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Gestational hypoxia and epigenetic programming of brain development disorders

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Abstract

Adverse environmental conditions faced by an individual early during its life, such as gestational hypoxia, can have a profound influence on the risk of diseases, such as neurological disorders, in later life. Clinical and preclinical studies suggest that epigenetic programming of gene expression patterns in response to maternal stress have a crucial role in the fetal origins of neurological diseases. Herein, we summarize recent studies regarding the role of epigenetic mechanisms in the developmental programming of neurological diseases in offspring, primarily focusing on DNA methylation/demethylation and miRNAs. Such information could increase our understanding of the fetal origins of adult diseases and help develop effective prevention and intervention against neurological diseases.

Keywords

brain; hypoxia; epigenetic programming; DNA methylation/demethylation; micro RNAs; neurological disease

Introduction

During the late 1980s, Barker and colleagues uncovered the correlation between the nutrition condition of early life (during the prenatal and postnatal periods) and the increased risk of adult ischemic heart disease [1], known as ‘Barker hypothesis’. Thereafter, numerous retrospective and prospective clinical and preclinical studies have supported this ‘fetal origins of adult disease’ theory [2–4], showing a substantial correlation between an adverse maternal environment and the development of various diseases in later life, including cardiovascular disease, diabetes and neurological disease [5–9]. One general mechanism by which maternal stress can be linked to phenotypic changes later in life is the epigenetic

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Teaser: Epigenetic mechanisms are important in the brain development, and have a key role in the programming of increased risk of neurological diseases resulting from early-life stress.

programming of genes, which has a central role in determining the functional output of the information that is stored in the genome.

Epigenetics is defined as heritable changes in gene expression that are not associated with concomitant alterations in the DNA sequence. The genetic information for the transcriptional program of gene expression is controlled by epigenetic mechanisms, including methylation/demethylation of DNA, post-translational modifications of histone, and noncoding RNAs (ncRNAs) such as miRNAs [10]. Epigenetic events are highly responding process[LM1] to alterations in endogenous and environmental signals; therefore, epigenetic instructions have crucial roles in the regulation of the expression of appropriate sets of genes, especially in particular tissues at specific time windows [10–12]. Accumulating evidence from both human and animal studies indicates that epigenetic modifications serve as a memory of early life events and can induce long-term changes in gene expression profiles, which potentially result in disease in later life [8,13,14] (Figure 1). For example, in a recent study, epigenetic programming of the glucocorticoid receptor (*GR*) gene after maternal hypoxia was found to be related to the enhanced susceptibility of neonate hypoxic-ischemic encephalopathy (HIE) brain injury [15].

Hypoxia is a common form of intrauterine stress, and the fetus might experience a period of hypoxic stress under a variety of conditions, including pregnancy at high altitude, pregnancy with anemia, placental insufficiency, cord compression, pre-eclampsia, heart, lung and kidney disease, or with hemoglobinopathy. Intrauterine hypoxia contributes significantly to developmental malformations in the fetal tissues and/or organs, particularly the brain. The brain represents only 2% of the body weight, but consumes 20% of the oxygen requirements of the body. The immature fetal brain is particularly sensitive to changes in oxygen level. Low levels of oxygen result in neurovascular development malformations, such as cerebral palsy and periventricular leukomalacia, in the developing brain and increases risk of brain injury in the newborn [16,17]. Maternal hypoxia also delays neuronal migration and alters neurotransmitters expression during embryogenesis [18], subsequently compromising neuronal circuits and affecting neural organization in the brain tissue [19]. As a result, maternal hypoxia leads to increased susceptibility to seizures, epilepsy [20], and cerebral insults in affected offspring [15,19]. However, the molecular mechanisms of gestational hypoxia in the programming of brain disorders in postnatal life remain largely elusive. Thus, here we summarize recent findings of the role of epigenetic mechanisms, specifically DNA methylation/demethylation and miRNAs, in the neural development and pathogenesis of neurological disorders, with a view to addressing whether fetal hypoxia might contribute to these neurological disorders in affected offspring.

Epigenetic mechanisms and the machineries

DNA methylation and the machineries

DNA methylation refers to the biological process whereby a methyl group is added to a DNA nucleotide, and occurs almost exclusively on cytosines occurring directly before guanine molecules (i.e., CpG dinucleotides). The major function of DNA methylation is to silence transcriptionally the expression of related genes, and the repression effect is generally correlated with the methylation level within the gene promoter and the extent of

interference of transcription factor binding [21,22]. DNA methylation can inhibit gene expression by several mechanisms. For example, it can interfere directly with the binding of transcription factors to the gene-regulating sequences. In other cases, DNA methylation does not directly inhibit transcription factor binding to the gene promoter. Instead, a family of proteins called methyl-CpG binding proteins (MBDs) bind to methylated CpG residues independent of the DNA sequence and mediate other epigenetic events, such as histone modifications that lead to further alteration of gene expression. MBDs can intimately link DNA methylation to patterns of histone post-translational modifications. Initially, methylated DNA promotes the recruitment of MBDs at specific sites in the genome, which in turn recruit histone-modifying enzymes and chromatin-remodeling complexes to the methylated DNA sites [23,24]. This helps to orchestrate DNA replication and repair, thus reinforcing and stabilizing transcriptional suppression.

DNA methylation is catalyzed by DNA methyltransferases (DNMTs); there are five DNMTs in mammals although only three (DNMT1, DNMT3a and DNMT3b) have methyltransferase activity and are generally grouped into *de novo* DNMTs (DNMT3a and DNMT3b) and maintenance DNMTs (DNMT1) [25]. The *de novo* DNMTs are highly expressed in embryonic stem (ES) cells and are downregulated in differentiated cells. DNMT3a and DNMT3b are believed to be responsible for establishing the pattern of methylation during development [23]. DNMT1, the most abundant DNMT in the cell, is transcribed chiefly during the S phase of the cell cycle, and is often needed to methylate hemimethylated sites that are generated during semiconservative DNA replication [26]. CpG islands are defined as short interspersed DNA sequences that deviate significantly from the average genomic pattern by a G+C content of at least 50% [27]. CpG islands are highly enriched at or near the gene promoter region. In mammalian genomes, approximately 60% of gene promoters are associated with CpG islands. In normal cells, most CpG islands are unmethylated, although some (approximately 6%) become methylated in a tissue-specific manner during early development or in differentiated tissues [28]. By contrast, 80% of CpGs located outside of CpG islands are methylated [29].

Although usually associated with transcriptional suppression, recent studies suggest that DNA methylation might also be involved in the transcriptional activation of some genes. However, these mechanisms remain to be determined. In mouse hypothalamus tissue, methyl CpG binding protein 2 (MeCP2) dysfunction was shown to induce changes in the expression levels of thousands of genes, and most genes (approximately 85%) appeared to be activated by MeCP2 [30]. MeCP2 was associated with the transcriptional activator CAMP responsive element binding protein 1 (CREB1) of an activated target but not a repressed target at the promoter [31].

DNA demethylation and the machineries

DNA demethylation is the reverse process of DNA methylation, leading to the loss of the methyl group from methylated cytosine (5meC) nucleotides. DNA demethylation, occurring through both passive and active mechanisms (Figure 2), has been observed in specific contexts, including both early development and somatic cells. Passive DNA demethylation refers to the loss of methyl group when the maintenance DNMT1 is absent or inhibited

during DNA replication. By contrast, active DNA demethylation is the enzymatic process that results in the removal of the methyl group from 5meC by breaking a carbon–carbon bond. Different tissues can use different demethylation pathways depending on whether demethylation is genome wide or local and on its targets in the genome. Current evidence indicates that genome-wide demethylation occurs at specific times during early development, whereas gene-specific demethylation serves as a response to specific signals in somatic cells. During development, previous paternal-specific methylation marks are removed in the zygote by extensive genome-wide DNA demethylation shortly after fertilization. Although passive demethylation might also be involved in this process, it is likely that active demethylation has a major role in mediating this process, because loss of methylation is detected before the completion of the first cell division [32–34]. Both methylation and demethylation are resisted by imprinted genes, which are epigenetically modified at a different crucial period of gametogenesis [35]. On specific genomic loci in somatic cells, active DNA demethylation also has been observed in response to certain signals. For example, in unstimulated neurons, the brain-derived neurotrophic factor (BDNF) is maintained in a repressed state through methylation of promoter and binding of MeCP2. When neuron was depolarized with KCl, demethylation of the promoter occurred, and MeCP2 was released, thereby leading to upregulation of BDNF [36]. These findings indicate that, in addition to the long-term regulation of gene expression, methylation and demethylation also function in the dynamic regulation of gene expression as a rapid response to specific stimuli.

Based on various studies, several mechanisms have been proposed to address molecular mechanisms of active DNA demethylation, including enzymatic removal of the methyl group of 5meC, base excision repair (BER) through direct excise of 5meC, nucleotide excision repair (NER), deamination of 5meC to T followed by BER, and oxidative demethylation [37]. In plants, BER is used to achieve demethylation of DNA, although evidence supporting a similar mechanism in mammals is less compelling. Some proteins, including methyl-CpG binding domain protein 4 (MBD4) and a human T DNA glycosylase (TDG), have been shown to have glycosylase activity against 5meC *in vivo* in mammals. However, their excision activity against 5meC is comparatively low and their function in active demethylation remains to be elucidated. Decisive genetic and biochemical evidence supporting their roles in active DNA methylation in mammals remains to be determined. *In vitro*, activity-induced deaminase (AID) has been shown to be capable of delaminating 5meC to T and, thus, is implicated in the DNA demethylation process. In addition, a recent large-scale bisulfite sequencing study indicated a slightly increased level of methylation in primordial germ cells (PGCs) derived from AID-knockout mouse embryos. This suggests a contribution of AID in PGC demethylation. However, given the comparatively high levels of methylation in either ESCs or somatic cells (70–80%) compared with AID-null PGCs (approximately 20%), other mechanisms might be responsible for DNA demethylation.

Oxidative demethylation is another possible mechanism by which DNA demethylation can be achieved, and has recently received extensive attention. The discovery that members of the Ten-eleven translocation (Tet) family of proteins can convert 5-mC to 5-hydroxymethylcytosine (5hmC) provides further evidence to support this hypothesis. The

Tet proteins family comprises three members, namely Tet1, Tet2 and Tet3, and shows broad expression patterns in different tissues [38]. In the mouse, all three Tets have the catalytic capacity to convert 5mC to 5hmC both *in vitro* and *in vivo*, and the presence of 5hmC depends on the presence of pre-existing of 5mC *in vivo*; this further suggests that 5mC is the only source of genomic 5hmC. Tet1 is expressed in ESCs and is required for the maintenance of embryonic status. The Tet1 mRNA level declines during ESC differentiation, accompanied by a decrease of the 5hmC levels [39]. Although Tet2 expression is observed in ESCs, the silencing of Tet2 fails to show obvious phenotypic changes in ESC biology. Tet3 expression is highest in oocytes and zygotes, where the 5hmC level increase dramatically in the male pronucleus. Moreover, zygotes with Tet3 depletion fail to demethylate the male pronucleus. Knockout of Tet1, but not Tet2 or Tet3, results in impaired ESC self-renewal and maintenance [40], and leads to embryonic lethality. The existence of three mammalian Tet enzymes raises the possibility that each has a distinct panel of genomic targets, such that their tissue specific expression might generate unique physiological effects.

DNA methylation and hypoxia

Under hypoxic conditions, hypoxia-induced transcription factors regulate a series of transcriptional responses, including cellular adaptation, angiogenesis, and cell survival. Accumulating evidence shows that these transcription factors, mainly hypoxia-inducible factor 1-alpha (HIF-1α), are controlled by, and cooperate with, epigenetic machineries, such as DNA methylation, and have an essential role in determining the cellular and molecular response to hypoxia [41]. Specifically, epigenetic changes at the DNA level influenced the HIF-1α expression level, its binding to target gene promoters and also HIF-1α protein stabilization [42].

The HIF-1α promoter contains CpG-rich regions, which might regulate HIF-1α expression by alteration of methylation status. In a hematopoietic cell line, Walczak-Drzewiecka and colleagues reported that the methylation of the HIF-1α promoter decreased its transcriptional activity, which was increased when cells were treated with a DNA demethylating agent, 5'-azacytidine [43]. Moreover, MeCP2 protein was also preferentially associated with the HIF-1α promoter in vivo [43]. Another study using tumor cells revealed a positive autoregulation mechanism of HIF-1α expression, in which demethylation of the HIF-1α promoter region enabled binding of HIF-1α to its own promoter, thereby increasing HIF-1α expression [44].

It is widely accepted that, in the presence of oxygen, HIF hydroxylases, prolyl hydroxylase domains (PHDs) destruct HIF-1α subunits by promoting von Hippel–Lindau tumor suppressor (VHL)-dependent ubiquitination and proteolysis. However, under hypoxic conditions, the HIF-1α degradation process is suppressed because of inactivation of the HIF hydroxylases complex [45]. Mounting evidence shows that an epigenetic mechanism is involved in controlling HIF-1α stability by affecting the activities of the degradation components. For example, in renal carcinomas, VHL gene expression was silenced when hypermethylation occurred in a normally unmethylated CpG island in the 5' region, resulting in the constitutive activation of HIF-1α [46]. Abnormal methylation in the CpG island of

VHL has been observed in various disorders, including bone disease [47], retinoblastoma [48], and non-small cell lung cancer [49]. One member of the HIF hydroxylase group, prolyl hydroxylase 3 (PHD3), was also regulated by DNA methylation [50,51]. It has been reported that some cancer cell lines displayed a failure of PHD3 mRNA induction when placed in a hypoxic environment [51]. Further studies investigated the major mechanism in multiple tumors, showing that the suppression of PHD3 expression was the result of a change in methylation level of the CpG island [41,50]. Based on these findings, hypermethylation of PHD3 and VHL promoter could be an important epigenetic mechanism in HIF stabilization during hypoxia.

HIF-1α acts by binding to HIF-responsive elements (HREs) in the promoter of target genes; therefore, methylation of CpG islands in HREs might also interfere with the actions of HIF-1α. For example, the expression of erythropoietin [52] and the class III beta-tubulin [53] genes induced by hypoxia was dependent upon the tissue-specific methylation status of an HRE in the 3' flanking region. Indeed, chronic hypoxia can result in increases in global levels of DNA methylation, thus affecting the methylation level of HRE regions [54,55].

In addition to HIF-1α, transcriptional actions of other transcription factors under hypoxic conditions are also regulated by DNA methylation. For example, methylation of specific transcription factor binding sites (Egr-1 and Sp1[LM2]) at the PKCε promoter was increased in heart tissue of animals exposed to fetal hypoxia, leading to decreased expression of PKCε and loss of protection to the heart during ischemia-reperfusion injury [56–58]. In a cohort study, children exposed to maternal smoking had dramatically lower methylation of the DNA repetitive elements of the long interspersed element 1 (*LINE-1*) and *AluYb8*, which are normally hypermethylated under normal conditions, when compared with controls [59]. These studies provided evidence that prenatal exposure of the pregnant mother to tobacco smoke might be responsible for the increased risk of disease in their offspring later in life.

miRNAs

In humans, only 1–2% of the genome encodes protein, whereas ncRNAs that do not produce protein represent most human transcripts. Moreover, ncRNAs have important post-transcriptional regulatory roles in gene expression. miRNAs are one type of ncRNA, comprising 18–25 nucleotides and usually leading to suppression of their target genes. In mammals, approximately 50% of all genes are currently regulated by miRNAs. To date, mirBase, the primary miRNA information database, has over 2000 human, 1300 mouse, and 700 rat annotated mature miRNAs. A large number of mammalian-specific miRNAs have been described in the regulation of various biological processes, including development, differentiation, apoptosis, and cell proliferation [60–64].

miRNAs arise from either intergenic or intragenic regions of a host protein coding or noncoding gene [65]. In the nucleus, miRNAs are chiefly transcribed from those genomic regions by RNA polymerase II as primary miRNAs (pri-miRNAs). These are long segments and typically contain 1–6 precursors of mature miRNAs. The hairpin structure portions in the pri-miRNAs are trimmed by a microprocessor complex containing RNase type III, named Drosha, and the double-stranded (ds)RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCR8) to pre-miRNAs. This latter contains a stem-loop structure

and is approximately 70–100 nucleotides long [66]. With a nuclear transport receptor complex, exportin-5-RanGTP, pre-miRNAs are transported to the cytoplasm and further processed by a dsRNase type III, Dicer to an approximately 22-nt ds miRNA duplex, which is incorporated into a RNA-induced silencer complex (RISC)-loading complex (RLC) in an ATP-dependent manner [67]. The sense strand is then deleted from the RLC by a helicase, whereas the antisense strand remains in the complex to form a mature RNA-induced silencer complex (mature RISC) and serves as a template for capturing target mRNAs. Both the transcription of pri-miRNA and the post-transcriptional process leading to mature miRNA are finely regulated. Alterations of the processing molecules can result in a global change of mature miRNA levels.

The RISC is a complex containing multiple factors that work together to regulate target mRNA expression. Based on the extent of complementary between miRNAs and target mRNAs, RISC has different roles in regulating gene expression [68]. For highly complementary target mRNAs, the mature RISC complex cleaves and leads to degradation of the target mRNAs. For partially complementary targets, the RISC complex can decap or deadenylate target mRNAs, thereby decreasing the stability of target mRNAs. In fact, most animal miRNAs are only partially complementary to their targets, and several recent reports have indicated that miRNAs can also induce significant degradation of target mRNAs despite imperfect mRNA–miRNA base pairing [69,70]. Additionally, some components of the RISC complex can also inhibit translation of the target genes.

MiR-210 and hypoxia

HIFs are one of the most sensitive physiological sensors of hypoxia and control the cellular response to the low oxygen condition. A large number of miRNAs have been implicated in regulating HIF pathways. For instance, miR-199a, miR-17–92 clusters, and miR-20b regulate HIF-1α signals during hypoxia [71–73], whereas miR-23, miR-24, miR-26, miR-107, miR-210, and miR-373 have been shown to be downstream molecules of HIFs [74,75]. A recent study further confirmed the effect of hypoxia on miRNA expression, showing that the epidermal growth factor receptor (EGFR) is the upstream regulator of miRNA biogenesis through phosphorylation of argonaute 2 (AGO2) in response to hypoxia [76].

Although multiple miRNAs are regulated by hypoxia, miR-210 is regarded as the master of hypoxia-induced miRNA because it is progressively and consistently increased under hypoxia in various cell types [77]. The stemloop of miR-210 is located in an intron of a ncRNA, which is transcribed from AK123483 on chromosome 11p15.5. The predicted size of human pri-miR-210 is 2927 base pairs, which is almost the same as genomic AK123483 [78]. Additionally, expression of AK123483 is increased under hypoxia compared with normoxia conditions [79]. The highly preserved HRE is responsible for the expression of miR-210. The functional HRE element is located in the miR-210 promoter approximately 40 base pairs upstream of the predicted transcription starting site, and can directly bind HIF-1α under hypoxia [75].

In addition to HIFs, nuclear factor (NF)-κB can also induce miR-210 expression in response to hypoxia [80]. Genomic mapping of miR-210 showed a conserved κB binding site located

on the 200-base pair core promoter region and NFκB p50 subunits can directly bind to and *trans*-activate the miR-210 promoter under hypoxia [80]. In hypoxic cardiomyocytes, Akt regulates miR-210 expression through an HIF-independent pathway [81]. A recent study also showed that DNA demethylation in neural progenitor cells (NPCs) regulates the expression of miR-210 in response to hypoxia through a HIF-1-independent pathway [82] (Figure 3).

One of the cellular responses to hypoxia is the upregulation of angiogenesis-related genes in endothelial cells to enhance new blood vessel formation and ensure survival. MiR-210 upregulation is a crucial element of the endothelial cell response to hypoxia, affecting cell survival, migration, and differentiation. Fasanaro and colleagues first found that miR-210 blockage inhibited the formation of capillary-like structures stimulated by hypoxia and decreased cell migration in response to vascular endothelial growth factor (VEGF). Further study showed that ephrin-A3 (EFNA3) was the direct target of miR-210 during hypoxia, and overexpression of EFNA3 significantly blocked the pro-angiogenic effect of miR-210 or hypoxia preconditioning [83]. Another study showed that miR-210 directly targeted protein-tyrosine PTP1B [84,85], which negatively regulated VEGF signaling in endothelial cells. Inconsistent with these results, bioinformatics analysis showed that miR-210 is correlated with hypoxia/VEGF and angiogenesis signaling in patients with breast cancer [86], indicating the involvement of miR-210 in angiogenesis.

Additionally, miR-210 has an important role in stem cell survival and differentiation under hypoxia conditions. It has been reported that ischemia preconditioning enhances bone marrow-derived mesenchymal stem cells (MSCs) survival via induced expression of miR-210, whereas miR-210 knockdown increases caspase-8-associated protein-2 (CASP8AP2) levels, a regulator in Fas-mediated apoptosis, resulting in enhanced cell apoptosis after ischemia [87]. Consistent with this finding, a recent study reported that upregulation of miR-210 during hypoxia decreased in NPC apoptosis as a result of inhibition of Bcl-2 adenovirus E1B 19kDa-interacting protein 3 (BNIP3) [88]. However, in contrast to the findings that miR-210 promotes cell survival, Chio and colleagues reported that miR-210 directly targeted the 3' untranslated region (UTR) of the antiapoptotic gene Bcl-2, thereby enhancing the hypoxia-induced apoptosis of neuroblastoma cells treated with oxygen/glucose deprivation [89]. Thus, it is possible that miR-210 has different roles in different cell types, and further studies are needed to elucidate its role in cell survival.

MiR-210 is involved in DNA damage repair by repression of RAD52, a key protein for DNA ds break repair and homologous recombination cooperating with other DNA repair components [90]. Using tumor cells, Crosby and colleagues reported that RAD52 is downregulated under hypoxia and this hypoxic-induced downregulation can be partially reversed by antisense inhibition of miR-210 [74]. Further luciferase reporter gene assays showed that miR-210 was capable of binding to the 3′ UTR of RAD52 [74]. This study uncovers a new mechanism by which miR- 210 regulates the DNA damage repair and genetic instability that occurs under hypoxia.

MiR-210 represses mitochondrial respiration, which represents an evolutionarily ancient cellular adaptation to hypoxia and profoundly influences cell survival and function. Under

hypoxia conditions, mitochondrial metabolism is suppressed and energy production is shifted from the tricarboxylic acid (TCA) cycle to glycolytic pathways. Recently, miR-210 has been suggested as a new player in regulating mitochondrial metabolism under hypoxia, through repressing the expression of iron-sulfur (FeS) cluster scaffold protein isoforms ISCU1 and ISCU2 [91] that facilitate the assembly of [4Fe-4S] and [2Fe-2S] iron-sulfur clusters. These are prosthetic groups that promote electron transport and oxidation–reduction reactions affecting various cellular processes, including ribosome biogenesis, DNA repair, and iron metabolism [92]. Moreover, miR-210 also disrupts the electron transport activity of mitochondria complex I under hypoxia by repressing ISCU1/2. Apart from ISCU1/2, miR-210 also targets cytochrome c oxidase assembly protein (COX10) [93] and SDHD, subunit D of succinate dehydrogenase complex (SDH) [94], which were key factors in electron transport chain.

Given the close correlation between miR-210 expression and hypoxia, circulating miR-210 could be a potential prognostic marker in patients. A recent study investigated miRNA levels derived from the placenta circulation in maternal blood in pregnancy complicated by fetal hypoxia, and found that several miRNAs, including miR-210, miR-424, miR-21, miR-373, miR-199a and miR-20b, were significantly increased throughout labor because of acute fetal hypoxia accompanied by each uterine contraction [95]. Levels of miR-210 increased 4.2-fold at the time of delivery compared with before the induction of labor in healthy term pregnancies. Moreover, a 3.6-fold upregulation of miR-210 was observed in pregnancy complicated by severe preterm fetal growth restriction, compared with gestation-matched controls. This study suggests that miR-210, with other miRNAs, such as miR-424, miR-199a, and miR-20b, could serve as non-invasive biomarkers for fetal hypoxia *in utero* [95]. Similarly, miR-210 can be detected in blood from patients with cancer and, thus, could act as a potential biomarker. For example, increased levels of miR-210 were detected in the blood of patients with pancreatic cancer [96] and diffuse large B-cell lymphoma [97].

Cross-talk between miRNA and DNA methylation/demethylation

Given that half of miRNA genes are associated with CpG islands, DNA methylation/demethylation is believed to have an important role in the regulation of miRNAs. Indeed, hypermethylation of CpG islands is a common feature of aberrant miRNA expression profiles. Administration of 5-aza-2′-deoxycytidine (5-Aza), a DNMT inhibitor, significantly induced the expression of miR-127, which is embedded in a CpG island promoter [98]. By contrast, miR-9-1, which is found to be hypermethylated and downregulated in breast cancer, can be induced by reduction of methylation and concomitant reactivation of expression by 5-Aza treatment [99]. The miR-34 family of miRNAs can be inactivated by the methylation of a CpG island at the transcription start site (TSS) of their host genes [100]. Similarly, methylation of a CpG island near the predicted TSS of the miR-200c/miR-141 gene is significantly correlated with its expression level [101]. Given that miRNA levels are tightly regulated by not only the transcription of pri-mRNA, but also the molecules involved in the post-transcriptional process, it is conceivable that epigenetic regulation of those processing proteins contributes to the alteration of miRNA profile.

miRNAs are involved in the establishment and/or maintenance of DNA methylation [102]. Intriguingly, a specific group of miRNAs, which is defined as epi-miRNAs, can regulate the expression of DNA methylation-related genes, such as those encoding DNMTs. miR-29 family members have been reported to downregulate directly DNMT3a and 3b by reactivating methylation-silenced tumor suppressor genes. This action can restore normal patterns of DNA methylation in non-small cell lung cancer cells [103]. Another study showed that short-hairpin RNA-mediated miR-148 repression led to an increase in DNMT3b1 expression, whereas overexpression of miR-148 resulted in decreased DNMT3b1 expression. Further experiments revealed that the miR-148 directly targeted DNMT3b1 coding sequences, because mutation of the putative miR-148 binding site in DNMT3b1 abolished the suppression by miR-148 [104].

Taken together, post-transcriptional regulation by miRNAs and transcriptional mechanisms by DNA methylation/demethylation cooperate with each other to organize the whole gene expression profile and to maintain cellular physiological functions. Once this epigenetic machinery is disrupted, normal physiological functions can be interfered with, thereby contributing to the development of various disease processes.

Environmental epigenetic programming and disease susceptibility

Traditionally, studies of disease susceptibility have often focused on the environmental influences on genetic factors, such as individual genotype variation, which is mostly a result of environmental exposure and germline mutations in the gene coding and promoter regions. However, environmental exposure encountered in early life also influences the health and risk of disease in adulthood. Thus, a general mechanism by which early environmental exposure could be linked to adult phenotypic changes is the alteration of epigenetic signatures, which are crucial in orchestrating the output of genomic information. Understanding of disease susceptibility will require consideration of both genetic and epigenetic information that indicates environmental interactions integrating with the genome. Indeed, it has been reported that prenatal and early postnatal environment factors, such as maternal hypoxia [15,105], maternal nutritional supplements [106], and xenobiotic chemicals [107], are responsible for altered reprogramming of epigenetic marks and subsequent changes of disease susceptibility in the developing fetus.

Some genomic targets, including gene promoter regions, transposable elements adjacent to genes with metastable epialleles, and regulatory elements of imprinted genes, are likely to be susceptible to gene-expression changes caused by environmental perturbations. These targets usually contain regions that are CpG-rich sequences with differentially methylation status, and some regions are also subject to histone modifications for the regulation of gene expression. Transposable elements are repetitive elements that are distributed throughout mammalian genomes, and comprise approximately 45% of the human genome. Although most of these are silenced by CpG methylation, the epigenetic state of a subset of transposable elements is metastable and could affect the expression of adjacent genes. Metastable epialleles are alleles that can be epigenetically modified in a variable and reversible manner and, therefore, are expressed variably in genetically identical individuals [108]. Importantly, epigenetic marks at metastable epialleles have been proved to be readily

altered by gestational exposure to nutritional agents and other environmental factors [106,109]; thus, they could be associated with health and disease susceptibility in later life. Being more susceptible to environmental influence, epigenetic deregulation of genes with these marks tends to be involved in enhanced disease susceptibility. One good example of such epigenetically based phenotype viability is the A^{vy} allele, the result of the insertion of a transposable element (transposon) upstream of the TSS of the mouse *Agouti* gene that encodes a paracrine signaling molecule involved in the production of a yellow pigment in mice melanocytes. Insertion of this transposon promotes constitutive expression of *Agouti*, leading to yellow fur, obesity, diabetes, and tumorigenesis. CpG methylation in the A^{vy} correlates inversely with expression of *Agouti*. The degree of methylation varies dramatically among individual $A^{vy/a}$ mice, causing a wide distribution of coat color from yellow (unmethylated) to brown (methylated) [110]. Moreover, supplementation of pregnant mice with betaine, choline, folic acid, and vitamin B12 can lead to a shift in coat color from yellow to brown and provide protection against obesity in the offspring. This effect has been shown to result from an increase in DNA methylation of the CpG sites in the transposon [111,112].

Recent findings by Zucchi and colleagues have demonstrated that the gestational stress can induce miRNAome and transcriptome changes, which are related to the psychiatric and neurological diseases in the offspring. For example, miR-103, which is involved in brain pathologies, and miR-145, a marker of multiple sclerosis in human, are both upregulated in response to gestational stress [105]. Such findings not only improve the understanding of disease susceptibility formation by specifying the important role of epigenetic mechanisms in this process, but also indicate that environmental-induced epigenetic changes during early development are associated with diseases in adulthood.

Gestational hypoxia and vulnerability of neonate hypoxic-ischemic encephalopathy

Etiology of fetal hypoxia

Although low oxygen tension is important for normal fetal organogenesis and growth, additional hypoxia during gestation contributes to developmental malformations in the fetus. Intrauterine hypoxia can be related to various complications occurring in the mother, placenta, or fetus, which can manifest differently and lead to different consequences. There are three types of hypoxic condition during pregnancy: preplacental hypoxia, in which both the mother and her fetus are hypoxic; uteroplacental hypoxia, in which the maternal oxygenation is normal but the fetus is hypoxic because of impairment of uteroplacental circulation; and postplacental hypoxia, in which only the fetus is hypoxic [113]. Preplacental hypoxia represents the chronic hypoxia, mostly caused by high-altitude environments and pre-existing maternal cardiovascular disease, such as cyanotic heart disease, heart failure, or pulmonary hypertension [114]. Chronic hypoxia is a prominent risk factor of intrauterine growth restriction [115]. It limits the ability of the fetus to achieve its genetically determined growth potential and is responsible for multiple complications, such as cognitive dysfunction and cerebral palsy. It can also lead to brain morphological and functional alterations during development, or can potentially result in fetal brain injury via cell death or

other cellular signals, owing to low oxygen. Uteroplacental hypoxia is associated with abnormal placental implantation and can be induced by gestational hypertension and preeclampsia. As a result, both mothers and fetus are at an increased risk for cardiovascular disease later in life [116,117]. Postplacental hypoxia is mainly related to fetal diseases, such as decreased uterine artery flow or genetic abnormalities, rather than to the direct effect of hypoxia on the fetus [114].

Hypoxia-induced brain injury occurs in both white matter and gray matter, which has been confirmed in animal models showing that severe acute hypoxia causes an equal injury in subcortical white matter and gray matter region in preterm fetal sheep at 0.6 and 0.7 of gestation [LM3][118–120]. Indeed, the cerebral cortex, hippocampus, and subventricular zone are the regions most vulnerable to hypoxic insults [121,122]. Brain injury caused by fetal hypoxia consequently leads to abnormal behavioral presentations. Using near-term intrauterine hypoxia in the spiny mouse, Ireland and colleagues reported that the acute hypoxic episode produced a mild neurological deficit during the early postnatal period [123]. A sequence of mechanisms is involved in fetal hypoxia-induced brain injury in the developing brain, including the loss of stem or progenitor cells, increased apoptotic cell death because of energy deprivation [124], accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and immature oligodendrocytes in the periventricular leukomalacia region [125,126]. The inflammatory response is also associated with hypoxia-induced fetal brain injury by enhancing the expression of some pro-inflammatory cytokines and proapoptotic proteins [127].

Fetal hypoxia and neonate hypoxic-ischemic encephalopathy

Neonatal hypoxia-ischemia (HI) is a crucial perinatal event that is characterized by exposure of the individual to low oxygen (hypoxia) and decreased blood flow (ischemia) before, during, or after labor. HI is the leading cause of acute mortality and chronic disability in newborns with an incidence of 1–8 cases per 1000 births, ultimately affecting 60% of preterm infants [128,129]. The chronic complications of cerebral palsy, mental retardation, and epilepsy induced by HI affect a staggering 80% of the survivors [130]. If occurrence of HI is severe enough to damage the brain, it leads within 12–36 h to hypoxic-ischemic encephalopathy (HIE), which is also called perinatal asphyxia [131]. In addition to short-term medical complications, HIE is also associated with mental and neurological diseases with delayed clinical onset [132]. The underlying mechanisms remain elusive and the etiology of brain damage secondary to HIE is complicated and multifaceted. Compared with the adult brain, the neonatal brain shows some differences in physiological structure organization, ontogeny, function, cellular composition, and signaling pathways related to gene expression and, therefore, demonstrates more sensitive and plastic features to challenges [14]. The clinical signs of HIE manifest in the progression of various cellular and molecular responses, such as delayed cell death, suppression and/or overactivation of gene expression, triggered by initial insults that probably occurred during gestation [131,132]. Fetal hypoxia is one of these initial insults, and can lead to abnormal brain development and increased susceptibility of neonates to HIE at the perinatal stage. However, little is known about the role of epigenetic programming in this process. A recent study showed that epigenetic programming of the GR in response to fetal hypoxia resulted in enhanced brain

vulnerability to hypoxic/ischemic brain injury in neonatal rats [133]. This finding demonstrated that fetal hypoxia resulted in repression of the GR via methylation of the GR promoter in the developing brain, which enhanced the vulnerability of neonates to HI brain injury. This suggests that fetal hypoxia-induced epigenetic programming of brain developing gene expression is, at least partly, responsible for neonate HIE. Using a gestational intermittent hypoxia rat model, Wang and colleagues observed a sex-dependent anxiety-like behavior in 90-day-old (P90) male offspring that were exposed to hypoxia during their prenatal stage. This behavior was linked to the activation of corticotropin-releasing hormone type-1 (CRHR1) transcription in P90 paraventricular nucleus and E19 embryo hypothalamus, which was controlled by the DNA methylation level at several specific CpG sites of the promoter [134]. These studies demonstrate that the epigenetic programming of gene expression induced by fetal hypoxia increases the risk of brain disorders in the offspring, further supporting the crucial role of epigenetic machinery in regulating brain development.

Epigenetic programming and neurological diseases

Epigenetic mechanisms and neural development

Epigenentic mechanisms together orchestrate CNS development and plasticity, as well as brain function throughout life, by contributing to promoting neural lineage commitment, and the determination or maintenance of neural cell identity. A range of evidence has shown that epigenetic factors, including DNMT [135], MBDs [136], histone- and chromatin-modifying enzymes [137], and miRNA biogenesis factors [138], are essential for the process of neurogenesis and gliogenesis. For exmple, epigenetic regulatory complexes, repressor for element-1 (RE1)- silencing transcription factor (REST) and co-RE1-silencing transcription factor (CoREST) complexes, are central players in regulating seminal neural cell fate decisions and neuronal or glial lineage specification, through targeting transcriptional and epigenetic regulation of developmental gene networks [139]. A large amount of miRNAs in the mammalian brain have specialized roles in neurodevelopment and adult homeostasis and/or plasticity. For instance, miR-184 promotes neural stem cell proliferation and inhibits neuronal differentiation. MiR-125b, miR-128, miR-132, miR134, and miR-138 are also associated with neuronal maturation and neural network integration [140].

Epigenetic mechanisms also regulate the synaptic plasticity, which is associated with long-term potentiation, depression, and learning and memory, via control of the expression of related genes. DNA methylation is responsible for silencing these genes, which are associated with pluripotency and alternative lineage programming, thereby regulating premature neural stem cell development and synaptic plasticity. DNMTs are expressed throughout brain development. Even in the adult brain, these enzymes also function in specific regions mediating ongoing neurogenesis. In animal models, genetic knockout of both the *Dnmt1* and *Dnmt3a* genes reduced the DNA methylation levels, which led to plasticity abnormalities in the hippocampal CA1 region and consequently damage learning and memory in the affected animals. Moreover, hippocampal Dnmt3a2 levels determine cognitive abilities in both young adult and aged mice [141]. MiRNAs also target genes encoding synaptic proteins, and have important roles in activity-dependent synaptic

plasticity and memory formation [142,143]. As is evident, the dysregulation of miRNAs lead to various neurological diseases.

Epigenetic programming of neurological disorders

Many epigenetic modifications have been associated with fetal brain development, and the abnormal expression of epigenetic factors results in neurological disease in adults. MeCP2 is a key transcriptional regulator of gene expression during brain development and is tightly regulated to ensure normal neurological function at different developing stages. For example, the expression level of MeCP2 is usually low at the fetal stage, but increases significantly during postnatal development [144]. X-linked *Mecp2* mutations are the primary cause of an autism spectrum disorder, Rett syndrome (RTT). *Mecp2* has multiple different types of mutation, including missense, nonsense, deletions, and insertions, all of which result in RTT-like symptoms [145]. In addition, *Mecp2* mutations are also found in patients with other neurological conditions, such as learning disabilities, neonatal encephalopathy, autism, and X-linked mental retardation [146,147]. The important role of MeCP2 has been confirmed by genetic ablation experiments showing that the symptom in *Mecp2*-null mice is rescued by reintroduction of *MeCP2* [147]. A recent study showed that miR-483-5p, an intragenic miRNA of the imprinted insulin-like growth factor 2 (IGF2) gene, has important roles in regulating MeCP2 expression in the fetal brain [148].

A growing body of evidence has demonstrated that alterations of DNA methylation are associated with the pathogenesis of Alzheimer's disease (AD) [149,150]. Mastroeni and colleagues evaluated the expression of two DNA methylation markers and eight methylation maintenance factors in entorhinal cortex layer II, a region exhibiting substantial effects of AD. Their findings indicated that the neuronal epigenetic factors were significantly decreased in AD cases, particularly in neurofibrillary tangle-bearing neurons [150]. The extracellular amyloid β (Aβ) plaque is the main mark of both familial and sporadic forms of AD. It has been reported that the promoter region of the *APP* gene, which encodes the Aβ precursor protein, is regulated by at least two GC elements [151] and exhibits differential expression patterns in human brain [152]. These observations implicate a role of methylation patterns in AD pathogenesis via regulation of *APP* expression.

miRNAs are also associated with various neurological diseases. For example, miRNAs are regarded as key players in leucine-rich repeat kinase 2 (LRRK2)-mediated Parkinson disease (PD) pathogenesis. Through negative regulation of miRNA-mediated translational repression, LRRK2 mutations can cause familial and sporadic forms that are characteristic of PD. Blockage of let-7 and miR-184* regenerated the effects in wild type LRRK2 cells, whereas overexpression of let-7 and miR-184* alleviated LRRK2 mutant-induced pathology [153]. Compelling evidence now indicates that miRNAs are differentially expressed in the AD brain and might be associated with AD pathogenesis [154,155]. The relation between miRNAs and AD is initially recognized by postmortem analyses indicating that beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) protein level, but not mRNA level, was upregulated in brains from patients with AD [156]. Wang and colleagues also found that miR-107 was significantly decreased in patients with the earliest stages of AD pathology when BACE1 mRNA levels tended to be increased. Bioinformatics analysis predicted that

the 3' UTR of BACE1 mRNA has multiple binding sites for miR- 107, and at least one of these sites was shown to be functionally regulated by miR-107 in reporter gene assays [155]. The miR-29a/b-1 cluster has also been reported to regulate BACE1 expression in brain development and in primary neuronal cells. Correspondingly, the miR-29a/b-1 cluster was significantly decreased in patients with AD and abnormally high levels of BACE1 protein [157]. APP was regulated by miR-101, which targeted the RNA responsive element (RE) at the 3' UTR of APP mRNA. Moreover, inhibition of endogenous miR-101 increased the level of APP, whereas overexpression of miR-101 significantly reduced APP and Aβ load in cultured hippocampal neurons [158].

Other neurological diseases, such as fragile X syndrome (FXS), an X chromosome-linked intellectual disability, resulted from the loss of fragile X mental retardation protein (FMRP), are also linked to miRNA machinery. FMRP interacts with many brain plasticity-associated miRNAs, including miR-132, miR-124, and miR-219, to control the spatial and temporal translation of mRNAs involved in synaptic plasticity [143]. Indeed, phosphorylation of FMRP is involved in the inhibition of postsynaptic density protein 95 (PSD-95), a key postsynaptic component of glutamatergic synapses via formation of a complex with miR-125a and Argonaute 2 (Ago2). FMRP dephosphorylation released PSD-95 mRNA from miR-125a-mediated repression and increased the PSD-95 protein level [159].

Environmental cues lead to activation of diverse epigenetic mechanisms, which mediate ESC maintenance and maturation, and neural circuit formation and synaptic plasticity. In addition to fetal hypoxia, as discussed above, maternal cigarette smoking is another well-known pathogen that contributes to the increased risk of neonatal morbidity. Although the underlying mechanisms remain unclear, the nicotine-induced downregulation of AT_2R in the developing brain might be associated with increased brain vulnerability to HI injury of neonatal offspring. Moreover, AT_2R downregulation is mediated by epigenetic mechanisms and methylation of a single CpG locus near the TATA-box at AT_2R promoter resulted in decreased binding affinity of TATA-binding protein (TBP), leading to repression of the *AT2R* gene in the developing brain [160]. In addition, BDNF has a vital role in brain development and its expression is regulated by alternations in DNA methylation [161]. The BDNF-4 promoter, BDNF-6 promoter, and 5′UTR were modulated by stress hormones, antidepressant treatment, and DNA methyltransferase inhibitors [162,163]. Studies in humans showed that cigarette smoking is associated with a reduced blood level of BDNF protein [164]; however, the extent to which maternal smoking is related to BDNF methylation in adolescent offspring remains unknown. Toledo-Rodriguez and and colleagues conducted an epidemiologic study in adolescents whose mothers smoked during pregnancy and revealed that nicotine exposure during the prenatal period increased the methylation level of the BDNF-6 promoter, which could have led to changes in the plasticity and development of the brain [165].

In addition to environment stressors, a continuous supply of methyl group donors in food might also regulate nervous system development and functioning via epigenetic mechanisms. Maternal diets deficient of methyl donors (MDD) were shown to increase anxiety and learning ability in aged female offspring. However, the DNA methylation patterns of the genes encoding GR, 11β-HSD2, neuronatin, and reelin in hippocampus of rat

offspring were not altered significantly when compared with offspring from mothers on a control diet [166]. Although a large number of candidate genes were related to the neurobehavioral function, only a small group of genes was investigated in this study; therefore, it is possible that other DNA regions or uninvestigated epigenetic mechanisms were involved. In rats, this notion was confirmed by a large-scale study in offspring exposed to maternal stress during gestational days 12–16. Over 700 genes in the frontal cortex and hippocampus were found to be differentially expressed following prenatal stress. The DNA methylation that is associated with behavioral changes in offspring rats was increased with both sex-dependent and region-specific profiles [167].

Potential therapeutic interventions for developmental programming disease

Understanding of epigenetic mechanisms in developmental programming of health and disease has a potential in revealing targets for therapeutic interventions and in for drug discovery. Recent laboratory studies have explored the reversibility of induced phenotypic effects by intervention on epigenetic factors. Several compounds have been shown to manipulate DNMT or histone deacetylase (HDAC) activity, and are capable of reversing programmed phenotypic effects. For example, infusion of the HDAC inhibitor trichostatin A (TSA) or the essential amino acid Lmethionine into the brain ventricles of adult animals reversed epigenetic modifications in the hippocampus, GR expression and HPA [LM4]response to stress in the female offspring of dams showing low levels of maternal care [168–170]. Another example is that of the plant-derived isoflavone genistein, which can reactivate methylationsilenced genes. Maternal dietary genistein supplementation during gestation increased methylation of six CpG sites in the transposon upstream of *Agouti* and led to a shift in the fur color of $A^{vy/a}$ mouse offspring to pseudoagouti [109]. Supplementation of the methyl donor folate to dams on a low-protein diet prevented the elevation of blood pressure, impairment of vasodilatation, and the decrease of nitric oxide synthase mRNA levels in the offspring. In addition, folate supplementation during pregnancy improved the cardiovascular function of offspring exposed to maternal protein restriction [171]. Exogenous leptin administration from day 3 to 13 in the neonatal offspring of undernourished rats rescued the phenotypic changes, gene expression, and associated methylation changes in the PPARα promoter, suggesting that developmental metabolic programming is potentially reversible by an intervention late during developmental plasticity [172]. In addition, Godfrey and colleagues revealed that the DNA methylation level of umbilical cord tissue at a specific site in the retinoid X receptor alpha (*RXRA*) promoter is associated with lower maternal carbohydrate intake in early pregnancy and higher childhood body fat [173]. RXRA is a transcription factor involved in fat metabolism and insulin sensitivity and, thus, the finding of epigenetic alteration to its promoter could provide utility in identifying individual vulnerability to later obesity and metabolic disease, and /or other conditions. The same principle has been extended to the developmental programming of brain disorders. Recent studies found that exposure to gestational hypoxia downregulated GR in the developing brain, which was associated with enhanced vulnerability of neonates to HI brain injury. Further experiments showed that gestational hypoxia-induced GR downregulation resulted from increased DNA methylation and

decreased binding of transcription factors Egr-1 and Sp1 to GR gene exon 1_7 and 1_{11} promoters [15]. These findings indicate that developmental epigenetics could provide new therapeutic targets against fetal programming of disease.

Recently, based on the extended studies of miRNAs, intervention on miRNAs has been developed step by step and has shown therapeutic potential [174–177]. Increasing amounts of modified oligonucleotide mimics or antisense oligonucleotide are being applied to diseases caused by epigenetic alterations [178]; moreover, newer biochemical modifications have been introduced to enhance the stability, specificity, and efficacy of these oligonucleotides. Locked nucleic acids (LNAs), a family of conformationally locked nucleotide analogs, have been designed and widely used to modulate miRNA function [179]. For instance, LNA-modified oligonucleotide (LNAantimiR) effectively antagonized the liver-expressed miR-122 in nonhuman primates, resulting in the depletion of mature miR-122 and dose-dependent lowering of plasma cholesterol [180]. In addition, other effects have been directed toward the development of small-molecule drugs that can intervene in the biogenesis process of miRNAs or directly influence their function [181]. However, there remains much uncertainty regarding the relative importance of developmental factors in the development of disease and, thus, more research needs to be done in contemporary cohorts to define a better early-life growth pattern.

Concluding remarks

Accumulating evidence from human epidemiological and laboratory animal studies suggests that one’s environment situation during early development sets the stage for one’s subsequent health. A growing body of evidence indicates that, at the molecular level, developmental programming is reflected by transcriptional and/or translational changes in neurodevelopment-related genes, and these changes are achieved, for the most part, through alteration of epigenetic modifications of the genes involved. Various complications during development, including maternal hypoxia and nutritional signals, are capable of altering the epigenetic marks surrounding the DNA and RNA. Although each epigenetic pattern has its own characteristics, they function as an orchestrated and complicated network to ensure integrated fine-tuning of gene regulation. Moreover, the detrimental effects, as well as altered epigenetic marks, resulting from prenatal insults can be inherited transgenerationally and affect more than one generation. Fortunately, the concept of developmental programming of health and disease has been demonstrated by numerous studies and has received extensive attention. In addition to avoiding possible prenatal insults, further understanding of the underlying molecular mechanisms of fetal programming is likely to promote timely intervention and valid therapy approaches to minimize the pathophysiological consequences induced by prenatal programming. Not only does such understanding have great implications for individual health, but the awareness of, and dedicated investigations for, preventative and therapeutic approaches also have enormous national and global relevance.

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Biographies

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Highlights

- Epigenetic mechanisms are important in the brain development
- Hypoxia is a common form of intrauterine stress
- Hypoxic-ischemic brain injury is a leading cause of neonatal mortality and subsequent neurological disorders
- Epigenetic mechanisms play a key role in programming of increased risk of ischemic brain injury

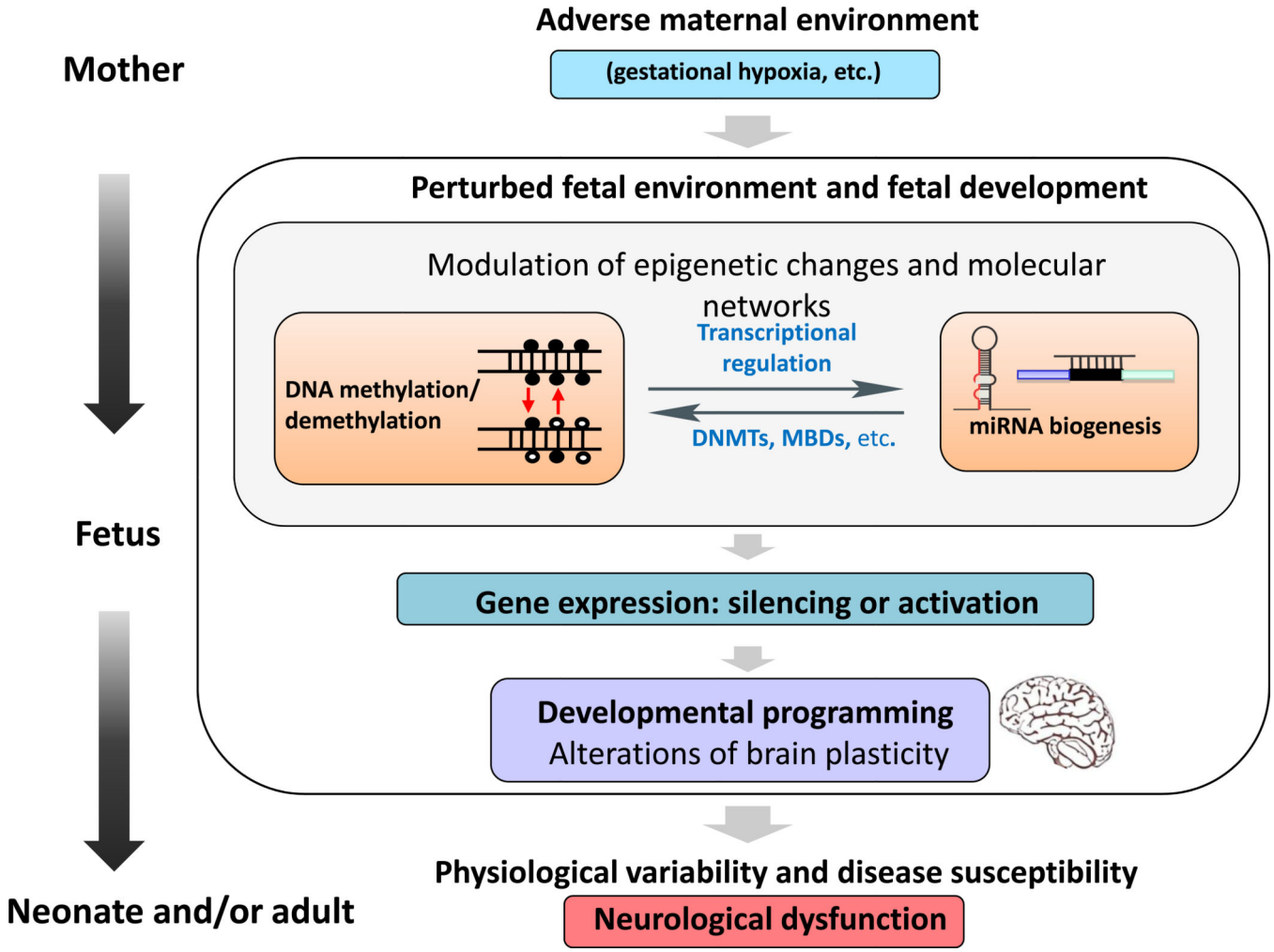

Figure 1.

Schematic diagram showing the correlation between adverse maternal environment (e.g., gestational hypoxia) and epigenetic mechanisms in developmental programming of neurological diseases in the offspring. A tightly controlled gene expression profile has a crucial role during brain development and plasticity. Exposure of the mother to adverse factors during pregnancy, such as maternal hypoxia, results in a perturbed maternal–fetal environment and can lead to modulated epigenetic changes that alter the gene expression profile as an adaption of the developing fetus to the adverse environment. Different epigenetic mechanisms corporately [LM6]orchestrate this process and also regulate each other. For example, DNA methylation/demethylation transcriptionally regulates miRNA biogenesis, and is conversely regulated by miRNAs modulating the expression of DNA methylation-related enzymes and proteins. Changes in physiology and neuroplasticity of the developing brain can permanently affect the structure and/or functionality, and result in increased disease susceptibility in the offspring. Abbreviations: DNMT, DNA methyltransferases; MBD, methyl-CpG binding protein.

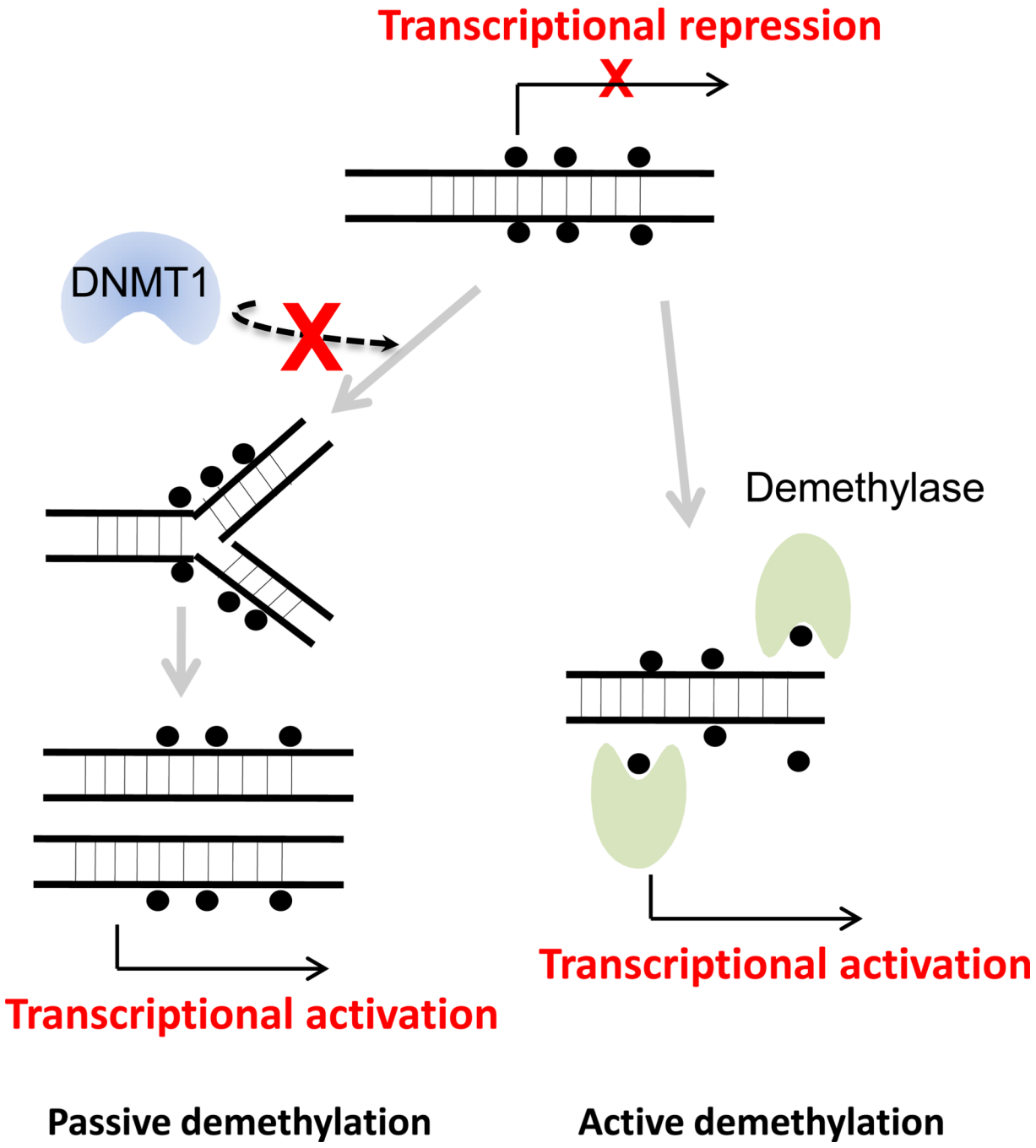

Figure 2.

Passive demethylation and active demethylation. Passive DNA demethylation occurs as a consequence of replication process in the absence of the maintenance DNA methyltransferase 1 (DNMT1). In contrast to passive demethylation, active demethylation is the enzymatic process by demethylases that results in the removal of the methyl group from 5-methylcytosine and subsequent transcriptional activation. Black filled circle: methylated CpG dinucleotides.

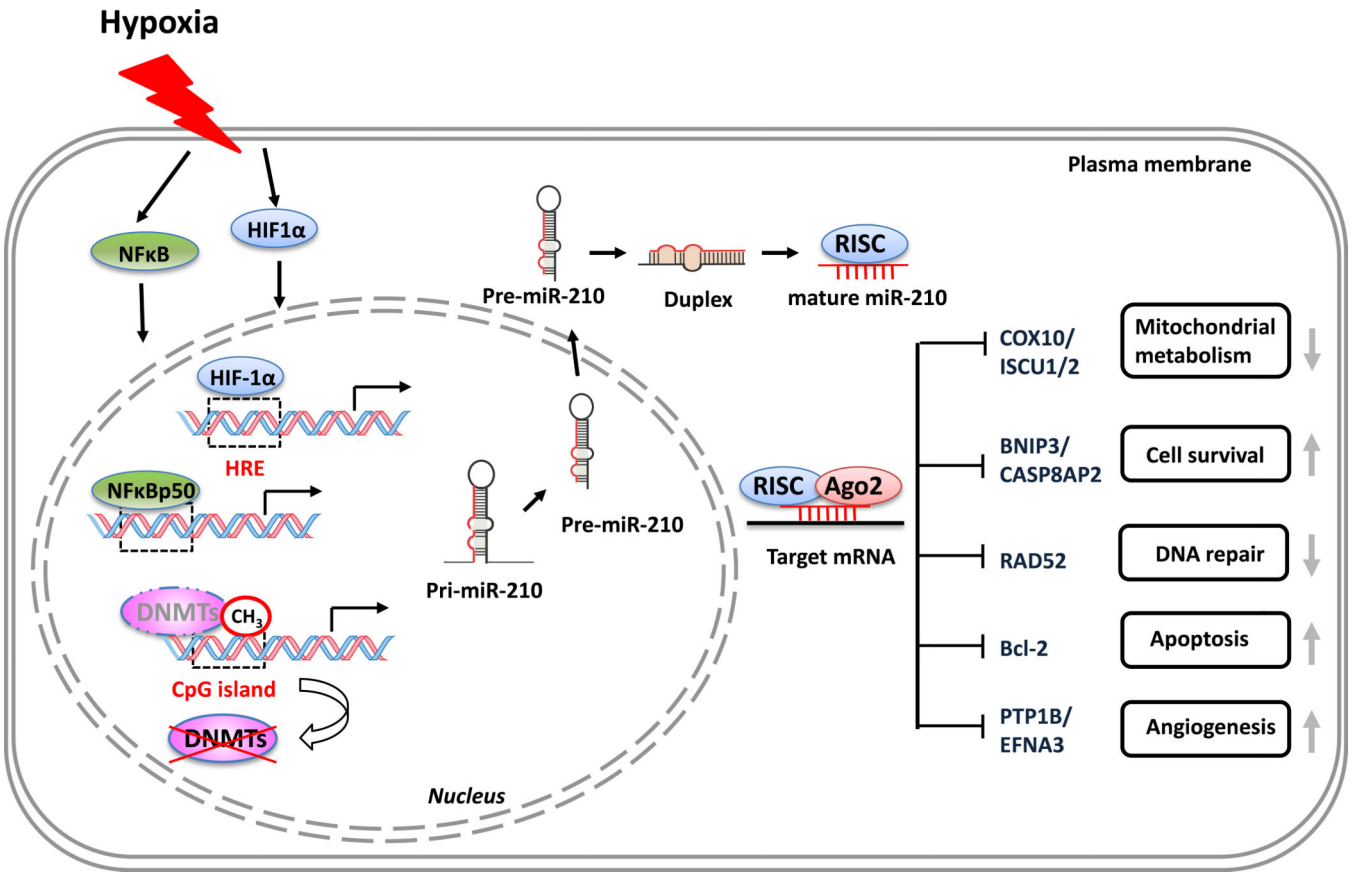

Figure 3.

Summary of miR-210 upregulation mechanisms under hypoxia and its target genes. Hypoxia induces miR-210 upregulation via hypoxia inducible factor (HIF)-1α-dependent and -independent pathways. HIF-1α binds to the hypoxia responsive element (HRE) region and initiates gene transcription: the so-called 'HIF-1α- dependent pathway'. Hypoxia activates nuclear factor (NF)-κB, which regulates miR-210 transcription by binding to the promoter of miR-210. Hypoxia-induced demethylation by releasing DNA methyltransferases (DNMTs) from CpG islands or inactivating DNMTs also increases miR-210 expression. These represent the HIF- 1α-independent pathway. In the cytosol, pre-miRNA-210 is processed to mature miR-210, binds to 3' untranslated region (UTR) and represses the expression of a subset of genes associated with a sequence of cellular functions, including mitochondrial metabolism (*COX10/ISCU1/2*), apoptosis (*Bcl2/BNIP3/CASP8AP2*), angiogenesis (*PTP1B/EFNA3*), and DNA repair (*RAD52*). Abbreviations: Ago2, Argonaute 2; RISC, RNA-induced silencer complex. For definitions of gene symbols, please see the main text.