Short Conceptual Overview

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Epigenetic regulation mechanisms of microRNA expression

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Abstract: MicroRNAs (miRNAs) are single-stranded RNAs of 18–25 nucleotides that regulate gene expression at the post-transcriptional level. They are involved in many physiological and pathological processes, including cell proliferation, apoptosis, development and carcinogenesis. Because of the central role of miRNAs in the regulation of gene expression, their expression needs to be tightly controlled. Here, we summarize the different mechanisms of epigenetic regulation of miRNAs, with a particular focus on DNA methylation and histone modification.

Keywords: CpG islands; DNA methylation; epigenetic regulation; histone modification; microRNAs.

Introduction

Epigenetic mechanisms are heritable changes in gene expression that occur in a cell without any modifications in the DNA sequence, indicating a change in the phenotype without any change in the genotype (1, 2). Epigenetic modifications can be mediated by three major mechanisms: DNA methylation, histone modifications and regulation by non-coding RNAs.

MicroRNAs (miRNAs) are non-coding, single-stranded RNAs of 18–25 nucleotides that generally bind to the 3′ untranslated regions (UTRs) of messenger RNAs (mRNAs) to suppress protein translation or cause mRNA degradation (3). An inverse function has also been described for miRNAs whereby they can increase the translation of specific targets. Although the underlying mechanism of this function is poorly understood, it is known that the miRNA binds to the 5′UTR region of the mRNA (3, 4). miRNAs have been classified by most authors as epigenetic modulators as they affect the protein levels of the target mRNAs without modifying their DNA sequence (5). Moreover, miRNAs can also be the target of epigenetic modifications that modulate their transcription levels (6–9), which will be the main focus of this review.

The biogenesis of miRNAs involves an initial process of precursor molecules in the nucleus (10) that ends in the cytoplasm with the formation of a mature miRNA. First, RNA polymerase II or III transcribes miRNA genes as large RNA molecules, named primary miRNAs (pri-miRNAs). The pri-miRNAs fold into hairpins, which are recognized by a microprocessor complex that contains an RNA-binding protein DGCR8 and RNase III Drosha (11). This complex cleaves the pri-miRNA and generates a precursor miRNA (pre-miRNA) of ~70 bp, which is exported to the cytoplasm by the complex Exportin 5-Ran-GTP. Once in the cytoplasm, the RNase III Dicer cuts the pre-miRNA into a form of ~20 bp miRNA/miRNA* coupled pair (12, 13). One strand represents the 5′ miRNA, whereas the other strand represents the 3′ miRNA. The last step of the process requires the participation of the RNA-induced silencing complex (RISC), which can unwind both the strands. In most cases, one of the strands will be degraded while the other will be incorporated into the RISC and guide it to its mRNA target (14).

In the last decades, miRNAs have been postulated as important regulatory molecules in many cellular and developmental pathways (15, 16). Numerous studies have focused on the assessment of miRNA expression and have shown considerable changes in their expression profiles in various diseases (17–20), although the most remarkable changes have been observed in cancer (21–24). The results of these studies point to the profiling of miRNA expression as an important tool for the prognosis, diagnosis and treatment of diseases (18, 25, 26).

Because of the central role of miRNAs in the regulation of gene expression, their expression needs to be

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tightly controlled by epigenetic mechanisms. DNA modifications and post-translational modifications (PTMs) of histones have been the two most widely studied of these mechanisms (27). Here, we review these two mechanisms of epigenetic regulation of miRNAs.

DNA modifications

DNA methylation

The most frequent DNA modification involves the covalent binding of a methyl group to the cytosine bases located in CpG dinucleotides (cytosine nucleotide followed by a guanine nucleotide in the linear sequence of bases in 5′→3′ direction linked by a phosphate, 5′-C-phosphate-G-3′). This CpG is underrepresented in the genome, except for the small regions known as CpG islands – regions with a minimum length of 0.5 kb with a higher proportion of CpGs (minimum 55% GC). CpG islands are mainly located in the proximal promoter regions of almost half of all human genes (28, 29). According to the UCSC human version GRCh37/hg19, 28 691 CpG islands are scattered throughout the genome. Interestingly, chromosome 19 is the one with the highest number of CpG islands (n = 2541) while chromosome Y harbors the lowest number (n = 181). A feasible explanation for this may lie in the fact that chromosome 19 is rich in biological and evolutionary significance with the highest gene density of all human chromosomes. In addition, the GC content of the chromosome is unusually high (48% vs. 41% of the median whole human genome) with two-thirds of genes having at least one CpG island (30). In contrast, chromosome Y has evolved by a gene loss leading to a paucity of genes, which correlates with the paucity of CpG islands (31).

DNA methylation is the major modification of eukaryotic genomes related to downregulation of gene expression. The overall methylation grade of CpGs in mammals can fluctuate at different developmental stages or under different pathological conditions. CpGs are mostly unmethylated at the early stages of development, allowing the expression of a particular gene if the appropriate transcription factors are present and the chromatin structure is accessible (32). When development progresses, DNA methylation helps to restrict embryonic lineages and prevent regression to an undifferentiated state (33). In non-embryonic cells, around 80% of the CpG residues that do not form a CpG island are methylated (34), while CpG islands are mostly unmethylated at all times (28).

In some pathological conditions, such as human cancers, two epigenetic events have been observed (35): global hypomethylation of the CpG residues that do not form a CpG island is linked to genomic instability, which may take the form of activation of mitotic recombination or activation of transposons (36, 37); and hypermethylation of promoter areas, especially CpG islands, associated with the corresponding silencing of tumor suppressor genes (38, 39). miRNA genes have been frequently found in the cancer-associated genomic regions (40) and can play two opposing roles in this disease, either as oncogenes or as tumor suppressors (24). Several studies have shown that tumor suppressor miRNAs, such as miR-203, can be silenced by aberrant methylation of CpG islands adjacent to their promoters (41, 42). miR-203 can be inactivated in human tumors by both genetic and epigenetic mechanisms. In hematopoietic malignancies, including chronic myelogenous leukemia, its methylation enhances ABL1 and BCR-ABL1 oncogene expression and its re-expression dramatically reduces the proliferation of tumor cells in an ABL1-dependent manner (43).

The frequency of human miRNA gene methylation is nearly one order of magnitude higher than that of the protein-encoding genes (44). To further shed light on this fact, we depicted the genomic distribution of human miRNAs (obtained from miRbase v21) (45) throughout the chromosomes and merged this chromosome map with the CpG island genomic density using karyoploteR (46) (Figure 1A). The results revealed the hotspots for miRNA methylation in chromosomes 1, 2, 9, 11, 16, 17 and 19, which are in accordance with a study by Kunej et al. (47). Among these chromosomes, chromosome 19 stands for which has the highest number of miRNAs overlapping a CpG island (n = 19). Remarkably, using regioneR (48), which assesses the relation between genomic regions using a permutation test, we observed that a higher proportion of miRNA genes than expected by chance is embedded in CpG islands susceptible to methylation (189 of 1881; z-score = 46.13; number of permutations = 1000; p-value = 0.0009, Figure 1B and C, Table 1 and shown in red in Figure 1A), which might explain why certain miRNAs are more prone to be epigenetically regulated by methylation than others. Furuta et al. (49) studied the methylation grade of CpG islands close to 39 miRNA genes in hepatocellular carcinoma cell lines and found that miR-124-1, miR-124-3, miR-203 and miR-375, which were completely embedded in a CpG island, underwent methylation-mediated silencing (Figure 2A).

miRNAs can be classified into two broad categories according to their genomic region: intergenic and intragenic (50, 51). Intergenic miRNAs (Figure 2A–C) are located

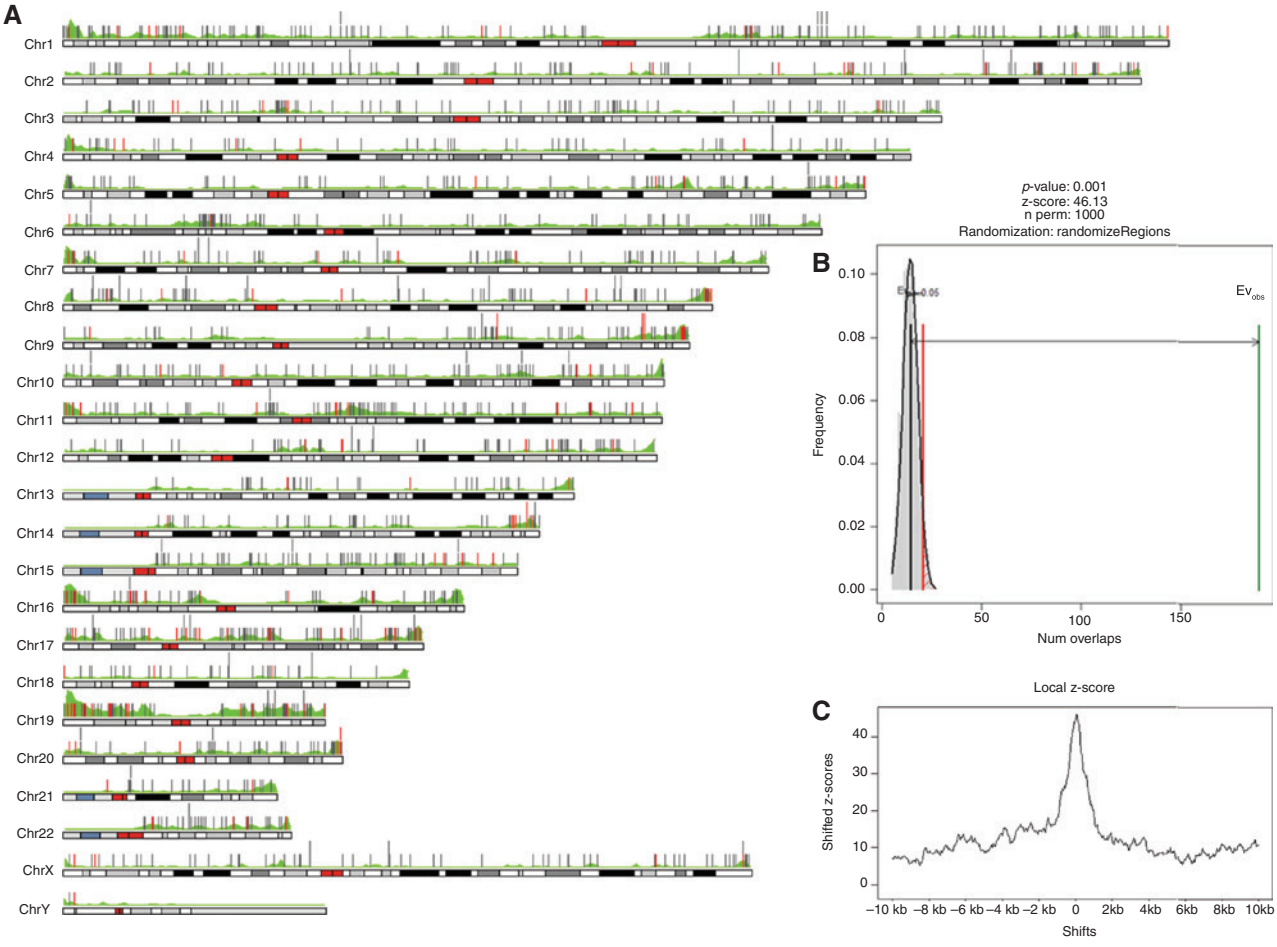

Figure 1: MicroRNAs overlapping CpG islands.
(A) MicroRNAs and CpG island distribution on different chromosomes. Chromosomes are represented as horizontal rectangles, in which red rectangles indicate the centromeres. Green density graph shows the distribution of CpG islands above chromosomes (UCSC human version GRCh37/hg19). Gray bars projected above the chromosomes indicate the genomic positions of the 1881 miRNAs (miRbase v21), 189 of which are depicted by red bars to highlight the miRNAs whose genomic position overlaps a CpG island. (B) Permutation test analysis (RegioneR, Bioconductor) showing association between miRNAs and CpG islands. The overlapPerm Test function was used for calculation with the following parameters: per.chromosome = FALSE and count.once = FALSE. (C) Local z-score graph to evaluate the strength of the association. The graph shows that the observed association is highly dependent on the exact position.

in the regions between annotated genes, while intragenic miRNAs (Figure 2D and E) are located within exons (exonic miRNAs) or introns (intronic miRNAs) of the protein-coding genes. In humans, according to the miRIAD database (52), a total of 1157 (61.5%) miRNAs are intragenic (169 exonic and 988 intronic), while 724 (38.5%) are intergenic.

The transcription of intergenic miRNAs is independent of coding genes as they are transcribed mostly by RNApol III (50, 51). They have their own transcription regulatory elements, including promoter, transcription start site (TSS) and terminator signals. Three patterns of epigenetic regulation of intergenic miRNAs have been observed. Firstly, as described above, a miRNA gene can directly overlap a CpG island, as is the case of miR-203 (49) (Figure 2A). Secondly, a miRNA can have a promoter region that is enriched in CpG residues but is not a CpG island, such as miR-34a, whose expression levels are modulated by methylation of the CpG residues located in the region comprising up to 2.5 kb of its TSS in its promoter (53) (Figure 2B). Finally, a miRNA can have a CpG island upstream of its TSS, such as the miR-17-92 cluster located in Chr13, which has a CpG island ~2 kb upstream of its TSS (54) (Figure 2C). Most of the CpG islands that are located close to the miRNA's TSS are involved in their transcription regulation. However, the maximum distance to the TSS from where a CpG island can regulate the miRNA transcription has not yet been defined and probably varies according to the size of each miRNA

Table 1: Pri-miRNAs overlapping a CpG island and their positions in chromosomes (Chr).

Chr.	n	miRNA genes overlapping a CpG island
chr1	11	mir-92b, mir-137, mir-760, mir-3124, mir-3917, mir-4632, mir-4695, mir-6084, mir-6727, mir-7846, mir-8083
chr2	13	mir-10b, mir-149, mir-375, mir-663b, mir-933, mir-4444-1, mir-4757, mir-4783, mir-4785, mir-5001, mir-5703, mir-7704, mir-7845
chr3	7	mir-564, mir-1224, mir-4442, mir-4787, mir-4792, mir-5787, mir-6872
chr4	6	mir-572, mir-574, mir-943, mir-4449, mir-4453, mir-5091
chr5	5	mir-2277, mir-3655, mir-3661, mir-4634, mir-4638
chr6	2	mir-219a-1, mir-6720
chr7	8	mir-196b, mir-339, mir-3147, mir-4285, mir-4285, mir-4285, mir-4651, mir-6836
chr8	9	mir-124-1, mir-320a, mir-937, mir-939, mir-3610, mir-4469, mir-4470, mir-4664, mir-6850
chr9	11	mir-24-1, mir-126, mir-219a-2, mir-219b, mir-2861, mir-3074, mir-3621, mir-3960, mir-4479, mir-4665, mir-4674
chr10	4	mir-1915, mir-2110, mir-3663, mir-4683
chr11	12	mir-34b, mir-210, mir-611, mir-675, mir-1237, mir-129-2, mir-1908, mir-3656, mir-4488, mir-4492, mir-4687, mir-6090
chr12	6	let-7i, mir-615, mir-3652, mir-4497, mir-6125, mir-8072
chr13	3	mir-3613, mir-3665, mir-8075
chr14	10	mir-127, mir-203a, mir-203b, mir-369, mir-409, mir-410, mir-412, mir-1247, mir-4707, mir-6765
chr15	5	mir-9-3, mir-1469, mir-3175, mir-4515, mir-7706,
chr16	16	mir-1225, mir-1538, mir-3176, mir-3178, mir-3180-1, mir-3180-2, mir-3180-3, mir-3180-4, mir-3180-5, mir-3181, mir-4519, mir-6511a-1, mir-6511a-2, mir-6511a-4, mir-6511b-1, mir-6511b-2
chr17	17	mir-132, mir-152, mir-193a, mir-212, mir-632, mir-636, mir-1180, mir-3185, mir-3615, mir-4522, mir-4523, mir-4734, mir-4737, mir-4740, mir-6080, mir-6776, mir-6787
chr18	3	mir-1539, mir-4741, mir-8078
chr19	19	mir-638, mir-639, mir-935, mir-1181, mir-1199, mir-1909, mir-3187, mir-4321, mir-4322, mir-4323, mir-4530, mir-4745, mir-4746, mir-4747, mir-4750, mir-4754, mir-6789, mir-6790, mir-7108
chr20	9	mir-124-3, mir-663a, mir-941-1, mir-941-2, mir-941-3, mir-941-4, mir-941-5, mir-1292, mir-6869
chr21	3	mir-3197, mir-3648-2, mir-3687-2
chr22	4	mir-658, mir-1281, mir-1306, mir-6821
chrX	5	mir-424, mir-503, mir-718, mir-4767, mir-6089-1
chrY	1	mir-6089-2

promoter. Our group performed an *in silico* analysis in order to determine the distances between pri-miRNAs and their nearest CpG islands in a range of distances from 1 to 10 000 bp (Figure 3). Like most authors, we considered only the CpG islands located at maximum 2000 bp away from the TSS of the analyzed gene. Among 1881 miRNAs analyzed, 116 had a CpG island within 1000 pb upstream of their promoters, 51 (44%) of which were intergenic miRNAs, and 80 miRNAs had a CpG island within 1001–2000 pb, 23 (29%) of which were intergenic.

Conversely, intragenic miRNAs, located in the same DNA strand and coexpressed with their host genes, are usually transcribed by RNApol II (55) (Figure 2D). However, some studies have found a low level of correlation between the expression of a miRNA and that of its host gene (56, 57), which is not what might be expected if their transcription is regulated by the same promoter. A possible explanation, as proposed by Ozsolak et al. (58) and Monteys et al. (59), could be that a considerable percentage of intronic miRNAs [~35%, using miRBasev9 (58) and v12.0 (59)] are associated with Pol II or Pol III promoters that could drive transcription independently of their host gene promoter (Figure 2E). More recently, Marsico et al. (60), using their ProMIRNA tool (http://promirna.molgen.mpg.de), detected up to 50% of intragenic miRNAs regulated by their own independent promoters (mirBase v 18.1). These authors also performed an analysis to identify the unique features of intronic miRNA promoters, such as miR-126 (Figure 2E). The results showed that (1) the CpG content changed according to the promoter type, where the intronic independent miRNA promoters were less enriched in CpGs than the host gene promoters; (2) intronic promoters were usually narrow and enriched in TATA box elements; and (3) intronic promoters tended to be regulated by a different set of transcription factors enriched in tissue-specific master regulators. The authors concluded that the intronic independent miRNA promoters may modulate miRNA expression in a tissue or cellular state-specific manner and that the observed differences in the regulatory elements point toward a different evolution of the two promoter classes.

Although most studies analyzing the role of methylation in miRNA regulation have focused on promoter

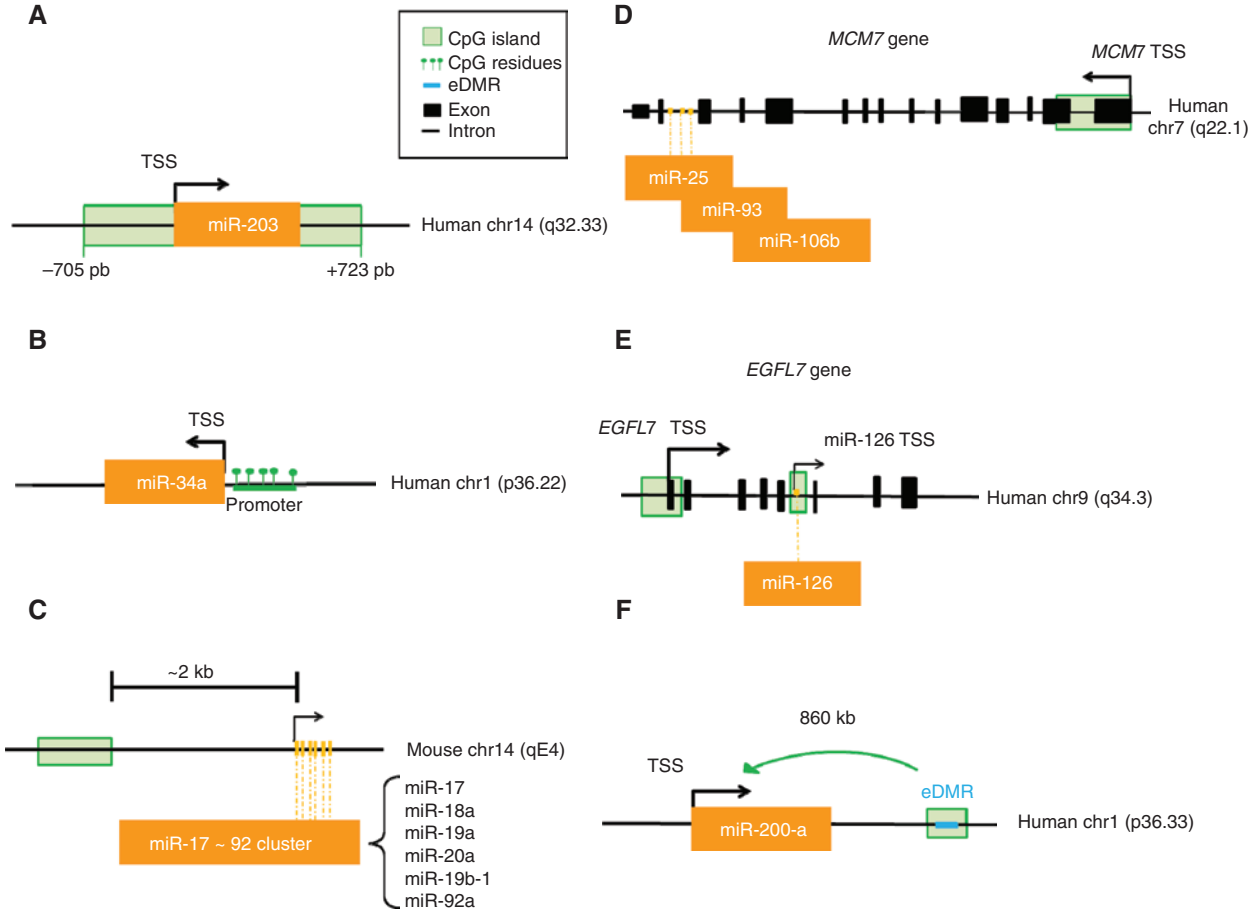

Figure 2: Summary of the different mechanisms involved in the methylation-dependent downregulation of the miRNA genes described in several cell lines, murine models and tumor tissues.
(A) miRNA gene overlapping a CpG island. (B) miRNA with a promoter region enriched in CpG residues susceptible to methylation. (C) miRNA cluster with a CpG island 2 kb upstream of its transcription start site (TSS). (D) Intronic miRNA whose transcription is regulated by the CpG island located in the promoter of the host gene. (E) Intronic miRNA whose transcription is independent of the host gene but is dependent on its own independent promoter. (F) miRNA whose expression is regulated by a distant enhancer that can be methylated. (eDMR, enhancer differential methylated region).

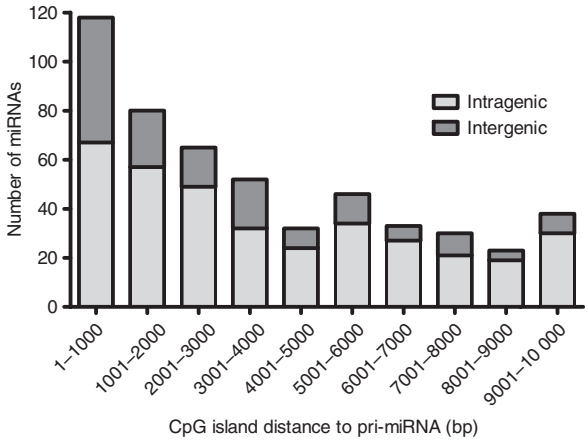

Figure 3: Number of miRNAs with an upstream CpG island located close to the gene.
A range of distances (1–10 000 bp) is shown and the number of intragenic and intergenic miRNAs is indicated for each distance.

regions and CpG islands, there is some evidence that methylation of distant regions of the genome, such as enhancers, can also modulate miRNA expression, as is the case of miR-200a/b and miR-9-1 (61) (Figure 2F). miR-9-1, in uterine and head and neck cancers, and miR-200a/b, in breast and colon cancer, respectively, are upregulated as a consequence of the hypomethylation of an enhancer differential methylated region (eDMR). In both cases, their functions involve E-cadherin and the epithelial-mesenchymal transition that is crucial for metastasis (62, 63). Methylation has the same effect on enhancers as on CpG islands, inhibiting their activity. Recently, it has been observed that CpG islands located far from any known transcript, known as orphan CpG islands, can display features of active enhancers with the presence of chromatin features H3K4me1 and H3K27Ac (64).

DNA hydroxymethylation

DNA hydroxymethylation is a recently discovered modification of CpG dinucleotides that involves the addition of a hydroxyl group on 5-methylcytosines (5mCs) to produce 5-hydroxymethylcytosine (5hmCs) (65, 66). This modification, mediated by the TET family of proteins (Tet1, Tet2, Tet3), is an intermediate step in the process of active demethylation of 5mCs, and as such, it is responsible for enhancing transcriptional efficiency (67) and is essential in a range of biological processes, such as embryonic development, stem cell function and cancer formation (68, 69).

The 5hmC marks are found at reduced levels (~1%) compared to the 5mC levels (4–5%) in the human genome. However, like 5mC alterations, the 5hmC patterns undergo considerable changes linked to genome instability (70, 71) across several forms of human cancers (72). Recent studies revealed that 5hmC is consistently found at significantly reduced levels (more than 50% reduction, $p \leq 0.01$) in various solid tumors (73–75). Recently, using a mouse model, Pan et al. (76) studied the role of hydroxymethylation in neurophysiological processes and provided the first evidence that miRNAs can be regulated by hydroxymethylation of their promoters. Working with a mouse model of formalin-induced acute inflammatory pain, they observed a significant increase in 5hmC and a decrease in 5mC in the miR-365-3p promoter that resulted in the enhancement of miR-365-3p transcription and in the subsequent increase of miR-365-3p expression (Figure 4). The TET-mediated hydroxymethylation of miR-365-3p played a key role in the regulation of nociceptive behavior through targeting the potassium channel KCNH2. This epigenetic modification in the nociceptive pathway contributes to pain processes and analgesia response. To date, the effect of hydroxymethylation of miRNAs has not been explored in humans, but given the findings in mouse models, it seems to warrant investigation in humans as well.

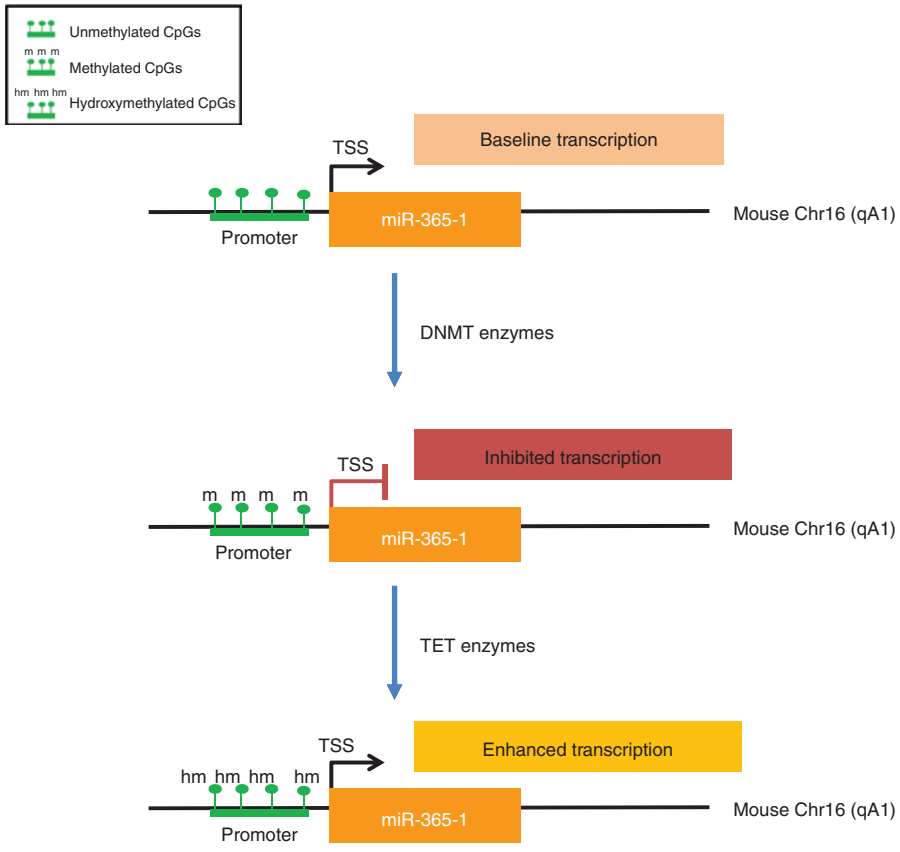

Figure 4: Hydroxymethylation (hm) of the miR-365-1 promoter enhances its transcription in mice.
When the promoter is unmethylated, the miRNA shows a baseline transcription that can be inhibited by the methylation (m) of the promoter by DNMT enzymes. The intermediate step in the demethylation of the promoter, mediated by the TET enzymes, produces a hm of the promoter that leads to enhanced transcription of the miRNA. DNMT, DNA methyl transferase; TSS, transcription start site.

PTM of histones

Repression of gene transcription can be associated with PTM in the amino terminal coils of histones, which leads to chromatin modifications impacting gene expression, usually by altering the chromatin structure. Chromatin is integrated by nucleosomes, which consist of DNA wrapped twice around a histone octamer, each containing two copies of four highly conserved histones (H2A, H2B, H3, H4), which are susceptible to PTMs, including methylation, acetylation, phosphorylation, ubiquitination, biotinylation, sumoylation and ADP-ribosylation (77, 78). In fact, the most well-studied types of modifications are lysine acetylation and methylation. While lysine acetylation can relax the chromatin structure and enhance transcription activation, lysine methylation can cause different effects on gene expression, depending on the positions and degree of methylation. Methylation at H3K4, K36 and K79 is associated with gene activation, whereas methylation at H3K9, H3K27 and H4K20 correlates with transcriptional repression (77, 78).

The EpimiR database (79) is a comprehensive repository that includes all histone modifications affecting miRNA expression in different species, including humans, described until 2013. Table 2 lists all miRNAs whose expression is regulated by lysine acetylation or methylation, based on EpimiR information ('high confidence' criterion) and a review of the literature (January 2013–June 2017; references are shown in Supplementary File 1). Table 2 includes well-known oncogenic miRNAs, such as miR-21, which has been shown to play an oncogenic role in most tumors by blocking cell differentiation and by promoting proliferation. For example, Zhou et al. (80) demonstrated that RBP2, a histone 3 lysine 4 (H3K4) demethylase, downregulated miR-21 expression by reducing H3K4 trimethylation at the proximal promoter region of miR-21 in chronic myeloid leukemia cells. Table 2 also includes tumor suppressor miRNAs, such as miR-34a, which is known to inhibit cell proliferation and induce cell cycle arrest, apoptosis and senescence, as well as to increase chemosensitivity. EZH2, a histone 3 lysine 27 (H3K27) methyltransferase, is a major player in the silencing of miR-34a in pancreatic ductal adenocarcinoma. Inhibition of EZH2 upregulated miR-34a expression in pancreatic ductal adenocarcinoma cells, while EZH2 overexpression repressed miR-34a expression. EZH2 was specifically guided to the promoter region by HOTAIR, a long noncoding RNA, to catalyze H3K27 trimethylation of the miR-34a promoter. The authors concluded that the identification of HOTAIR-guided miR-34a silencing opened

Table 2: Different post-translational modifications (PTMs) of histones affecting miRNA genes and their subsequent effect on gene expression.

PTM	Effect on miRNA expression	miRNAs
H3ac	↑	let-7a-1, let-7d, let-7f-1, miR-9-1,miR-10a, miR-10b, miR-17, miR-18a,miR-19a, miR-19b, miR-20a, miR-22, miR-23a, miR-26a, miR-30a, miR-92, miR-124a, miR-126,miR-129-2, miR-130b, miR-137,miR-199a-2,miR-200a, miR-205,miR-223, miR-376a, miR-512-5p
H4ac	↑	let-7a-1, let-7d, let-7f-1, miR-23a, miR-26a, miR-29, miR-30a, miR-124a, miR-223
H3K4ac	↑	miR-29b
H3K9ac	↑	miR-21, miR-29b, miR-132, miR-139, miR-212,miR-224, miR-452
H3K14ac	↑	miR-21, miR-224, miR-452
H3K4me2	↑	miR-15a, miR-16, miR-29b, miR-132
H3K4me3	↑	let-7a-1, let-7d, let-7e, let-7f-1,miR-10a, miR-10b, miR-17, miR-18a, miR-19a,miR-19b, miR-20a, miR-21,miR-23a, miR-26a, miR-30a, miR-31, miR-92a-1, miR-150, miR-193b, miR-199a-2, miR-200b/200a/429, miR-200c/141, miR-205, miR-223, miR-365, miR-451
H3K9me2	↓	miR-9-3, miR-96, miR-182, miR-183, miR-199a-2, miR-205, miR-212, miR-302, miR-302c
H3K9me3	↓	miR-124a, miR-329, miR-622
H3K27me3	↓	let-7b, miR-9, miR-10a, miR-10b, miR-21, miR-22, miR-23a, miR-26a, miR-27a, miR-29, miR-31, miR-34a, miR-34b/c, miR-101, miR-124a, miR-139, miR-143, miR-150, miR-181a/b,miR-193b, miR-199b, miR-200b/200a/429, miR-200c/141, miR-205, miR-212, miR-223, miR-329, miR-365, miR-429, miR-449a,miR-449b, miR-451, miR-568, miR-622,miR-1275

All modifications included in this table are in lysines.
K, lysine; ac, acetylation; me, methylation; me2, dimethylation; me3, trimethylation.

a new avenue in miR-34a-oriented therapy against pancreatic ductal adenocarcinoma (81).

These findings highlight the relevance of the transcriptional regulation of miRNAs mediated by changes of the chromatin states through modifications in the methylation/acetylation patterns of amino terminal coils of histones. Taken together, they suggest new avenues of research on drugs targeting genes involved in the PTM of histones that could affect miRNA expression.

Conclusions

A notable number of miRNA genes are epigenetically regulated and among the epigenetic mechanisms involved, DNA methylation and PTM of histones have been the most widely studied. miRNA genes can be silenced following different DNA methylation patterns that in most cases involve CpG islands located in the promoter regions. Moreover, an important number of miRNAs are embedded in a CpG island susceptible to methylation, which will then inhibit the miRNA expression. Recent findings have highlighted the importance of orphan CpG islands located far from the miRNA genes that act as enhancers to modulate the miRNA expression. In addition, in the process of active demethylation of CpG residues, the intermediate products formed by TET enzymes are hydroxymethylated cytosines, which play a role as enhancers of the transcription of some miRNAs over their basal levels with unmethylated promoters. Through PTM of histones, miRNA expression can be subjected to either enhanced or repressed transcription activation depending on whether amino terminal coils of histones are acetylated or methylated.

All the mechanisms described here are usually deregulated in the pathological processes, including cancer. The epigenetic mechanisms underlying the regulation of miRNA expression provide a potential therapeutic target, as both CpG methylation and histone modifications can be pharmacologically modulated. Demethylating agents and histone deacetylase inhibitors are currently used in several diseases, especially in cancer, in order to reactivate the expression of epigenetically silenced genes that are key elements of pathogenesis. Several studies have shown that treatment with epigenetic agents is effective, as it leads to reexpressing epigenetically silenced miRNAs with metastasis suppression activity. We can therefore consider miRNAs as potential 'druggable' targets for this type of therapies. However, we cannot ignore the fact that one miRNA can regulate several target genes and multiple cellular processes and modulating a miRNA with an epigenetic agent may well affect several different pathways with unforeseen effects.

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List of abbreviations

eDMR	Enhancer differential methylated region
5hmCs	5-hydroxymethylcitosines
5mCs	5-methylcytosines
miRNAs	MicroRNAs
mRNA	Messenger RNA
pre-miRNA	Precursor miRNA
pri-miRNAs	Primary microRNAs
PTM	Post-translational modification
RISC	RNA-induced silencing complex
TSS	Transcription start site
UTR	Untranslated region

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