
- Visvanathan J, Lee S, Lee B, Lee JW, Lee SK. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev*. 2007; 21:744–749. [PubMed: 17403776]
- Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S. A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proc Natl Acad Sci U S A*. 2005; 102:16426–16431. [PubMed: 16260724]
- Vogel G. Developmental biology. The unexpected brains behind blood vessel growth. *Science*. 2005; 307:665–667. [PubMed: 15692030]
- Volpe JJ. Perinatal brain injury: from pathogenesis to neuroprotection. *Ment Retard Dev Disabil Res Rev*. 2001; 7:56–64. [PubMed: 11241883]
- Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 2009; 8:110–124. [PubMed: 19081519]
- Walczak-Drzewiecka A, Ratajewski M, Pulaski L, Dastyk J. DNA methylation-dependent suppression of HIF1A in an immature hematopoietic cell line HMC-1. *Biochem Biophys Res Commun*. 2010; 391:1028–1032. [PubMed: 19995553]
- Wang F, Xiong L, Huang X, Zhao T, Wu LY, Liu ZH, Ding X, Liu S, Wu Y, Zhao Y, Wu K, Zhu LL, Fan M. miR-210 suppresses BNIP3 to protect against the apoptosis of neural progenitor cells. *Stem Cell Res*. 2013a; 11:657–667. [PubMed: 23688833]
- Wang H, Flach H, Onizawa M, Wei L, McManus MT, Weiss A. Negative regulation of Hif1a expression and TH17 differentiation by the hypoxia-regulated microRNA miR-210. *Nature immunology*. 2014; 15:393–401. [PubMed: 24608041]

- Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R, Olson EN. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Developmental Cell*. 2008a; 15:261–271. [PubMed: 18694565]
- Wang X, Meng FS, Liu ZY, Fan JM, Hao K, Chen XQ, Du JZ. Gestational hypoxia induces sex-differential methylation of *Crhr1* linked to anxiety-like behavior. *Mol Neurobiol*. 2013b; 48:544–555. [PubMed: 23529784]
- Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, Zhao K. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell*. 2009; 138:1019–1031. [PubMed: 19698979]
- Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet*. 2008b; 40:897–903. [PubMed: 18552846]
- Watson JA, Watson CJ, McCann A, Baugh J. Epigenetics, the epicenter of the hypoxic response. *Epigenetics : official journal of the DNA Methylation Society*. 2010; 5:293–296. [PubMed: 20418669]
- Watson JA, Watson CJ, McCrohan AM, Woodfine K, Tosetto M, McDaid J, Gallagher E, Betts D, Baugh J, O'Sullivan J, Murrell A, Watson RW, McCann A. Generation of an epigenetic signature by chronic hypoxia in prostate cells. *Hum Mol Genet*. 2009; 18:3594–3604. [PubMed: 19584087]
- Wayman GA, Davare M, Ando H, Fortin D, Varlamova O, Cheng HY, Marks D, Obrietan K, Soderling TR, Goodman RH, Impey S. An activity-regulated microRNA controls dendritic plasticity by down-regulating p250GAP. *Proc Natl Acad Sci U S A*. 2008; 105:9093–9098. [PubMed: 18577589]
- Wegner M, Stolt CC. From stem cells to neurons and glia: a Soxist's view of neural development. *Trends in neurosciences*. 2005; 28:583–588. [PubMed: 16139372]
- Wellmann S, Bettkober M, Zelmer A, Seeger K, Faigle M, Eltzhig HK, Buhrer C. Hypoxia upregulates the histone demethylase JMJD1A via HIF-1. *Biochemical and biophysical research communications*. 2008; 372:892–897. [PubMed: 18538129]
- Wenger RH, Kvietikova I, Rolfs A, Camenisch G, Gassmann M. Oxygen-regulated erythropoietin gene expression is dependent on a CpG methylation-free hypoxia-inducible factor-1 DNA-binding site. *European journal of biochemistry / FEBS*. 1998; 253:771–777. [PubMed: 9654078]
- Westenskow PD, Kurihara T, Aguilar E, Schepke EL, Moreno SK, Wittgrove C, Marchetti V, Michael IP, Anand S, Nagy A, Cheresch D, Friedlander M. Ras pathway inhibition prevents neovascularization by repressing endothelial cell sprouting. *J Clin Invest*. 2013
- Wheldon LM, Abakir A, Ferjentsik Z, Dudnakova T, Strohbuecker S, Christie D, Dai N, Guan S, Foster JM, Correa IR Jr, Loose M, Dixon JE, Sottile V, Johnson AD, Ruzov A. Transient accumulation of 5-carboxylcytosine indicates involvement of active demethylation in lineage specification of neural stem cells. *Cell reports*. 2014; 7:1353–1361. [PubMed: 24882006]
- Williams K, Christensen J, Helin K. DNA methylation: TET proteins-guardians of CpG islands? *EMBO Rep*. 2012; 13:28–35. [PubMed: 22157888]
- Wu SC, Zhang Y. Active DNA demethylation: many roads lead to Rome. *Nat Rev Mol Cell Biol*. 2010; 11:607–620. [PubMed: 20683471]
- Xiong F, Zhang L. Role of the hypothalamic-pituitary-adrenal axis in developmental programming of health and disease. *Frontiers in neuroendocrinology*. 2013; 34:27–46. [PubMed: 23200813]
- Xiong L, Wang F, Huang X, Liu ZH, Zhao T, Wu LY, Wu K, Ding X, Liu S, Wu Y, Zhao Y, Zhu LL, Fan M. DNA demethylation regulates the expression of miR-210 in neural progenitor cells subjected to hypoxia. *The FEBS journal*. 2012; 279:4318–4326. [PubMed: 23039253]
- Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, Wang G, Wu Q, Wei C, Bi Y, Jiang L, Cai Z, Sun H, Zhang K, Zhang Y, Chen J, Fu XD. Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. *Cell*. 2013; 152:82–96. [PubMed: 23313552]
- Yamamoto K, Takahashi T, Asahara T, Ohura N, Sokabe T, Kamiya A, Ando J. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *J Appl Physiol* (1985). 2003; 95:2081–2088. [PubMed: 12857765]

- Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem*. 2005; 280:9330–9335. [PubMed: 15613470]
- Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell*. 1998; 93:361–372. [PubMed: 9590171]
- Ye F, Chen Y, Hoang T, Montgomery RL, Zhao XH, Bu H, Hu T, Taketo MM, van Es JH, Clevers H, Hsieh J, Bassel-Duby R, Olson EN, Lu QR. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. *Nat Neurosci*. 2009; 12:829–838. [PubMed: 19503085]
- Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S. Hypoxia enhances the generation of induced pluripotent stem cells. *Cell stem cell*. 2009; 5:237–241. [PubMed: 19716359]
- Yu IT, Park JY, Kim SH, Lee JS, Kim YS, Son H. Valproic acid promotes neuronal differentiation by induction of proneural factors in association with H4 acetylation. *Neuropharmacology*. 2009; 56:473–480. [PubMed: 19007798]
- Zeng L, He X, Wang Y, Tang Y, Zheng C, Cai H, Liu J, Wang Y, Fu Y, Yang GY. MicroRNA-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. *Gene therapy*. 2014; 21:37–43. [PubMed: 24152581]
- Zeng L, Xiao Q, Margariti A, Zhang Z, Zampetaki A, Patel S, Capogrossi MC, Hu Y, Xu Q. HDAC3 is crucial in shear- and VEGF-induced stem cell differentiation toward endothelial cells. *J Cell Biol*. 2006; 174:1059–1069. [PubMed: 16982804]
- Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, Xin H, Sun S. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. *Journal of cellular and molecular medicine*. 2012; 16:249–259. [PubMed: 21388517]
- Zhang Y, Teng F, Luo GZ, Wang M, Tong M, Zhao X, Wang L, Wang XJ, Zhou Q. MicroRNA-323-3p regulates the activity of polycomb repressive complex 2 (PRC2) via targeting the mRNA of embryonic ectoderm development (Eed) gene in mouse embryonic stem cells. *The Journal of biological chemistry*. 2013; 288:23659–23665. [PubMed: 23821546]
- Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, Bruggen N, Chopp M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *The Journal of clinical investigation*. 2000; 106:829–838. [PubMed: 11018070]
- Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, Wang X, Lo EH. Role of matrix metalloproteinases in delayed cortical responses after stroke. *Nature medicine*. 2006; 12:441–445.
- Zhao Q, Rank G, Tan YT, Li H, Moritz RL, Simpson RJ, Cerruti L, Curtis DJ, Patel DJ, Allis CD, Cunningham JM, Jane SM. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. *Nat Struct Mol Biol*. 2009; 16:304–311. [PubMed: 19234465]
- 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. [PubMed: 12748381]
- Zhong Q, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. *J Cell Biochem*. 2010; 110:1306–1313. [PubMed: 20564224]
- Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JA, Weitz CJ, Greenberg ME. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. *Neuron*. 2006; 52:255–269. [PubMed: 17046689]
- Zhu N, Zhang D, Chen S, Liu X, Lin L, Huang X, Guo Z, Liu J, Wang Y, Yuan W, Qin Y. Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. *Atherosclerosis*. 2011; 215:286–293. [PubMed: 21310411]
- Ziemin-van der Poel S, McCabe NR, Gill HJ, Espinosa R 3rd, Patel Y, Harden A, Rubinelli P, Smith SD, LeBeau MM, Rowley JD, et al. Identification of a gene, MLL, that spans the breakpoint in

11q23 translocations associated with human leukemias. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88:10735–10739. [PubMed: 1720549]

Zucchi FC, Yao Y, Ward ID, Illynskyy Y, Olson DM, Benzie K, Kovalchuk I, Kovalchuk O, Metz GA. Maternal stress induces epigenetic signatures of psychiatric and neurological diseases in the offspring. PloS one. 2013; 8:e56967. [PubMed: 23451123]

Abbreviations

AID/APOBEC	activation-induced cytidine deaminase/apolipoprotein B mRNA-editing enzyme complex
BDNF	brain-derived neurotrophic factor
5caC	5-carboxylcytosine
CBP	CREB binding protein
CREB	cAMP response binding protein
DNMT	DNA methyltransferases
ECs	endothelial cells
FMRP	fragile X mental retardation protein
GFAP	Glial fibrillary acidic protein
GR	glucocorticoid receptor
HATs	histone acetyltransferases
HDAC	histone deacetylase
HIE	hypoxic-ischemic encephalopathy
HIFs	hypoxia-inducible factors
5hmC	5-hydroxymethylcytosine
H3K4me3	Histone H3 lysine 4 trimethylation
HMTs	histone methyltransferases
HRE	hypoxia response elements
JMJD	jumonji-domain
MBD	methyl-CpG-binding proteins
5mC	5-methylcytosine
MLL	mixed lineage leukemia
Ngn2	Neurogenin2
NMDA	N-methyl-D-aspartate receptors
NPCs	neural progenitor cells
NSC	neural stem/progenitor cell
PHDs	prolyl hydroxylase domains

PRC2	polycomb repressor complex2
pre-miRNA	precursor miRNA
pri-miRNA	Primary miRNA
PVL	periventricular leucomalacia
REST	Repressor element 1-silencing transcription factor
SIRT1	sirtuin 1
SAHA	suberoylanilide hydroxamic acid
SVZ	subventricular zone
TDG	thymine DNA glycosylase
TET	ten-eleven translocation proteins
TSS	ranscriptional start site
UHRF1	ubiquitin-like, containing PHD and RING finger domains 1
3'UTR	3' untranslated region
VEGF	vascular endothelia growth factor
VHL	von-Hippel-Lindau-tumour-suppressor

Highlights

Epigenetic mechanisms regulate neuronal and vascular development

Fetal hypoxia impacts epigenetic machineries

Epigenetic programming may play an important role in fetal stress-induced hypoxic/
ischemic-sensitive phenotype in the developing brain

Figure 1. DNA methylation controls switching of neural progenitor cells (NPCs) differentiation

At neurogenesis stage (E8.5-11.5), neuronal genes are expressed and neurogenesis process is dominative; whereas the astrocytic-related genes, such as *Gfap* are highly methylated by DNMTs binding with MeCP2 and MBDs proteins. The methylation is maintained by DNMT1, and thereby prevents astrocytic-related gene transcription and astroglialogenesis (upper box). At the astroglialogenesis stage (E14.5-postnatal), the expressions of neuronal genes are silenced by histone methylation, such as K27me3, and neurogenesis process is inhibited. The release of DNMT1 from *Gfap* promoter leads to the demethylation and gene transcription. The astroglialogenesis is initiated (lower box).

Figure 2. DNA methylation and histone modification cooperatively regulate gene expression
Transcription active DNA wraps around histone proteins to form active chromatin architecture, which is associated with unmethylated CpGs (blue filled ovals) and specific modifications of histone tails, including acetylated histone 3 lysine 27 (H3K27) and trimethylated H3K4. A group of enzymes, including histone acetyltransferases (HATs), histone demethylases (HDMs) and DNA demethylases (DMEs) promote the formation of this resultant DNA. On the contrary, another group of proteins, including methyl-CpG binding domains (MBDs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs) promote and maintain the inactive chromatin conformation, which is characterized by methylated CpGs on the DNA (red filled ovals) and specific histone modifications, such as trimethylation of H3K9 and H3K27, subsequently inhibiting the genes transcription.

Figure 3. Histone modification regulates motor neuron differentiation

CBP and p300 are co-activators with HAT activity. Neurogenin-2 (Ngn2) and retinoic acid receptor (RAR) form a complex binding to the promoter of neuron-related genes in motor neuron progenitor cells. Without RAR ligand binding, HDAC deacetylates neuron-related genes and leads to an inactive chromatin. When the extrinsic RAR activator, retinoic acid (RA) is introduced, CBP/p300 bind with the Ngn2/RAR complex and recruit HAT to the Ngn2 target in motor neuron, leading to transcriptionally active open chromatin structure by acetylation of H3/4 and motor neuron gene expressions, and differentiation to motor neurons.

Figure 4. HDACs in vascular development

HDACs regulate expression of homeobox transcription factor HoxA9, which regulates the expression of endothelial-committed genes in progenitor cells, including endothelial nitric oxide synthase (eNOS), VEGFR2, etc., promotes endothelial cell differentiation and neovascularization. HDAC3 expression is induced by VEGF activated Flk-1-PI3K-Akt pathway. During embryogenesis HDAC3 deacetylates p53, which activates p21 and promotes endothelial progenitor cell differentiation. SIRT1, a member of class III HDACs, negatively regulates forkhead transcription factor Foxo1 transcriptional activity and rescues the expression of Foxo1 target genes, such as VEGFR2. HDAC7 inhibits myocyte enhancer factor-2 (MEF2) activity, which regulates MMP10 expression in endothelial cell, and is related to the vascular remodeling.

Figure 5. MiRNA-124 in neuronal development

MiR-124 regulates neuronal development by suppressing several pathways that are associated with neuronal differentiation. For example: It targets the mRNAs polypyrimidine tract-binding protein 1 (PTB; neural variant, nPTB), and promotes neural-specific splicing. It regulates neural gene expression by repression of REST/SCP1 complex. In contrast, REST/SCP-1 complex also block miR-124 expression during neuronal development. MiR-124 also promotes adult neurogenesis in mice by repression of SRY-box transcription factor SOX9. Moreover, in Aplysia patient, miR-124 is upregulated in brain and promotes synaptic plasticity by repressing transcriptional activator CREB.

Figure 6. MiRNAs in the vascular development

MiRNAs promote VEGF-dependent vasculogenesis and angiogenesis by interfering with multiple VEGF downstream signal pathways. MiR-126 activates VEGF-dependent Akt and Erk signaling by targeting p85 β subunit of PI3 kinase (*Pik3r2*) and SPRED1 (*Spred1*) respectively, thereby promoting angiogenesis. MiR-132 increases Ras activity by directly suppressing its endogenous RAS inhibitor, p120RasGAP (*Rasa1*) expression, and promotes VEGF-dependent vasculogenesis and angiogenesis.

Figure 7. Potential effects of initial insults, such as fetal hypoxia on the hypoxia-ischemic encephalopathy (HIE) risk in offspring

Fetal hypoxia modulates DNA methylation/demethylation, histone modification and miRNAs regulating circuit. As a result, genomic expression program in control of neuronal and neurovascular development patterns in the developing brain is altered, consequently leading to the central nervous system (CNS) developmental malformations and increased vulnerability of fetal brain to (HIE). DNA methylation/demethylation and histone modification transcriptionally regulates miRNAs biogenesis, and is conversely regulated by miRNAs modulating the expression of DNA methylation-related enzymes and proteins, and chromatin remodeling factors.