

Epigenetics and MicroRNAs

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ABSTRACT: Epigenetics is defined as mitotically and meiotically heritable changes in gene expression that do not involve a change in the DNA sequence. Two major areas of epigenetics—DNA methylation and histone modifications—are known to have profound effects on controlling gene expression. DNA methylation is involved in normal cellular control of expression, and aberrant hypermethylation can lead to silencing of tumor-suppressor genes in carcinogenesis. Histone modifications control the accessibility of the chromatin and transcriptional activities inside a cell. MicroRNAs (miRNAs) are small RNA molecules, ~22 nucleotides long that can negatively control their target gene expression posttranscriptionally. There are currently more than 460 human miRNAs known, and the total number is predicted to be much larger. Recently, the expression of miRNAs has been definitively linked to cancer development, and miRNA profiles can be used to classify human cancers. miRNAs are encoded in our genome and are generally transcribed by RNA polymerase II. Despite the growing evidence for their importance in normal physiology, little is known about the regulation of miRNA expression. In this review, we will examine the relationship between miRNAs and epigenetics. We examine the effects of miRNAs on epigenetic machinery, and the control of miRNA expression by epigenetic mechanisms. Epigenetics is defined as heritable changes in gene expression that do not involve a change in DNA sequence. (*Pediatr Res* 61: 24R–29R, 2007)

Epigenetics is defined as heritable changes in gene expression without a change in the DNA sequence itself (1). DNA cytosine methylation and histone modifications are two important mechanisms in the area of epigenetics that have profound roles in gene regulation, development, and carcinogenesis (2–5).

DNA methylation is a normal process used by mammalian cells in maintaining a normal expression pattern; it is involved in the regulation of imprinted gene expression and X-chromosome inactivation, among others (6–8). DNA methylation occurs almost exclusively on a cytosine in a CpG dinucleotide, and is achieved by the addition of a methyl group to the 5 position of a cytosine ring mediated by DNMTs (Fig. 1). CpG sites are roughly 80% depleted in the genome, and are asymmetrically distributed into CpG poor regions and dense regions called CpG “islands” (9,10), which are often located in the promoter regions of roughly half of all the protein-coding genes. The majority of the genome is rather CpG-poor due to

the mutagenicity of a methylated cytosine; a methylated cytosine can undergo spontaneous deamination to become a guanine (Fig. 2) (1, 11). CpG islands normally remain unmethylated, whereas the sporadic CpG sites in the rest of the genome are normally methylated. There is a gradual reversal of this pattern during aging that leads to sporadic methylation in the CpG islands and a global loss of methylation, but this change is particularly pronounced during carcinogenesis (Fig. 3) (1,12). Methylation of CpG islands in promoter regions is often associated with gene silencing, and aberrant DNA methylation occurs in most cancers, leading to the silencing of some tumor suppressor genes (Fig. 2) (2,12). There are three major enzymes involved in establishing and maintaining DNA methylation patterns: DNMT 3A and 3B are *de novo* methyltransferases, and DNMT1 is the maintenance DNMT that ensures that methylation patterns are copied faithfully throughout each cell division (3). They cooperate with each other to establish and maintain the cellular DNA methylation patterns (Fig. 4) (1).

Histone modifications, especially the posttranslational modifications of amino-terminal tail domains, are also important epigenetic mechanisms in controlling gene expression (1). Certain histone modifications, such as histone acetylation, are associated with active gene transcription, whereas others such as the methylation of histone H3 lysine 9 (H3K9) is an indicator of condensed and inactive chromatin (1,13,14).

The relationships between DNA methylation and histone modifications have recently become clearer, although much is still to be learned. It is now believed the two mechanisms cooperate in controlling gene expression. For example, methylation of histone H3 lysine 9 can be triggered by DNA methylation (15–17). DNA methyltransferases have also been shown to interact with histone deacetylases (HDAC), histone methyltransferases, and methyl-cytosine-binding proteins in a complex network (18–20).

The association between abnormal epigenetic changes such as DNA hypermethylation and human diseases, including cancer, has become increasingly clear (1). In contrast to genetic changes that cause cancer, epigenetic modifications of gene expression are more general and usually involve more than one gene. Many drugs have been found to have effects in reversing the abnormal epigenetic changes that occur in carcinogenesis, and they can be divided into two classes—DNA methylation inhibitors and HDAC inhibitors (1,21). These

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Abbreviations: 5-Aza-CdR, 5-Aza-2'-deoxycytidine; DNMT, DNA methyltransferase; miR and miRNA, microRNA; PBA, 4-phenylbutyric acid

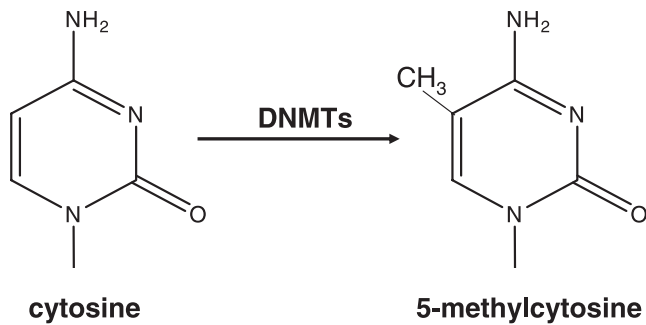


Figure 1. Mechanism of DNA methylation. DNA methylation involves the addition of a methyl group onto the 5 position of a cytosine residue, mediated by the enzymes DNMTs. DNA methylation happens almost exclusively on cytosines in front of a guanine in a CpG dinucleotide.

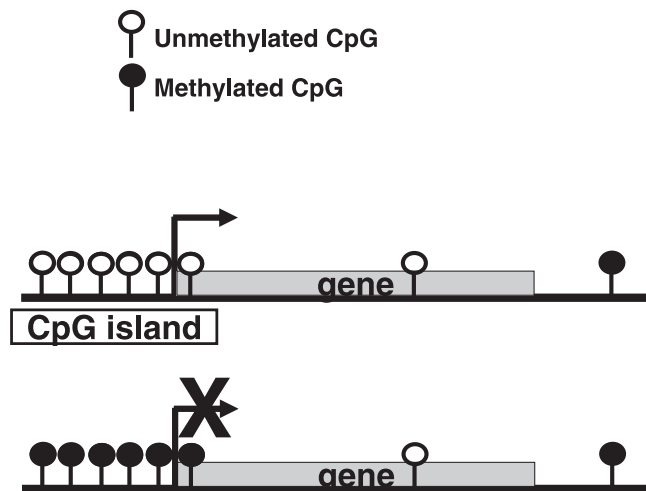


Figure 2. CpG sites in the genome are highly unevenly distributed. CpG islands can be found in the promoter regions of roughly half of the genes and normally remain unmethylated. When they become aberrantly hypermethylated, as can happen in many cancers, they lead to the silencing of downstream genes.

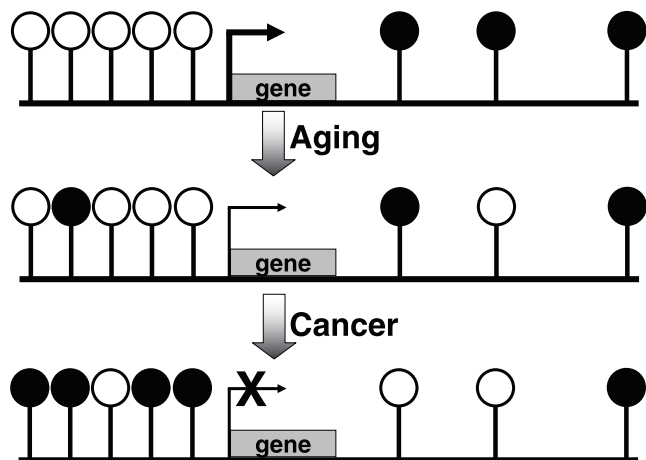


Figure 3. CpG islands normally remain unmethylated, whereas the sporadic CpG sites located in the rest of the genome often are methylated. With aging, there is a gradual reversal of this phenomenon. During carcinogenesis, this change is much more dramatic, leading to a global hypomethylation and hypermethylation of CpG islands. The results are chromosomal instability and silencing of some important tumor-suppressor genes.

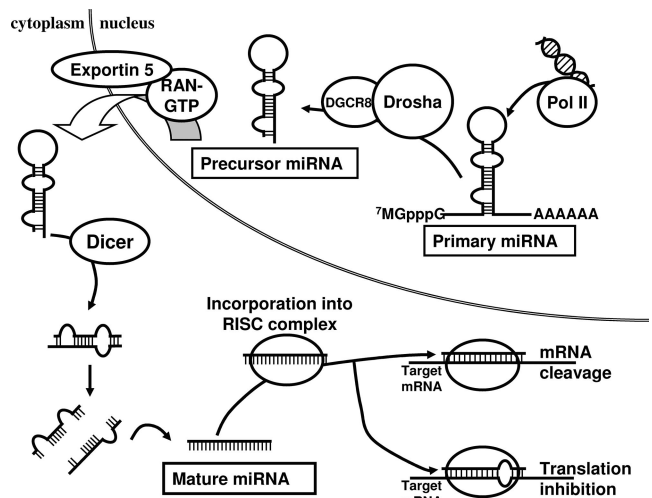


Figure 4. Biogenesis of miRNAs. miRNAs are endogenously encoded in the genome. They are generally transcribed by RNA polymerase II (PolII) into the primary miRNAs (pri-miRNAs), which therefore have 5' caps and poly-A tails. The pri-miRNAs are processed by RNase III Drosha with its partner DGCR8 (DiGeorge syndrome chromosomal region 8; also known as Pasha in invertebrates) into the precursor miRNAs (pre-miRNAs), which are then exported by the nuclear export factor Exportin 5 and its cofactor RAN-GTP into the cytoplasm. It is in the cytoplasm that the pre-miRNAs are further processed by another RNase III Dicer into the mature miRNAs. The mature miRNA is incorporated into the RISC complex and negatively regulates its target mRNA by one of two ways. When it binds its target with complete complementarity, it leads to the target mRNA cleavage. On the other hand, it can also bind to its target with incomplete complementarity, and leads to translational repression of the latter by a yet poorly understood mechanism.

agents hold great promises in improving the treatment of cancer.

miRNAs ARE ENDOGENOUS SMALL RNA MOLECULES THAT CAN CONTROL GENE EXPRESSION

miRNAs are ~22 nucleotides-long RNA molecules encoded in the genome that can have a profound effect in controlling gene expression. They are transcribed by RNA polymerase II (Pol II) into primary miRNAs (22,23), and are then processed in the nucleus by the RNase III Drosha and DGCR8 (microprocessor complex) into the precursor miRNAs. Precursor miRNAs are structured as imperfect stem-loops, and they are exported into the cytoplasm by Exportin-5. The precursor miRNAs are further processed in the cytoplasm by another RNase III Dicer into the final functional mature miRNAs (24–27).

miRNAs bind to their target mRNAs and down-regulate their stabilities and/or translation. When binding to its target mRNA with complete complementarity, the miRNA can lead to degradation of the target. MiRNAs can also bind to their targets with incomplete complementarity, often in the 3' UTR regions, and this leads to the translational suppression of their target genes by a mechanism that has yet to be completely elucidated (24–26). Each miRNA is predicted to have many targets, and each mRNA may be regulated by more than one miRNA (28–30). Currently, there are more than 460 human miRNAs known (31).

Table 1. Oncogenic MiRNAs

miRNA	Regulation	Role in cancer	References
miR-17-92 cluster	c-MYC induces the expression	Overexpressed in B-cell lymphomas and can accelerate tumor progression in a mouse B-cell lymphoma model. Plays a role in tumor angiogenesis. Overexpressed in lung cancers.	(46,47,62,63)
miR-372, miR-373		Overexpressed in testicular germ cell tumors. Permit proliferation and tumorigenesis of primary human cells that have both oncogenic RAS and active wild-type p53.	(54)
miR-21		Expressed in glioblastomas and breast cancers. An anti-apoptotic factor.	(34,36,37)
miR-155		Overexpressed in breast, colon, and lung cancers, as well as in B-cell lymphomas. Overexpression correlates with poor survival in lung cancers and poor prognosis in DLCLBL. High expression in children with Burkitt's lymphoma.	(34,55,56,60,64–66)
miR-146	NF- κ B	Overexpressed in breast, pancreas, and prostate cancers.	(66,67)

Table 2. Tumor-suppressor MiRNAs

miRNA	Regulation	Role in cancer	References
miR-127	DNA methylation and histone modifications	Decreased expression in bladder and prostate cancers. Targets proto-oncogene <i>BCL6</i> .	(49)
miR-15a, miR-16-1		Often down-regulated in B-cell chronic lymphocytic leukemias. Targets <i>BCL2</i> .	(32,33)
let-7		Targets RAS. Reduced expression in human lung cancer associates with poor prognosis.	(57–60)
miR-145		Reduced expression in colon and breast cancers.	(34,61)

miRNAs have recently been shown to be definitely linked to cancer, and they can act as either oncogenes (Table 1) or tumor-suppressor genes (Table 2) in carcinogenesis. For example, miR-15a and miR-16-1 can target the anti-apoptotic *BCL2*, and they are often down-regulated in chronic lymphocytic leukemia (32,33). miR-21 is found to be antiapoptotic, and it is up-regulated in glioblastomas and breast cancers (34–37). Lu *et al.* (38) showed that the expression profiles of miRNAs are able to classify human cancers.

miRNA CONTROL OF EPIGENETIC MECHANISMS

Small interfering RNAs (siRNAs), often considered to be closely related to miRNAs, have been shown to be involved in both DNA methylation and histone modifications. The processing pathways of siRNAs and miRNAs share many of the enzymes involved in the RNA interference (RNAi) pathway (39). Recent evidence also suggest that they affect histone modifications. Maison *et al.* (40) showed that RNase treatment can abolish the localization of methylated H3 lysine 9 and HP1 to pericentromeric chromatin. Fukagawa *et al.* (41) showed that Dicer-related RNAi machinery is necessary for the formation of heterochromatin structure. Because siRNAs and miRNAs are closely related, miRNAs could also play important roles in controlling DNA methylation and histone modifications.

miRNAs can be involved in establishing DNA methylation. miR-165 and miR-166 have been shown to be required for the methylation at the *PHABULOSA* (*PHB*) gene in *Arabidopsis*. They interact with the newly processed *PHB* mRNA to change the chromatin of the template *PHB* gene (42). This presents an exciting new mechanism by which miRNAs can control gene

expression in addition to the RNAi pathway. Similar findings in mammalian cells have yet to be shown. In addition, key DNA methylation enzymes DNMT1, 3a, and 3b all are predicted to be potential targets of miRNAs (28), although it remains to be experimentally determined whether the DNMTs can indeed be regulated by miRNAs.

In addition, miRNAs may regulate chromatin structure by regulating key histone modifiers. miR-140, which is cartilage-specific, can target histone deacetylase 4 in mice (43). Costa *et al.* (44) suggested that miRNAs may be involved in meiotic silencing of unsynapsed chromatin in mice. Taken together, miRNAs can be considered important players in the epigenetic control of gene expression.

EPIGENETIC CONTROL OF miRNA EXPRESSION

Since their initial discovery, miRNAs had been assumed to be transcribed by RNA polymerase III (Pol III) due to their small sizes (45), yet the biogenesis of miRNAs has only been elucidated in recent years. Lee *et al.* (27) showed that miRNAs are transcribed from long primary transcripts in 2002, and two years later miRNAs were proven to be generally transcribed by Pol II (22,23). We are now only beginning to understand how miRNA expression is regulated. Because miRNAs are generally transcribed by Pol II, they can be spatially and temporally regulated (25). In addition to negatively regulating their target mRNAs, miRNAs themselves can be regulated by other factors. *c-myc* has been shown to activate transcription of the miR-17-92 cluster, which has a role in tumor neovascularization (46,47). NF- κ B can induce the expression of miR-146a, which can then down-regulate IRAK1 and TRAF6 and thus acts as a component in a negative feedback loop that

controls TLR signaling. Fazi *et al.* (48) showed that the transcription factors NFI-A and C/EBPalpha compete for binding to the miR-223 promoter, leading to low and up-regulated expression of miR-223, respectively. In addition, miR-223 participates in its own feedback loop and favors the C/EBPalpha binding by repressing the NFI-A translation (48). Despite mounting evidence for the importance of miRNAs, the regulation of their expression is still poorly understood.

An exciting new discovery by Saito *et al.* (49) showed that epigenetic mechanisms, such as DNA methylation and histone modifications, can affect the expressions of miRNAs. In particular, miR-127 was found to be remarkably up-regulated in cancer cell lines after the treatment with 5-Aza-CdR, a potent DNA methylation inhibitor, and 4-phenylbutyric acid (PBA), a histone deacetylase inhibitor (1). Together, 5-Aza-CdR and PBA lead to reduced DNA methylation levels and more open chromatin structures, and therefore induce the re-expression of genes that had been silenced epigenetically (Fig. 5) (1). The finding that miR-127, among many other miRNAs, can be expressed after treatment with 5-Aza-CdR and PBA, suggests that epigenetic mechanisms can control the expression of miRNAs. Scott *et al.* (50) also showed that treatment of breast cancer cell line SKBr3 with HDAC inhibitor LAQ824 led to a rapid change in miRNA expression profile.

Many miRNAs are located in the introns of protein-coding genes (25). It is believed that such miRNAs are co-regulated with their host genes (51). However, it is possible that these miRNAs can have their own promoters. The finding that CpG islands within introns can act as promoters suggests that perhaps intronic miRNAs that have CpG islands upstream within the same intron could be transcribed from their own promoters that are regulated by DNA methylation (52,53).

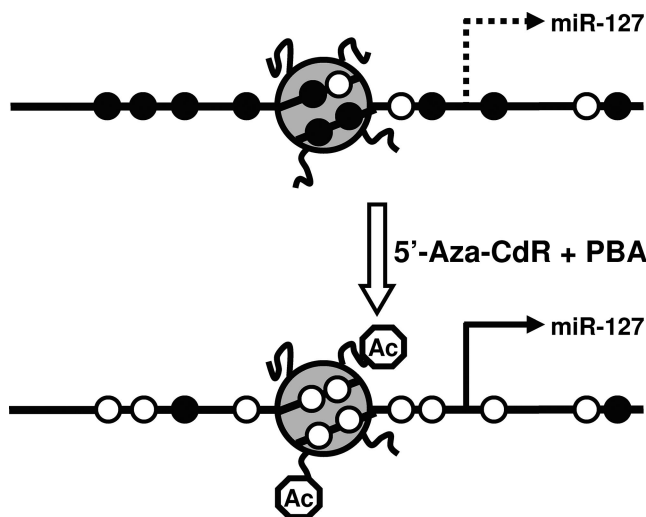


Figure 5. The expression of miRNAs can be controlled by epigenetic mechanisms. Epigenetic mechanisms such as DNA methylation and histone modifications can contribute to the transcriptional control of miRNA expression. In the case of miR-127, methylation of the CpG sites and deacetylation of the histones around its promoter region contribute to its silencing in tumor cell lines. Treatment with 5'-Aza-CdR and PBA leads to reduced DNA methylation and increased histone acetylation, allowing the miRNA to be expressed. The gray circle depicts a nucleosome with histone tails. Open circles on the DNA strand represent unmethylated CpG sites, and the filled circles methylated CpG sites. Octagons on the histone tails represent acetyl groups.

Much still needs to be learned before a definite role for epigenetic mechanisms in controlling the expression of miRNAs can be established. Knowing that epigenetics can control the expression of many protein-coding genes, and that miRNAs are also generally transcribed by Pol II, it is reasonable to hypothesize that epigenetics can play fundamental roles in controlling the miRNA expression.

CLINICAL SIGNIFICANCE AND FUTURE DIRECTIONS

Epigenetics and miRNAs are two important subjects of study that warrant significant growth in their fields in the future, and the relationship between epigenetics and miRNA is just beginning to be understood (Fig. 6). Some miRNAs have been found to play important roles in carcinogenesis. These miRNAs can serve as therapeutic targets in future cancer therapies. For example, knockdown of the oncogenic miRNA miR-21 can trigger apoptosis in cultured glioblastoma cells (36). Other examples of oncogenic miRNAs exist. MiR-372 and 373 are up-regulated in testicular germ cell tumors (54). MiR-155 is overexpressed in B-cell lymphomas and breast cancers (34,55,56). These, and other oncogenic miRNAs, can all serve as important targets in cancer therapy; knocking down of these miRNAs may stunt the cancer growth. On the other hand, restoring tumor-suppressor miRNAs can also be a powerful approach in treating cancer. The finding that epigenetic drugs 5-Aza-CdR and PBA are able to lead to the up-regulation of miR-127, which can down-regulate *BCL6*, is especially exciting (49). It demonstrates that epigenetics drugs may exert their antitumor effects on two fronts: they not only turn on the tumor-suppressor genes that were aberrantly si-

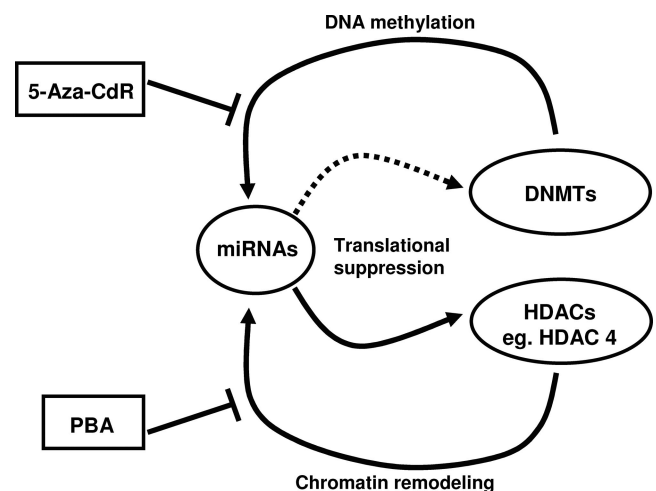


Figure 6. The interplay of epigenetics and miRNAs. Recent evidence has shone light on the relationship between miRNAs and epigenetics. miRNAs can affect the epigenetic mechanisms by targeting key enzymes involved in establishing epigenetic memory. For example, miR-140 has been shown to target HDAC4. It is likely that miRNAs can target other epigenetic players such as DNMTs. On the other hand, epigenetics can control the expression of miRNAs. DNMTs and HDACs can affect the expression of some miRNAs, and DNA methylation and histone modifications have been shown to control the expression of miR-127. Epigenetic drugs such as 5-Aza-CdR and PBA can reverse the changes done by DNMTs and HDACs, and this adds a new layer of understanding to the pharmacological actions of these drugs.

lenced epigenetically, but they also turn on tumor-suppressor miRNAs that down-regulate target oncogenic mRNAs. 5-Aza-cytidine and 5-Aza-CdR, both DNA methylation inhibitors, have been approved by the FDA to treat myelodysplasia (21). Knowing that these drugs can affect the expression of miRNAs helps us further understand the mechanisms of action of these agents. More studies are needed in these areas to further illuminate the therapeutic potential of epigenetic modifiers and miRNAs.

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