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Epigenetic Alterations and MicroRNA Misexpression in Cancer and Autoimmune Diseases: a Critical Review

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Abstract

Epigenetic markers such as DNA methylation and histone modifications around promoter regions modify chromatin structure and regulate expression of downstream genes. In fact, aberrant epigenetic modifications are common events in human disease including tumorigenesis and autoimmunity. Small non-coding RNAs named microRNAs (miRNAs) are modulators of gene expression and play critical roles in various cellular processes. Several miRNAs have been characterized as tumor suppressors or oncogenes in cancer, and recent reports implicate certain miRNAs in the pathogenesis of autoimmune diseases. Epigenetic investigations have shown that distinct miRNAs are directly regulated by DNA methylation and histone modifications at their promoters. Moreover, miRNAs themselves are key participants in regulating the chromatin modifying machinery. Chromatin-modifying drugs such as DNA methylation inhibitors and histone deacetylase inhibitors have shown efficacy in human malignancies and there is some evidence that these drugs may be useful in autoimmune disease. The benefits of these drugs are at least partially mediated by restoring expression of epigenetically silenced tumor suppressor genes, including miRNAs. The complex layers regulating gene expression have yet to be fully elucidated, but it is clear that epigenetic alterations and miRNA misexpression are essential events in pathologic processes, especially cancer and autoimmune disease, and represent promising therapeutic targets.

Keywords

Cancer; Autoimmunity; Epigenetics; MicroRNA; Methylation

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DNA Methylation and Histone Modifications

Epigenetic alterations such as DNA methylation and histone modification are critical in chromatin remodeling and regulation of gene expression in mammalian development [1]. Aberrant DNA methylation is one of the most consistent epigenetic changes in human cancers [2]. DNA methylation patterns are generated and heritably maintained by three active DNA methyltransferases DNMT1, DNMT3A, and DNMT3B. The de novo methyltransferases DNMT3A and DNMT3B act independently of replication and show equal preference for both unmethylated and hemimethylated DNA, whereas the maintenance DNA methyltransferase DNMT1 acts during replication and preferentially methylates hemimethylated DNA [3]. DNA hypermethylation refers principally to the gain of methylation at specific sites that are unmethylated under normal conditions. This aberrant methylation occurs mainly in short CpG-rich DNA stretches called CpG islands. DNA methylation can lead to gene silencing by either preventing or promoting the recruitment of regulatory proteins to DNA. It can provide binding sites for methyl-binding domain proteins, which can mediate gene repression through interactions with histone deacetylases (HDACs). This phenomenon of aberrant promoter CpG island hypermethylation is associated with the stabilization of transcriptional repression and loss of gene function, and often occurs in tumor suppressor genes. In contrast, DNA hypomethylation is associated mainly with the loss of DNA methylation in genome-wide repeat regions. DNA hypomethylation has been reported in several tumor types, such as colorectal and gastric cancers, and occurs in many gene-poor genomic areas, including repetitive elements, retrotransposons, and introns, where it leads to genomic instability [4].

The N-terminal tails of histones can undergo a variety of posttranslational modifications including methylation and acetylation on specific residues. These histone modifications regulate transcription of genes that play important roles in cellular processes. Unlike DNA methylation, histone modifications can lead to either activation or repression depending upon which residues are modified and the type of modifications present. For example, tri-methylation of lysine 4 on histone H3 (H3K4) is enriched at transcriptionally active gene promoters [5], whereas di-and tri-methylation of H3K9 and tri-methylation of H3K27 is present at gene promoters that are transcriptionally repressed [6, 7]. As shown in Fig. 1, transcriptionally active chromatin in normal cells is characterized by acetylation of histone H3 and tri-methylation of H3K4. Epigenetic silencing of tumor suppressor genes during carcinogenesis is generally mediated by two distinct histone modifications: methylation of H3K9 and tri-methylation of H3K27. The Polycomb Repressive Complex 2 (PRC2) mediates epigenetic gene silencing by tri-methylating H3K27. Methylation of H3K9 works in concert with DNA methylation, whereas tri-methylation of H3K27 occurs independently of DNA methylation [8]. HDAC induces deacetylation of histone H3 in both of these pathways of epigenetic silencing.

Biogenesis of MicroRNA (miRNA)

MicroRNAs (miRNAs) are ~22 nucleotide (nt) non-coding RNAs that usually post-transcriptionally downregulate expression of various target genes. Currently, ~1,500

miRNAs have been identified in the human genome, each of which potentially controls hundreds of target genes and therefore play important roles in cell proliferation, apoptosis, differentiation, and tumorigenesis [9, 10]. As shown in Fig. 2, miRNA genes are generally transcribed from transcription start sites (TSSs) by RNA polymerase II (pol II) to form primary transcripts (pri-miRNAs). Pol II-transcribed pri-miRNAs are capped with 7-methylguanosine and are polyadenylated. The nuclear RNase III enzyme Drosha and its co-factor DGCR8 process pri-miRNAs into ~60-nt precursor miRNAs (pre-miRNAs), which form an imperfect stem-loop structure. Pre-miRNAs are transported into the cytoplasm by exportin 5 and are subsequently cleaved by Dicer into mature miRNAs, which are then loaded into the RNA-induced silencing complex (RISC). The miRNA/RISC complex downregulates specific gene products by translational repression via binding to partially complementary sequences in the 3′-untranslated regions (UTRs) of the target mRNAs or by directing mRNA degradation via binding to perfectly complementary sequences [11].

Epigenetic Regulation of miRNAs in Cancer

Because miRNAs can have large-scale effects through regulation of a variety of target genes, understanding the regulatory mechanisms controlling miRNA expression is important. Many miRNAs are expressed in a tissue and tumor-specific manner, implying that some miRNAs are under epigenetic control. We reported that ~5 % of human miRNAs are upregulated more than 3-fold by treatment of T24 bladder cancer cells with the DNA demethylating agent 5-aza-2′-deoxycytidine (5-Aza-CdR) and the histone deacetylase (HDAC) inhibitor 4-phenylbutyric acid (PBA). In particular, *miR-127*, which is embedded in a CpG island, was remarkably induced by a decrease in DNA methylation and an increase in active histone marks around the promoter region of the *miR-127* gene. In addition, activation of *miR-127* by epigenetic treatment resulted in the downregulation of its target oncogene BCL6 [12]. We also demonstrated that treatment of gastric cancer cells with 5-Aza-CdR and PBA induced activation of *miR-512-5p* which is located at Alu repeats on chromosome 19. Activation of *miR-512-5p* by epigenetic treatment caused suppression of *MCL1*, resulting in apoptosis of gastric cancer cells [13]. These results indicate that chromatin remodeling by epigenetic treatment directly activates specific miRNA expression.

Lujambio et al. [14] compared miRNA expression profiling between the wild-type HCT116 colon cancer cell line and HCT116 after genetic disruption of both *DNMT1* and *DNMT3b* (DKO cells). They found that 18 out of 320 miRNAs are significantly upregulated in DKO cells. In particular, *miR-124a* is silenced by its own CpG island hypermethylation in human tumors, but can be activated by inhibition of DNA methylation. They also demonstrated that the oncogene *CDK6* is a target of *miR-124a* and that epigenetic silencing of *miR-124a* in cancer cells modulates *CDK6* activity. Brueckner et al. [15] showed that methylation of the *let-7a-3* gene, which is embedded in a CpG island, is cooperatively maintained by *DNMT1* and *DNMT3B*. The *let-7a-3* gene is heavily methylated in normal human tissues, but hypomethylated in lung adenocarcinomas. DNA hypomethylation causes increased expression of *let-7a-3* leading to oncogenic changes. Recent studies have reported that *miR-9-1* and *miR-9-3* are potential tumor suppressor miRNAs and are inactivated by epigenetic mechanisms in human cancers [16, 17]. *MiR-34a* was identified as a target of p53 and induces cell cycle arrest, senescence and apoptosis [18, 19]. *MiR-34a* expression is

silenced in several types of cancer due to aberrant CpG methylation of its promoter. Re-expression of *miR-34a* induced senescence and cell cycle arrest in pancreatic cancer cells, at least in part by targeting *CDK6*, indicating that *miR-34a* represents a tumor suppressor gene, which is inactivated by CpG methylation in pancreatic cancer [20]. *MiR-34b* and *miR-34c* are also silenced by aberrant CpG island methylation in colorectal cancer [21]. *MiR-1* expression is markedly reduced by aberrant CpG island methylation in primary human hepatocellular carcinomas (HCCs) compared with matching liver tissues. Reactivation of *miR-1* by the DNA methylation inhibitor 5-azacytidine (5-AzaC) with downregulation of its target genes *FoxP1*, *MET* and *HDAC4* suppressed proliferation of HCC cells [22]. As summarized in Table 1, we and other groups have reported that specific miRNAs can be directly regulated from their own promoters by epigenetic alterations induced by chromatin modifying drugs or by genetic disruption of key DNMTs in cancer.

Some miRNAs reside within the intronic regions of either coding or non-coding transcription units [23]. It is believed that intronic miRNAs are coordinately expressed with their host gene mRNA [23]. Kim et al. [24] revealed the processing mechanism for intronic miRNAs, in which intronic miRNAs are processed from unspliced intronic regions before splicing catalysis, indicating that both intronic miRNAs and their host genes are generated from a common transcript. This suggests the expression of intronic miRNAs depends on the regulation of their host genes. We have found that the tumor suppressor *miR-126*, which is located within an intron of the *EGFL7*gene, is downregulated in human cancer cell lines and in primary bladder and prostate tumors. *MiR-126* and one of the transcripts of *EGFL7* that has a CpG island promoter are concomitantly upregulated in cancer cell lines by inhibitors of DNA methylation and histone deacetylation. These findings suggest that epigenetic changes can control expression of tumor suppressor intronic miRNAs by directly controlling their host genes [25]. *MiR-342* is also located within an intron of the *EVL* gene, and is silenced in colorectal cancer. The expression of *miR-342* is coordinated with the expression of *EVL* by CpG island methylation upstream of *EVL*. This supports a mechanism for silencing intronic miRNAs in cancer by epigenetic alterations of cognate host genes [26]. These findings indicate that epigenetic treatment activates miRNA expression in two ways: by directly activating miRNAs such as *miR-127* from their own promoters and/or by activating intronic miRNAs such as *miR-126* together with their host genes (Fig. 3).

Chromatin-Modifying Factors Regulated by miRNAs in Cancer

Recent studies have reported that miRNAs directly regulate key chromatin-modifying factors such as *DNMT1*, *DNMT3A*, *DNMT3B*, and *EZH2*, suggesting that these miRNAs have important roles in the epigenetic control of gene expression (Table 2). *MiR-152* is downregulated in human cancers, including HCC and endometrial cancer, and targets *DNMT1*[27, 28]. In fact, *miR-152* may act as a tumor suppressor via suppression of *DNMT1*. Fabbri et al. showed that the *miR-29* family target *DNMT3A* and *DNMT3B*, and expression levels of the *miR-29* family were suppressed in lung cancer. The reduced expression of the *miR-29* family induced overexpression of *DNMT3A* and *DNMT3B*, resulting in aberrant DNA methylation in lung cancer [29]. PRC1 and PRC2-mediated epigenetic regulation is critical for maintaining homeostasis [30]. PRC2 mediates epigenetic gene silencing by trimethylation of H3K27 and aberrantly silences tumor suppressor genes in cancer. *EZH2*,

the catalytic subunit of PRC2, enhances tumorigenesis and is commonly overexpressed in several types of cancers. *MiR-101* is downregulated in bladder cancer, and *miR-101* directly represses *EZH2*. This suggests that abnormal downregulation of *miR-101* could lead to the overexpression of *EZH2* frequently seen in cancer. *MiR-101* may be a potent tumor suppressor by altering global chromatin structure through repression of *EZH2* [31, 32]. Cao et al. have identified several miRNAs such as *miR-181a, miR-181b, miR-200b, miR-200c*, and *miR-203* that are repressed by *EZH2*. These miRNAs, in turn, regulate the expression of PRC1 proteins *BMI1* and *RING2*, indicating a coordinate regulation of PRC1 and PRC2 activities that is mediated by miRNAs [33].

Epigenetics and Autoimmune Diseases

Autoimmune diseases are complex, display a spectrum of severity, and their etiology is poorly understood. In addition, there are many different cell types that may participate in autoimmune pathogenesis, which complicates the elucidation of distinct molecular mechanisms for disease initiation and progression. Despite the difficulties in studying autoimmune disease, reports have isolated distinct cell populations in specific diseases and have shown clear molecular alterations that likely contribute to pathogenesis. Although there is a genetic predisposition to developing autoimmune disease, the story does not stop there. This is highlighted by the discordance in monozygotic twins for some autoimmune diseases being as high as 85 % (in rheumatoid arthritis). Clearly, in many cases of aberrant autoimmunity, sequence independent changes in gene regulation are most important [34].

The major pathologic phenomena of rheumatoid arthritis (RA) are cartilage and bone damage from synovial hyperplasia caused by elevated levels of inflammatory cytokines produced by activated B and T cells, amongst other cell types. Therefore, studies have primarily focused on synovial fibroblasts (SF) and lymphocytes. RA synovial fibroblasts (RASF) differ in both morphology and gene expression from their healthy tissue counterparts. A role for DNA hypomethylation in the pathogenesis of RA was first suggested by the aberrant expression of L1 elements in RA tissues. L1 are normally silenced retrotransposoms, which were induced by treatment of normal SFs with 5-Aza-CdR [35]. Moreover, global hypomethylation was observed in RASF along with a decrease in DNMT1, and normal fibroblasts treated with 5-AzaC acquired an activated phenotype [36]. There is also evidence of epigenetically upregulated markers of inflammation in RA. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that correlates with RA disease activity, and was hypomethylated with corresponding upregulated mRNA levels in peripheral blood mononuclear cells (PBMC) from RA patients [37].

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease resulting from widespread tissue damage caused by autoreactive T lymphocytes and the production of autoantibodies. A decrease in enzymatic activity of DNMTs is associated with defective DNA methylation and aberrant gene regulation in SLE. Specifically, T cells isolated from SLE patients had decreased DNA methylation, and normal T cells treated with 5-AzaC showed functional and phenotypic similarities to T cells from SLE patients [38–41]. In addition, white blood cells from monozygotic twin SLE patients had marked DNA hypomethylation when compared to their healthy siblings [42]. CD4+ T cell studies have

shown there is overexpression and hypomethylation of genes involved in signaling pathways associated with cell-cell adhesion, T cell proliferation and clonal expansion, and B cell IgG overproduction [43–46]. IL-6 is associated with SLE disease severity and a recent report noted hypomethylation of an IL-6 promoter in SLE B cells [47]. The study also showed that increased IL-6 in B cells decreased DNMT1 expression and led to impaired B cell receptor signaling, which is relevant to SLE pathogenesis.

miRNA Misexpression in Autoimmune Diseases

Several studies have shown that miRNAs are an integral part of regulating the immune response and immune cell development [48]. Therefore, it was expected that miRNAs were also involved in the pathogenesis of autoimmune diseases. Examining T cells from SLE patients revealed several dysregulated miRNAs. *MiR-21* and *miR-148a* were overexpressed in CD4+ T cells from patients with SLE, which contribute to DNA hypomethylation by repressing *DNMT1* expression (Table 2). This in turn leads to the overexpression of autoimmune-associated methylation-sensitive genes, such as *CD70* and *LFA-1*, via promoter demethylation. *MiR-148a* directly suppressed *DNMT1* expression by targeting the protein coding region of its transcript, whereas *miR-21* indirectly downregulated *DNMT1* expression by targeting an important autoimmune gene, *RASGRP1*, which mediated the Ras-MAPK pathway upstream of *DNMT1* [49]. Another report has shown that *miR-126* was upregulated and suppressed *DNMT1* as a direct target in SLE CD4+ T cells. Expression of the *miR-126* host gene *EGFL7* was also upregulated in CD4+ T cells from patients with SLE, possibly in a hypomethylation-dependent manner (Tables 1 and 2). Thus, *miR-126* regulates DNA methylation in CD4+ T cells and contributes to Tcell autoreactivity in SLE by directly targeting *DNMT1* [50]. These findings demonstrated a critical functional link between miRNAs and aberrant DNA hypomethylation in SLE CD4+ T cells. Inquiries into the functional significance of *miR-142-3p/5p* downregulation in SLE CD4+ T cells showed it directly suppressed members of the signaling lymphocytic activation molecule (SLAM) family, IL-10 and CD84, and SLAM-associated protein (SAP). Enforced expression of *miR-142-3p/5p* in SLE CD4+ T cells restored IL-10, CD84, and SAP levels, and decreased both T cell activity and IgG production, while inhibiting *miR-142-3p/5p* expression in normal CD4+ T cells induced a SLE-like phenotype. Studies of PBMCs in SLE patients revealed several miRNAs were misexpressed when compared to controls [51]. Specifically, *miR-146a*, which was inhibited in SLE, targeted key signaling proteins in the interferon pathway [52].

Expression levels of *miR-34a** were found to be reduced in RASFs compared to SFs from osteoarthritis (OA) patients, where levels of *miR-34a*, *miR-34b/b**, and *miR-34c/c** did not differ. The promoter of *miR-34a/34a** was methylated and transcription of the *miR-34a* duplex was induced upon treatment with demethylating agents. Enforced expression of *miR-34a** led to an increased rate of FasL- and TRAIL-mediated apoptosis in RASFs. Moreover *miR-34a** directly suppressed *X-linked inhibitor of apoptosis protein (XIAP)*, which was upregulated in RASF (Table 1). These findings suggest that a methylation-specific down-regulation of proapoptotic *miR-34a** in RASFs results in up-regulation of its direct target *XIAP*, thereby contributing to the resistance of RASFs to apoptosis [53].

Stanczyk et al. analyzed differential miRNA expression in RASFs treated with TNFα and found that *miR-146a* and *miR-155* were upregulated. Both of these miRNAs also had higher levels of expression than in OASFs. Functional studies showed that *miR-155* expression led to repression of matrix metalloproteinase (MMP)-1 and -3 [54]. A subsequent report from this group revealed that *miR-203* was upregulated in RASFs when compared to OASFs or healthy controls. Interestingly, *miR-203* levels in normal SFs did not change with TNFα or IL-1β treatment, but were increased by treatment with 5-azaC. Enforced *miR-203* expression increased levels of MMP-1 and IL-6, though the IL-6 induction was dependent on an intact NF-κB pathway [55]. Studies using synovial tissue (not RASF) from RA patients showed that *miR-146* was upregulated when compared to OA patients, and *miR-146* expression in RASFs was induced by TNFα and IL-1β [56]. When PBMCs of RA patients were analyzed, *miR-146a*, *miR-155*, *miR-132*, and *miR-16* were overexpressed compared to controls, showing similarities to the miRNA misexpression patterns seen in RASFs [57].

Altered miRNA expression has also been found in multiple sclerosis (MS). MS is a chronic, inflammatory, autoimmune disease characterized by demyelination from myelin-specific CD4+ T cells. Otaegui et al. found that *miR-18b* and *miR-599* were associated with relapse, while *miR-96* was associated with remission and the interleukin and WNT pathways [58]. Interleukin 17 (IL-17)-producing T helper cells (T(H)-17 cells) play an important role in autoimmune pathogenesis including MS. *MiR-326*, a T(H)-17 associated miRNA, levels correlated with disease severity, and *miR-326* promoted T(H)-17 differentiation by targeting Ets-1, a negative regulator of T(H)-17 differentiation [59].

Perspectives

A promising option for cancer treatment is the use of epigenetic drugs which inhibit tumor growth by several mechanisms including restoring the expression of epigenetically silenced tumor suppressor genes and miRNAs [60]. Inhibitors of DNA methylation and histone deacetylation can work synergistically to suppress growth of cancer cell lines both in vitro and in vivo. This effect may be caused by inducing cancer cells to differentiate, re-expressing aberrantly silenced tumor suppressor genes, and re-expressing tumor antigens which would aid immune surveillance [60]. Many epigenetic drugs have shown promising results in clinical trials for cancer [61, 62]. Applying these epigenetic drugs to autoimmune diseases such as SLE and RA, which show aberrant epigenetic alterations, may lead to clinical benefits. In fact, a HDACi was used in a clinical trial for juvenile idiopathic arthritis [63]. Another intriguing therapeutic avenue would enforce expression of multiple miRNAs that may act synergistically for a specific disease [64, 65]. Taken together, alterations in epigenetic marks and miRNAs are critical to the molecular mechanisms underlying carcinogenesis and autoimmunity, and further elucidating the complex layers of regulation may lead to novel treatments for these diseases.

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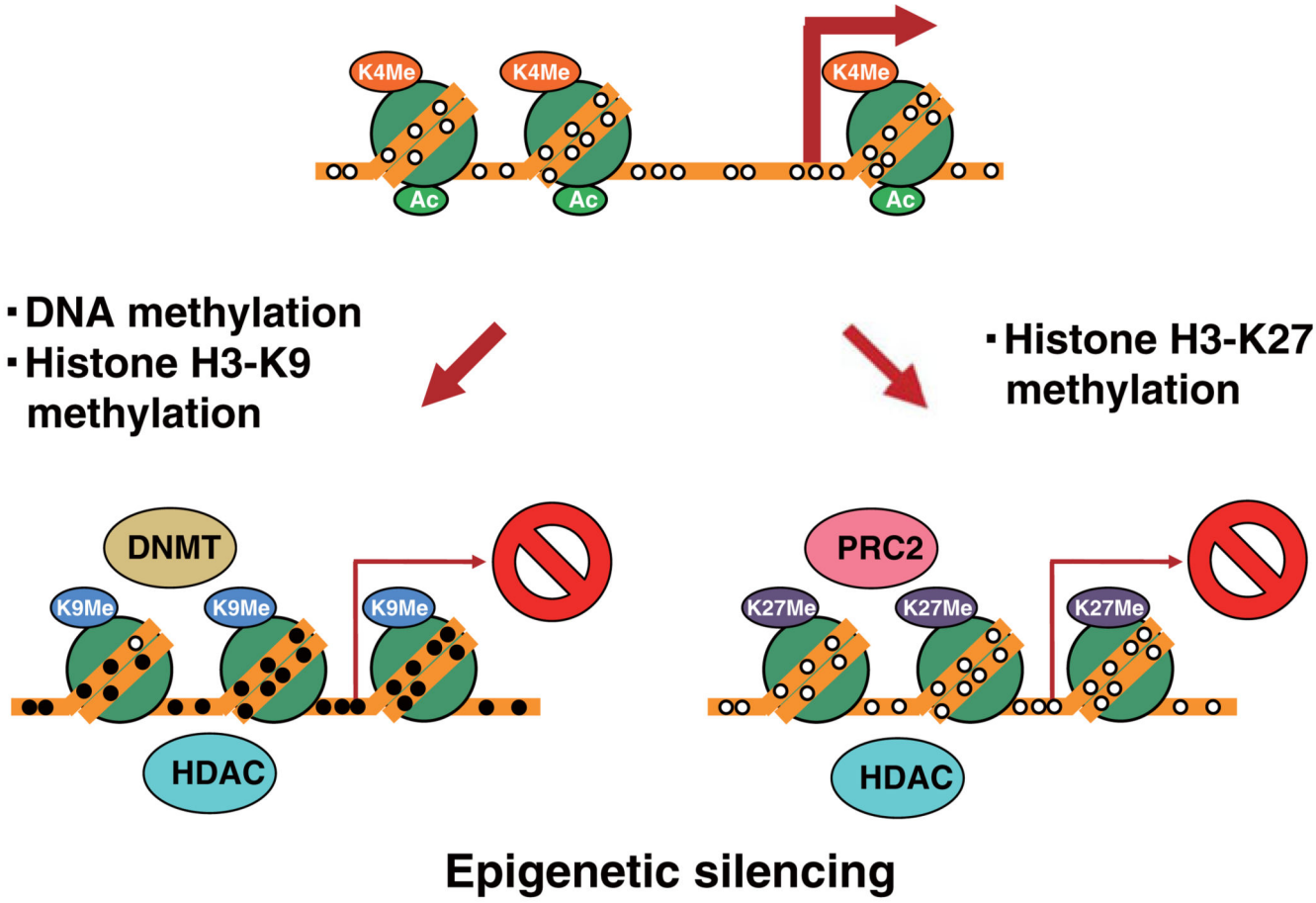

Fig. 1.

Epigenetic gene silencing in cancer cells. Transcriptionally active chromatin in normal cells is characterized by acetylation of histone H3 (*Ac*) and tri-methylation of H3K4 (*K4Me*). Epigenetic silencing of tumor suppressor genes during carcinogenesis is generally mediated by two distinct histone modifications: methylation of H3K9 (*K9Me*) and trimethylation of H3K27 (*K27Me*). PRC2 mediates epigenetic gene silencing by tri-methylating H3K27. Methylation of H3K9 works in concert with DNA methylation induced by DNMTs, whereas trimethylation of H3K27 works independent of DNA methylation. HDAC induces deacetylation of histone H3 in the both pathways of epigenetic silencing. *Open circle* unmethylated DNA; *filled circle* methylated DNA

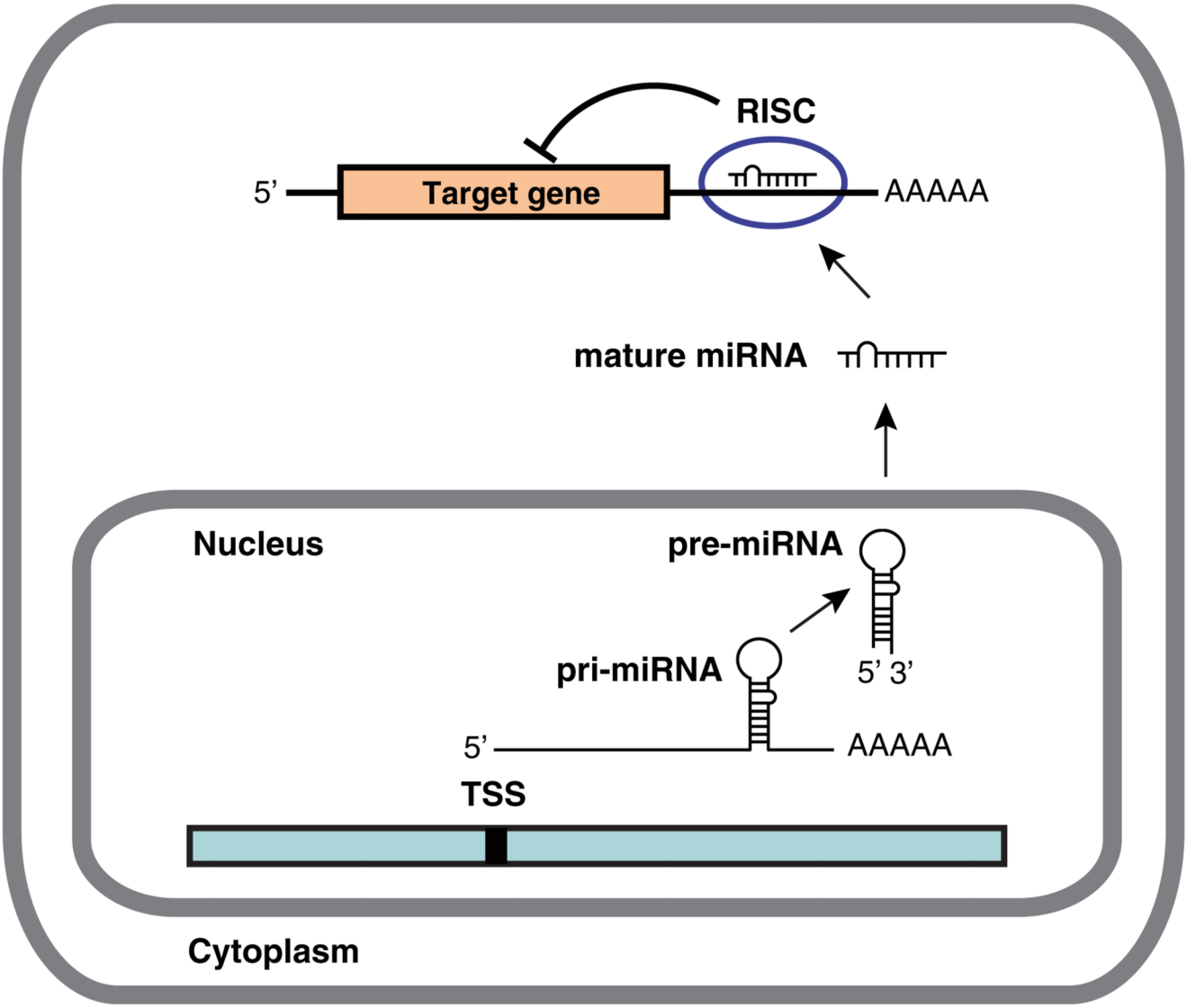

Fig. 2.

Biogenesis of miRNA. MiRNA genes are transcribed from TSS by RNA pol II to form pri-miRNAs which are capped with 7-methylguanosine and polyadenylated (*AAAAA*). Drosha and its co-factor DGCR8 process pri-miRNAs into pre-miRNAs. Pre-miRNAs are transported into the cytoplasm and are subsequently cleaved by Dicer into mature miRNAs. Mature miRNAs are then loaded into RISC, where miRNAs downregulate specific gene products by translational repression via binding to partially complementary sequences in the 3′ UTR of the target mRNAs or by directing mRNA degradation via binding to perfectly complementary sequences

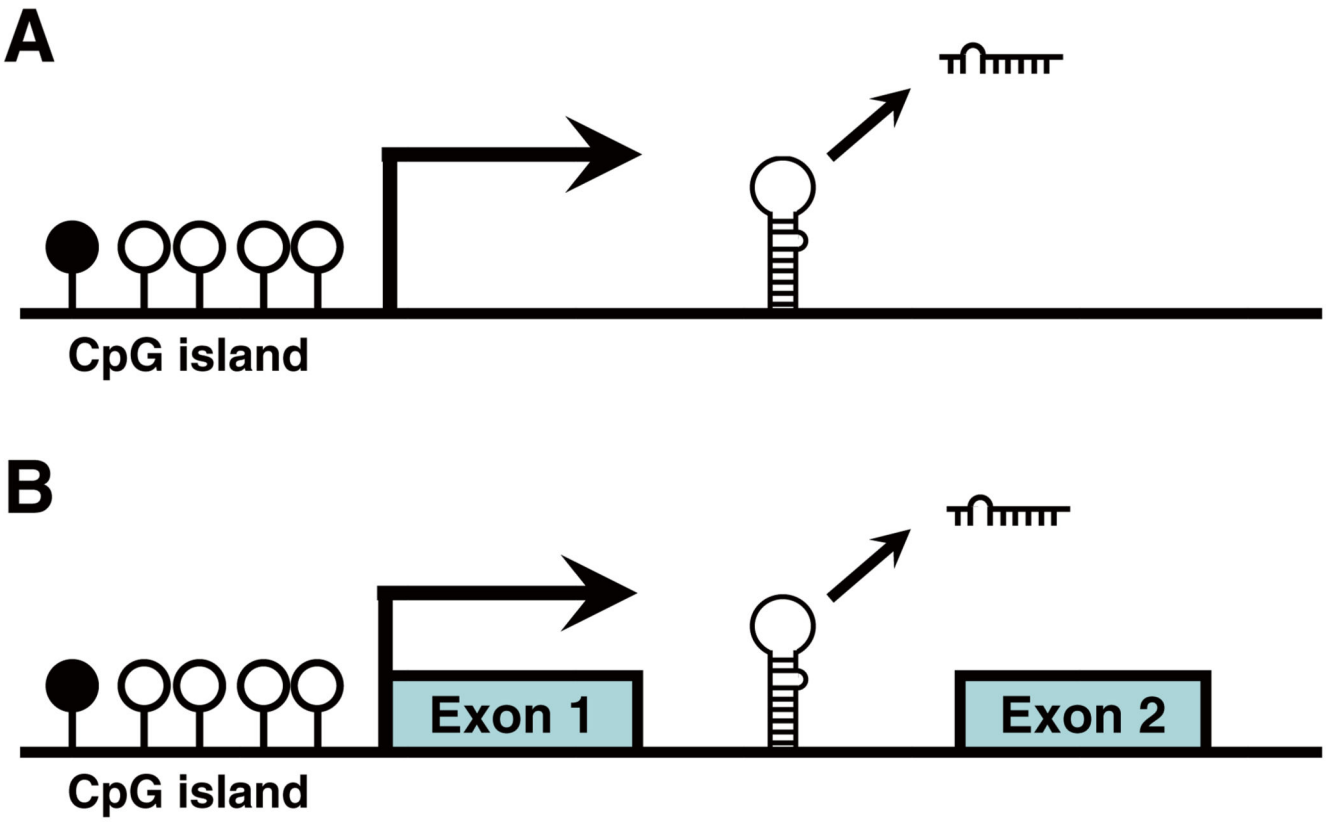

Fig. 3.

Epigenetic activation of miRNAs. Epigenetic treatment activates miRNA expression in two ways: by directly activating miRNAs such as *miR-127*from their own promoters (**a**) and by activating intronic miRNAs such as *miR-126* together with their host genes (**b**). *Open circle* unmethylated CpG site, *filled circle* methylated CpG site

Table 1

miRNAs regulated by epigenetics

miRNA	Host gene	CpG island	Target gene	Expression	References
let-7a-3	−	+	−	Overexpressed in lung cancer	[15]
miR-1	C20orf166	+	*FoxP1, MET, HDAC4*	Silenced in hepatocellular carcinoma	[22]
miR-9-1	−	+	−	Silenced in breast cancer	[17]
miR-9-3	−	+	−	Silenced in cancer metastasis	[16]
miR-34a	−	+	*CDK6*	Silenced in cancer	[20]
*miR-34a**	−	+	*XIAP*	Downregulated in rheumatoid arthritis synovial fibroblasts	[53]
miR-34b/c	BC021736	+	*MET, CCNE2, CDK4, CDK6*	Silenced in colon cancer	[16,21]
				Silenced in cancer metastasis	
miR-124a	−	+	*CDK6*	Silenced in cancer	[14]
miR-126	*EGFL7*	+	*DNMT1*	Silenced in bladder and prostate cancer	[25, 50]
				Overexpressed in lupus CD4+ T cells	
miR-127	−	+	*BCL6*	Silenced in bladder and prostate cancer	[12]
miR-342	*EVL*	+	−	Silenced in colon cancer	[26]
miR-512-5p	−	Alu repeats	*MCL1*	Activated by epigenetic tharapy of gastric cancer	[13]

Table 2

Chromatin modifying factors regulated by miRNAs

Chromatin-modifying factors	miRNAs	Expression	References
DNMT1	*miR-21*, *miR-148a*, *miR-126*, *miR-152*	*MiR-21*, *miR-148a*, and *miR-126* are over-expressed in lupus CD4+ T cells with downregulation of *DNMT1*.3 *miR-152* is silened in cancer with upregulation of *DNMT1*.	[27, 28, 49, 50]
DNMT3A, *DNMT3B*	*miR-29a*, *29b*, *29c*	*miR-29* family are silened in lung cancer with upregulation of *DNMT3A* and *DNMT3B*.	[29]
EZH2	*miR-101*	*miR-101* is silenced in cancer with upregulation of *EZH2*.	[31, 32]