

Chapter 2

Role of MicroRNAs in Cancer Epigenetics

Kishore B. Challagundla, Petra Wise, and Muller Fabbri

Abstract MicroRNAs (miRNAs) are small non-coding RNAs (ncRNAs) with gene expression regulatory functions. Increasing evidence shows that, despite not translated, miRNAs undergo the same regulatory mechanisms of any other protein coding gene (PCG). In particular, they undergo epigenetic regulation. Intriguingly, cancer cells are able to epigenetically regulate the expression of selected miRNAs, therefore granting an overall shift of the transcriptome towards an oncogenic phenotype. In parallel, miRNAs also directly target the expression of key effectors of the epigenetic machinery, therefore indirectly modulating the expression of epigenetically controlled PCGs. This intertwined relationship between the miRNome and the epigenome is further complicated by the existence of other categories of ncRNAs, also modulated by miRNAs and their epigenetic interactions. Overall, the complex layers of reciprocal regulation between ncRNAs and epigenetics are discussed in this chapter and represent a fundamental aspect of the biology of cancer cells.

Keywords Epigenetics • Cancer • MicroRNAs • Non-coding RNAs • Methylation • Chromatin • Histone • Oncogene • Tumor suppressor gene • Promoter • DNA methyltransferases • Histone deacetylases • Polycomb • Post-transcriptional regulation • Gene expression

K.B. Challagundla • P. Wise • M. Fabbri, MD, Ph.D. (✉)
Children's Center for Cancer and Blood Diseases, Norris Comprehensive Cancer Center,
Children's Hospital Los Angeles, University of Southern California,
4650 Sunset Blvd, MS #57, Los Angeles, CA 90027, USA
e-mail: mfabbri@chla.usc.edu

2.1 Introduction

MicroRNAs (miRNAs) are small non-coding RNAs (ncRNAs) which regulate gene expression at a post-transcriptional level [1]. MiRNA aberrant expression is involved in the genesis of several human diseases, including cancer [2]. Interestingly, it has been shown that the genes encoding for miRNAs undergo the same epigenetic regulation of any other protein coding gene (PCG), namely promoter methylation, histone acetylation and chromatin changes [3]. In addition, miRNAs can modulate the expression of key effectors of the epigenetic machinery, such as DNA methyltransferases (DNMTs), Histone deacetylases (HDACs), Polycomb genes, etc.... [4]. Recently, it has been shown that other ncRNAs, namely the transcribed ultraconserved regions (T-UCRs), are also dys-regulated in cancer, and their expression is controlled by miRNAs [5]. This discovery has provided the first evidence of a reciprocal epigenetic control between two different categories of ncRNAs. We define this interaction as direct epigenetic control of ncRNAs. Moreover, increasing evidence is showing that miRNAs are involved in feedback and feedforward regulatory loop, responsible for key steps in human carcinogenesis and drug resistance development. In some cases, it even has been shown that specific miRNAs can regulate the expression of other miRNAs through a common molecular pathway involving transcription factors [6]. We define this interaction as indirect epigenetic control of ncRNAs. This chapter will focus on these interactions by showing at first which miRNAs undergo an epigenetic control in some of the most common human malignancies, followed by a description of which miRNAs directly target key effectors of the epigenetic machinery. Finally, we will describe the direct and indirect mechanism through which miRNAs modulate other ncRNA expression.

2.2 Epigenetic Regulation of MicroRNAs in Human Cancer

2.2.1 *Breast Cancer*

Epigenetic regulation is responsible for aberrant miRNA expression in several malignancies. One of the first studies in this field was conducted in a breast cancer cell line by Scott et al. [7] who were able to demonstrate that 27 miRNA expression levels are rapidly modified by treatment with the HDAC inhibitor LAQ824, indicating that indeed epigenetic factors are involved in miRNA regulation [7]. In breast cancer cell lines treated with 5-aza-2'-deoxycytidine (5-AZA), a DNA demethylating agent, a reactivation of miR-9-1 occurred, without changes in the levels of the other aberrantly methylated miRNAs [8], suggesting that different epigenetic processes can control epigenetically regulated miRNAs in different types of cancer. Tavazoie et al. showed that miR-335, miR-206 and miR-126 act as metastasis suppressors and their expression levels are significantly reduced in primary breast neoplasms of patients who developed metastases [9]. In the case of miR-335 this reduction of expression was

partially due to a locus deletion in combination with hypermethylation of the miR-335 promoter region. The “maintenance” DNA methyltransferase 1 (DNMT1) was found to be aberrantly upregulated in breast cancer and was responsible for hypermethylation of miR-148a and miR-152 promoter regions. DNMT1 expression, one of the targets of miR-148a/152, was inversely correlated with the expression levels of miR-148a/152 in breast cancer tissues, suggesting a negative feedback regulatory loop [10]. Interestingly, IGF-IR and IRS1, often overexpressed in breast cancer, were also targets of miR-148a/152. Overexpression of miR-148a or miR-152 significantly inhibited cell proliferation, colony formation, and tumor angiogenesis *via* targeting IGF-IR and IRS1 and suppressing their downstream AKT and MAPK/ERK signaling pathways [10]. Chang and Sharan reported that BRCA1 recruits the HDAC2 complex to the miR-155 promoter, which is consequently epigenetically silenced through the deacetylation of H2A and H3 histones [11]. The study also showed the up-regulation of miR-155 in BRCA1 deficient or BRCA1 mutant human tumors. The knockdown of miR-155 in a BRCA1 mutant tumor cell line attenuates *in vivo* tumor growth. However, a knockdown of BRCA1 results in a twofold to threefold increase in miR-155 levels *in vitro*. In contrast, a 50 to 150-fold increase in miR-155 in human breast cancer cell lines or tumor samples was observed suggesting that this increase may not be caused only by BRCA1 loss; other transcription factors may also activate the miR-155 promoter after it is epigenetically activated due to the loss of BRCA1 [11].

2.2.2 Colorectal Cancer

Lujambio et al. created a double knockout (DKO) for DNMT1 and DNMT3b in the colorectal cancer cell line HCT-116 and compared the miRNA expression profile of DKO and wild-type cells [12]. About 6 % of the 320 analyzed miRNAs were upregulated in the DKO cells. Among the dysregulated miRNAs, only miR-124a was embedded in a CpG island that is densely methylated in the cancer cell line, but not in normal tissue. This miRNA directly targets CDK6, and its restoration reduces the levels of CDK6 and has an impact on the phosphorylation status of the CDK6 downstream effector Rb protein [12]. The miR-34b/c cluster as well is epigenetically regulated in colorectal cancer; Toyota et al. [13] demonstrated a promoter hypermethylation in 90 % of primary colorectal cancer tumors versus normal colon mucosa. The relationship between miRNA and cognate host gene epigenetic regulation was addressed by Grady et al. [14] by studying miR-342, located in an intron of the EVL (Ena/Vasp-like) gene. EVL promoter hypermethylation occurs in 86 % of colorectal cancers and is already present in 67 % of adenomas, suggesting that it is an early event in colon carcinogenesis. A combined treatment of 5-AZA with the HDAC inhibitor trichostatin A restores the synchronized expression of EVL and miR-342 [14]. In samples from patients with colorectal cancer, 5 miRNAs were identified that were down-regulated and located around/on a CpG island. Treatment with 5-AZA and the HDAC inhibitor 4-phenylbutyric acid restored expression of 3 of the 5 microRNAs (namely miR-9, miR-129 and miR-137) in 3 CRC cell lines.

Expression of miR-9 was inversely correlated with methylation of their promoter regions [15]. Further, methylation of the miR-9-1, miR-129-2 and miR-137 CpG islands were observed in CRC cell lines and in primary CRC tumors, but not in normal colonic mucosa. The methylation of miR-9-1 was associated with the presence of lymph node metastasis [15]. After screening 64 potential epigenetically regulated miRNAs in colon cancer cells, Yan et al. identified miR-941, miR-1237 and miR-1247 as upregulated after treatment of the cells with 5-AZA and transcriptionally independent from their respective putative host genes [16]. Functional studies of miR-941 and miR-1247 revealed that both miRNAs suppress cell growth and migration in CRC cells. Ectopic expression of miR-1247 significantly reduced cancer cell proliferation and migration in colon cancer cells, suggesting that miR-1247 may function as a tumor suppressor gene [16].

DNA methylation also regulates the expression of the miR-1-1 and miR-133a-2 cluster in CRC cell lines. After examining the expression of miR-1 and miR-133a in 64 paired tissue samples (CRC tumor and adjacent normal mucosa), Chen et al. found that the miR-1-133a cluster displayed significantly lower expression in CRC tissue compared to adjacent normal mucosa [17]. The results indicated frequent hypermethylation of the CpG islands upstream of miR-1-133a; liver metastatic tissues exhibited significantly lower miR-1 and miR-133a expression compared to adjacent normal mucosa. The expression of the miR-1-133a cluster is inversely correlated with TAGLN2 in the tested tumor specimens; therefore, epigenetic repression of the miR-1-133a cluster may play a critical role in colorectal cancer metastasis by silencing TAGLN2 [17]. Vinci et al. evaluated the expression of the miR-9-1 and miR-34b/c in CRC paired tissue samples from 160 patients and reported in all cases a significantly reduced expression miR-34c and miR-9-1 [18]. Subsequently, the analysis of the level of methylation in CRC and normal tissues revealed significant hypermethylation in tumor tissues for both miR-34b/c and miR-9-1 [18].

2.2.3 Lung Cancer

In HCT-116 cells deficient in DNMT1 and DNMT3B, Brueckner et al. demonstrated increased expression of let-7a-3, a miRNA normally silenced by promoter hypermethylation in the wildtype cell line [19]. In lung adenocarcinoma primary tumors, let-7a-3 promoter was found hypomethylated with respect to the normal counterpart [19], whereas hypermethylation of let-7a-3 promoter was described in epithelial ovarian cancer, paralleled the low expression of insulin-like growth factor-II expression, and was associated with a good prognosis [20]. Therefore, DNA methylation could act as a protective mechanism by silencing miRNAs that have oncogenic function.

The above-mentioned studies demonstrate that epigenetic factors can control human carcinogenesis, not only by directly affecting the expression of oncogenes (OGs) and tumor suppressor genes (TSGs), but also by affecting the expression of miRNAs involved in oncogenic pathways. MiRNA epigenetic control might be tissue-specific because no variation in miRNA expression was observed in lung cancer cells treated with either demethylating agents or HDAC inhibitors or their combination [21].

Besides via DNA methylation, epigenetic silencing in mammalian cells can also be mediated by histone modifications. For instance, increased levels of H3K27 trimethylation and H3K9 dimethylation as well as H3K9 acetylation in the promoter region of miR-212 in lung cancer cells compared to normal cells was observed [22], leading to a reduced expression of miR-212 in lung cancer compared to the normal lung tissue counterpart [23].

2.2.4 Hepatocellular Carcinoma (HCC)

In HCC miR-1 is frequently silenced by promoter hypermethylation. However, in DNMT1-null HCT-116 cells (but not in DNMT3B-null cells), hypomethylation and re-expression of miR-1-1 were observed [24], revealing a key role for the maintenance DNMT in the regulation of this miRNA. Aberrations in histone acetylation have been observed in HCC. In their study, Yuan et al. [25] determined that miR-200a and the level of histone H3 acetylation at its promoter region were reduced in human HCC tissues in comparison with adjacent noncancerous hepatic tissues. Furthermore, histone deacetylase 4 (HDAC4) inhibited the expression of miR-200a and its promoter activity and reduced the histone H3 acetylation level at the miR-200a promoter region through a Sp1-dependent pathway. Interestingly, the miR-200a directly targeted the 3'-untranslated region of the HDAC4 messenger RNA and repressed expression of HDAC4. This means that miR-200a ultimately induced its own transcription and increased the histone H3 acetylation level at its own promoter. After screening 78 HCC patient tissue samples, He et al. found miR-191 to be highly expressed in tumor tissues and the adjacent noncancerous tissues compared to normal liver [26]. This elevated expression was associated with poor prognosis: miR-191 overexpression led to a mesenchymal-like transition, and increased cell invasion. The miR-191 locus is located in the gene *DALRD3*, with which miR-191 is co-expressed. The *DALRD3* promoter region contains a CpG rich region that is hypomethylated in HCC. Treatment of normal liver cells with 5-AZA showed an increase in miR-191 expression, which suggests that miR-191 is involved in HCC progression [26]. Also, miR-224 is commonly upregulated in HCC, and regulates apoptosis and cell proliferation. Wang et al. [27] examined the expression of miR-224, neighboring miR-452 and genes on chromosome Xq28 in paired tissues from patients with HCC, finding that miR-224 is coordinately upregulated with its neighboring miRNAs and genes. The introduction of histone deacetylase (HDAC) inhibitors in non-transformed human liver cells resulted in a corresponding increase in histone H3 acetylation in this region. MiR-224 locus in Xq28 resulted reciprocally regulated by HDAC1, HDAC3, and histone acetylase protein, E1A binding protein p300 (EP300). Notably, in HCC tumors significantly overexpressing miR-224, EP300 is also overexpressed and displays increased binding to the Xq28 locus. Through inhibition of EP300 the high miR-224 expression in transformed HCC cells can be attenuated [27]. Liu et al. reported that a large Chromosome 19 miRNA cluster (C19MC) is upregulated in HCC cells after combined treatment with 5-AZA and trichostatin A [28]. Specifically, miR-517a and miR-517c were strikingly different from the

remaining 41 miRNAs in C19MC. Ectopic expression of miR-517a and miR-517c inhibited cell proliferation by blocking G2/M transition, whereas downregulation of miR-517a and miR-517c facilitated cell growth. The group showed that Pyk2 is a target of miR-517a/517c and both miRNAs are downregulated in HCC samples. These data collectively suggest that downregulation of both miR-517a and miR-517c contributes to HCC development by regulating Pyk2 [28].

2.2.5 *Melanoma*

Mazar et al. studied which miRNAs were upregulated upon treatment of a melanoma cell line with demethylating agents [29]. Among the 15 miRNAs silenced by promoter hyper-methylation, they showed that miR-375 and miR-34b are also involved in melanoma progression [29]. To investigate the epigenetic regulation of miRNAs in melanoma, Liu et al. [30] found that miR-182, a miRNA with oncogenic properties, was significantly upregulated in human melanoma cells after epigenetic modulation with 5-AZA and trichostatin A. Genome sequence analysis revealed the presence of a prominent CpG island 8–10 kb upstream of miR-182, whereas methylation analysis showed that this genomic region was exclusively methylated in melanoma cells but not in human melanocytes, skin, or peripheral blood mononuclear cells. This increased expression of the oncogenic miR-182 could be a concern for melanoma patients after epigenetic therapy [30].

The genomic region on chromosome 9p21 where miR-31 is located, is frequently deleted in solid cancers including melanoma. Asangani et al. [31] found that downregulation of miR-31 was a common event in melanoma primary tumors and cell lines and was associated with genomic loss in a subset of samples as well as with epigenetic silencing by DNA methylation and EZH2-mediated histone methylation. Ectopic overexpression of miR-31 in various melanoma cell lines inhibited cell migration and invasiveness. MiR-31 target genes included oncogenic kinases such as SRC, MET, NIK (MAP3K14) and the melanoma specific oncogene RAB27a. Furthermore, miR-31 overexpression resulted in downregulation of EZH2 and a repression of its target gene rap1GAP. The increased expression of EZH2 was associated with melanoma progression and poorer overall survival. Taken together, these data support a tumor suppressor role for miR-31 in melanoma and might identify potential novel therapeutic targets [31].

2.2.6 *Leukemias*

Prosper's group analyzed 353 acute lymphoblastic leukemia (ALL) patients and identified a signature of 13 miRNAs embedded in CpG islands, with high heterochromatic markers (namely, high levels of K9H3me2 and/or low levels of K4H3me3) [32, 33]. Treatment with 5-AZA induced upregulation of at least one miRNA of the signature

in 65 % of ALL patients [33]. Among these, miR-124a was methylated in 59 % of ALL patients, and its promoter hypermethylation was associated with a higher relapse and mortality rate versus non-hypermethylated cases [32]. Additionally, the impact of miR-124a in the CDK6-Rb pathway was demonstrated in ALL by showing that miR-124a directly silences CDK6 [32]. Rodríguez-Otero et al. analyzed the methylation status of the members of the miR-9 family, miR-9-1, miR-9-2 and miR-9-3, in a uniformly treated cohort of 200 newly diagnosed ALLs [34]. MiR-9 was methylated in 54 % of the patients and was associated with downregulation of miR-9 expression. Hypermethylation of miR-9 was an independent prognostic factor for disease-free survival, overall survival and event-free survival in a multivariate analysis. Epigenetic downregulation of miR-9 induced upregulation of its targets, FGFR1 and CDK6, while treatment of ALL cells with FGFR1 and CDK6 inhibitors induced a decrease in cell proliferation and increased apoptosis of ALL cells [34]. Transcription factors are able to recruit epigenetic effectors at miRNA promoter regions and contribute to the regulation of their expression as shown by Fazi et al. [35]. The AML1/ETO fusion oncoprotein is the aberrant product of t(8;21) translocation in acute myeloid leukemia (AML) and can bind to the pre-miR-223 region. The oncoprotein recruits epigenetic effectors (i.e., DNMTs, HDAC1, and MeCP2), leading to aberrant hypermethylation of the CpG in close proximity to the AML1/ETO binding site and H3-H4 deacetylation of the same chromatin region [35]. Finally, Chim et al. studied miR-34a, miR-124-1 and miR-203 in a panel of hematological malignancies [36–38] including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL). All three of the investigated miRNAs were found to be epigenetically silenced in a tumor specific manner: miR-34a methylation was detected in a percentage of CLL, MM and NHL samples at diagnosis but not at all in ALL, AML and CML. Amongst lymphoid malignancies, was miR-34a preferentially methylated in NHL, in particular in natural killer (NK)/T-cell lymphoma. Methylation of miR-124-1 as well as miR-203 could not be detected in CML but in ALL, AML, CLL and NHL, with varying percentages in all examined samples. Moreover, hsa-miR-203 methylation was associated with hypermethylation of hsa-miR-34a, -124a and -196b in NHL but not CLL [36–38].

2.2.7 Metastatic Cancers

Several studies have demonstrated that miRNAs affect the metastatic process by targeting metastasis-related genes [9, 39, 40]. Lujambio et al. [41] investigated whether epigenetic factors determine miRNA expression in metastatic cancer. By treating three lymph node metastatic cell lines with 5-AZA and performing a miRNA microarray analysis, followed by CpG island analysis and bisulfite genomic sequencing, the authors identified three miRNAs that showed cancer-specific CpG island hypermethylation: miR-148a, miR-34b/c, and miR-9 [41]. The reintroduction of miR-148a and miR-34b/c in cancer cells with epigenetic inactivation inhibited the cells'

Table 2.1 Epigenetically regulated microRNAs

miRNA	Regulation	Cell type	Year of discovery	References
let-7a-3	Hypermethylation	Lung cancer	2007	[64, 65]
miR-1	Hypermethylation	Hepatocellular carcinoma	2008	[69]
miR-1/133a cluster	Hypermethylation	Colorectal cancer	2012	[52]
miR-21	Hypomethylation	Ovarian cancer	2007	[43]
miR-34a	Hypermethylation	Leukemia	2010, 2011	[61-63]
miR-34b/c cluster	Hypermethylation	Metastatic cancers Colorectal cancer	2008 2008	[48, 53]
miR-107	Hypermethylation	Pancreatic cancer	2009	[55]
miR-1224	Hypermethylation	Bladder cancer	2011	[46]
miR-124	Hypermethylation	Leukemia	2010, 2011	[61-63]
miR-124a	Hypermethylation	Colorectal cancer Gastric cancer Leukemia	2007 2009 2009	[47] [54] [57, 58]
miR-126	Hypermethylation	Breast cancer	2008	[39]
miR-127	Hypomethylation, Histone Acetylation	Bladder cancer	2006	[44]
miR-129	Hypermethylation	Gastric cancer	2010	[56]
miR-130b	Hypermethylation	Ovarian cancer	2012	[42]
miR-148a	Histone deacetylation	Breast cancer Metastatic cancers	2012 2008	[45] [40]
miR-152	Hypermethylation	Bladder cancer Breast cancer	2011 2012	[46] [40]
miR-155	Histone deacetylation	Breast cancer	2012	[41]
miR-191	Histone deacetylation	Hepatocellular carcinoma	2011	[71]
miR-200a	Histone acetylation	Hepatocellular carcinoma	2011	[70]
miR-201	Hypomethylation	Ovarian cancer	2007	[43]
miR-203	Hypermethylation	Leukemia	2010, 2011	[61-63]
miR-205	Hypomethylation	Ovarian cancer	2007	[43]
miR-206	Hypermethylation	Breast cancer	2008	[39]
miR-212	Histone methylation Histone acetylation	Lung cancer	2006, 2011	[67, 68]
miR-223	Hypermethylation Histone deacetylation	Leukemia	2007	[60]
miR-224	Histone deacetylation	Hepatocellular carcinoma	2012	[72]
miR-335	Hypermethylation	Breast cancer	2008	[39]
miR-342	Hypermethylation	Colorectal cancer	2008	[49]
Mir-357	Hypermethylation Histone deacetylation	Melanoma	2011	
miR-517	Hypermethylation Histone deacetylation	Hepatocellular carcinoma	2012	[73]
miR-9	Hypermethylation	Leukemia Metastatic cancers	2011 2008	[59]
	Histone deacetylation	Colorectal cancer	2009	[50,53]
miR-137	Hypermethylation	Colorectal cancer	2009	[50, 53]
miR-129	Hypermethylation	Colorectal cancer	2009	[50, 53]
miR-941	Hypermethylation	Colon cancer	2011	[51]
miR-1237	Hypermethylation	Colon cancer	2011	[51]
miR-1247	Hypermethylation	Colon cancer	2011	[51]

motility and their metastatic potential in xenograft models and was associated with downregulation of miRNA oncogenic target genes such as c-MYC, E2F3, CDK6, and TGIF2. Finally, promoter hypermethylation of these three miRNAs was significantly associated with metastasis in human malignancies [41].

In summary, an abundance of studies (listed in Table 2.1) show that miRNAs undergo epigenetic regulation, similar to any other PCG. MiRNAs represent an

indirect mechanism through which epigenetics affect the expression of OGs and TSGs and ultimately impact on human carcinogenesis. The complexity of the miRNA-epigenetics relationship is refined by the discovery of a subset of miRNAs, the so-called “epi-miRNAs”, that can regulate the expression levels of effectors of the epigenetic machinery.

2.3 MicroRNAs Regulating Effectors of the Epigenetic Machinery

2.3.1 MicroRNAs Regulating DNMTs

The first evidence showing the regulation of DNMTs by miRNAs was provided by Fabbri et al. in 2007 in lung cancer cells. We showed that miR-29 family (29a, 29b and 29c) directly binds to the 3'UTR region of DNMT3A and 3B (*de novo* methyl transferases), two key enzymes involved in DNA methylation [42]. The miR-29 family comprises three isoforms arranged in two clusters: miR-29b-1/miR-29a on chromosome 7q32 and mir-29b-2/miR-29c on chromosome 1q23. MiR-29 family members have been shown to be downregulated in lung cancer [42, 43], and restoration of individual miR-29s induces a marked reduction of DNMT3A and 3B mRNA and protein levels leading to a global DNA hypomethylation, which in turn causes reactivation of epigenetically silenced TSGs such as FHIT and WWOX in cancer cell lines. Interestingly, the same group has also discovered another mechanism of DNMT regulation by miR29s in AML [44]. MiR-29b expression is dysregulated in primary AML blasts and restoration of miR-29b in AML cells results in a marked reduction of DNMT1, 3A, and 3B expression levels, which in turn causes a decrease in overall DNA methylation and re-expression of TSGs such as p15INK4b and ESR1 via promoter DNA hypomethylation. MiR-29b directly targets DNMT3A, and 3B, whereas targeting of DNMT1 is indirect and mediated by SP1, a trans-activator of DNMT1. The overexpression of miR-29 induces apoptosis in lung cancer cell lines and reduced tumorigenicity in a xenograft model of lung cancer and AML [42]. These discoveries explored an unknown functional link between microRNAs and aberrant DNA methylation via targeting DNMTs in lung cancer and AML models.

In 2008 Duursma et al. have revealed that miR-148 regulates DNMT3B expression by binding to its coding sequence (CDS) and not to its 3'UTR [45]. In the same year, Benetti et al. discovered a previously unknown DNA methylation mechanism involving the mammalian Dicer-dependent miR-290 cluster that is predicted to target Rbl2 [46, 47]. A substantial down-regulation of the miR-290 cluster was found in Dicer1-null cells compared to wild-type controls [46]. Rbl proteins epigenetically repress DNMT promoters by decreased abundance of AcH3K9 at the promoter regions of the DNMT1, DNMT3A and DNMT3B genes. Over-expression of Rbl2 protein causes decreased expression of DNMTs in Dicer1-null cells, concluding

that increased levels of Rbl2 protein in Dicer1-null cells is responsible for decreased DNMT expression and less DNA methylation in these cells [48]. The miRNA-290 family is highly expressed in pluripotent ES cells and repressed upon differentiation [49]. Altogether, these findings suggest that in the absence of Dicer, downregulation of the miR-290 cluster leads to increased mRNA levels of the miR-290 cluster's target gene Rbl2, whose product in turn inhibits DNMTs expression. Decreased DNMT expression, in part mediated by Rbl2, is leading to a significant hypomethylation of the genome, including the subtelomeric regions, as well as to the appearance of telomeric phenotypes such as increased telomere recombination and increased telomere length [46, 47, 49, 50].

IL-6 has been shown to regulate the activity of DNMT1 and the expression of TSGs by modulation of miR-148a, miR-152 and miR-301, which have a 3'UTR complementarity sequence to DNMT1 [51]. These miRNAs have been found to have decreased levels in IL-6 overexpressing malignant cholangiocytes and in tumor cell xenografts with concomitant decrease in expression of TSGs such as RASSF1A and p16INK4a. Over-expression of miR-148a and miR-152 in cholangiocytes causes decreased DNMT1 protein expression, increased Rassf1a and p16INK4a expression, and reduced cell proliferation [51, 52] providing a link between this inflammation-associated cytokine and oncogenesis in cholangiocarcinoma.

In 2010, Das et al. have explored the role of miR-152 mediated DNMT repression in all-trans-retinoic acid (ATRA) induced neuroblastoma cell line differentiation [53]. ATRA treatment causes downregulation of MYCN, hence leading to overexpression of MYCN repressed miRNAs such as miR-152, miR-26a/b, and miR-125a/b. This downregulates DNMT1 and DNMT3B expression and in turn leads to the demethylation and activation of NOS1, which promotes neural cell differentiation in SK-N-BE cells. Overexpression of miR-152 causes downregulation of DNMT1 that negatively regulates cell invasiveness and anchorage-independent growth, contributing to the differentiated phenotype [53]. These findings illustrate the dynamic nature of the miR mediated epigenome alterations during not only cancer cell proliferation, apoptosis but also during the differentiation process. Also, the expression of miR-152 was normally down-regulated with concurrent increase of DNMT1 expression in HBV induced HCCs [48]. Overexpression of miR-152 resulted in a significant reduction of the expression of DNMT1 via its 3'UTR, which in turn leads to a decrease in global DNA methylation. Moreover, inhibition of miR-152 causes overall DNA hypermethylation and increases promoter DNA methylation of TSGs such as glutathione S-transferase pi 1 (GSTP1) and E-cadherin 1 (CDH1) in HepG2 cells [48].

In 2010, viral miRNAs have been shown to control the epigenetic machinery of host cells through DNMTs [54]. K12-4-5p, a Kaposi sarcoma-associated herpesvirus (KSHV) miRNA was found to regulate the expression of DNMT1, 3A and 3B indirectly, by targeting the expression of Rbl2, a known repressor of DNMT1, 3A and 3B transcription. Ectopic expression of miR-K12-4-5p reduces Rbl2 protein expression and increases DNMT1, -3A, and -3B mRNA levels in 293 cells, thus affecting the overall epigenetic reprogramming of the host cell [54].

Table 2.2 Regulation of DNMTs by microRNAs

miRNA	Target	Cell type	Year of discovery	References
miR-29a,b,c	DNMT3a,3b	Lung cancer	2007, 2009	[1]
miR-29b	DNMT1,3a,3b	AML	2006	[2, 3]
	DNMT3b	Hela cells	2008	[4]
miR-290 cluster	DNMT1,3a,3b	Dicer null cells, Pluripotent ES cells	2008	[5-7]
miR-148a, 152, 301	DNMT1	Cholangiocytes	2010	[9, 10]
K12-4-5p	DNMT1,3a,3b	Viral infection	2010	[11]
miR-152, 26a/b,125a/b	DNMT1, 3b	Neuronal differentiation	2010	[13]
miR-21, 148a	DNMT1	SLE	2011	[14]
miR-342	DNMT1	Colorectal cancer	2010	[15]
miR-152	DNMT1	NiS induced tumorigenesis	2012	[16]
miR-29	DNMT3a,3b	Influenza infection	2012	[17]

More recently, Wang et al. showed that DNMT1 is regulated by miR-342 in CRC [55]. Low expression of miR-342 and high expression of DNMT1 were observed in CRC tissues and cell lines. Downregulation of DNMT1 expression through miR-342 caused reactivation of TSGs such as ADAM23, Hint1, RASSF1A and RECKS through promoter hypomethylation. Restoration of miR-342 resulted in a reduction of DNMT1 expression, reduced cell proliferation, and invasiveness in CRC cells and inhibition of tumor growth and lung metastasis formation in nude mice [55].

Nickel (Ni) compounds are well described human carcinogens. Recently an important regulatory double-negative feedback loop has been discovered between miR-152 and DNMT1 in nickel sulfide (NiS)-transformed human bronchial epithelial (16HBE) cells [56]. Expression of miR-152 was specifically downregulated by promoter hypermethylation, whereas ectopic expression of miR-152 resulted in a remarkable reduction of DNMT1 expression in transformed cells. Interestingly, treatment with 5-AZA or knock down of DNMT1 reversed this process. Further, inhibition of miR-152 expression in 16HBE cells was found to increase DNMT1 expression and DNA methylation. Moreover, ectopic expression of miR-152 caused a significant decrease of cell growth, whereas inhibition of miR-152 reversed this process in 16HBE cells, suggesting the existence of an important functional negative feedback loop between miR-152 and DNMT1, likely to play an important role in NiS induced carcinogenesis [56]. The series of studies showing miRNAs regulating DNMTs is listed in Table 2.2.

Table 2.3 Regulation of HDACs by microRNAs

miRNA	Target	Cell type	Year of discovery	References
miR-140	HDAC4	Muscular differentiation, colon cancer	2006, 2009	[1, 4]
miR-1	HDAC1	Skeletal muscle differentiation	2006	[2]
miR-449a,b	HDAC1	Prostate cancer, lung cancer	2009, 2012	[3, 6]
miR-9	HDAC4,5	Waldenstrom macroglobulinemia	2010	[5]

2.3.2 *MicroRNAs Regulating HDACs*

The first evidence of miRNA involvement in regulating histone deacetylases (HDACs) expression levels was provided in 2006. Two groups showed first that miR-140 plays an important role in promoting differentiation by suppressing HDAC4 levels, a known co-repressor of Runx2, a transcription factor essential for chondrocyte hypertrophy during skeletogenesis [57]. In the same year it was published that miR-1 promotes differentiation during muscle development by also suppressing HDAC4 [58]. In 2009, Noonan et al. provided a mechanistic insight on the regulation of HDAC1 by miR-449a in prostate cancer [59]. Overexpression of HDAC1 and a low expression of miR-449a were found in prostate cancer cells and tissue samples from patients when compared to their respective controls. MiR-449a binds and targets HDAC1 directly via the 3'UTR transcript. Overexpression of miR-449a resulted in cell-cycle arrest, apoptosis and a senescent-like phenotype by reducing the level of HDAC1 in PC-3 prostate cancer cell line, thus providing a link between miR-449a and HDAC1 that in turn alters the cellular epigenetic program to promoting cell proliferation and survival [59]. MiR-140 has also been shown to be involved in chemoresistance mechanisms by targeting HDAC4 [60]. Inhibition of endogenous miR-140 by locked nucleic acid-modified anti-miRs partially sensitized resistant colon cancer stem-like cells to 5-FU treatment by increasing HDAC4 levels, leading to a G₁ and G₂ phase arrest [60]. Low expression of miR-9 along with high expression levels of HDACs (HDAC4 and 5) were discovered in Waldenstrom macroglobulinemia (WM) [61]. Mir-9 targets HDAC4 and HDAC5 in WM cells. Overexpression of miR-9 causes downregulation of HDAC4, 5, leading to an up-regulation of acetylated-histone-H3 and -H4. This provides evidence that the loss of miR-9 might be responsible for up-regulation of HDAC4 and HDAC5 in WM cells, contributing to the pathogenesis of WM disease [61]. Recently, Jeon et al. showed that miR-449a, b regulate HDAC1 expression by directly targeting its 3'UTR transcript, indicating that this might be one of the reasons for the low miR-449a, b expression and the high expression of HDAC1 in lung cancer [62]. The series of studies showing miRNAs regulating HDACs is listed in Table 2.3.

2.3.3 *MicroRNAs Regulating Polycomb Group Proteins (PcG)*

The main function of polycomb group proteins (PcG) is the transcriptional repression of various TSGs through chromatin modifications. PcG proteins act together in polycomb repressive complexes (PRC). PRC2 includes the enhancer of zeste 2 (EZH2), the suppressor of zeste 12 (SUZ12), and embryonic ectoderm development (EED). EZH2, a mammalian histone methyltransferase, is the catalytically active component of PRC2 that contributes to the epigenetic silencing of target genes and regulates the survival and metastasis of cancer cells. EZH2 mediates the trimethylation of lysine 27 of histone H3 (H3K27me3) at target gene promoters, leading to the epigenetic silencing of the target genes. This modification of H3 is necessary for the repression of various TSGs.

In 2008, Varambally et al. showed that loss of miR-101 expression with concomitant elevation of EZH2 is most pronounced in metastatic prostate cancer [63]. This reduction in miR-101 expression inversely correlates with increased expression of EZH2 and dysregulation of epigenetic pathways which results in silencing target gene promoters and subsequent cancer progression. Overexpression of miR-101 inhibits the expression and function of EZH2 in cancer cell lines [63]. Inverse correlation between miR-101 and EZH2 was also observed in transitional cell carcinoma [64], glioblastoma [65], gastric cancer [66], and non-small cell lung cancer [67]. In prostate cancer it has been shown that miR-101 can be inhibited by androgen receptor and HIF-1 α /HIF-1 β [68]. Sander et al. showed that miR-26a was down regulated in a murine lymphoma model and in human Burkitt lymphoma samples [69]. Ectopic expression of miR-26a targets EZH2, inhibits cell proliferation, increases percentage of cells in G₁-phase, and induces apoptosis in Raji and Namalwa cells. Intriguingly, they also found that c-Myc negatively regulates miR-26a, therefore maintaining high EZH2 expression levels in cells and significantly contributing to c-Myc induced tumorigenesis [69]. In 2009, Juan et al. analyzed a regulatory double-negative feedback loop between miR-214 and EZH2 in controlling PcG dependent gene expression during differentiation [70]. PcG proteins suppress the transcription of miR-214 in undifferentiated skeletal muscle cells (SMC). Ectopic expression of miR-214 directly targets EZH2 via its 3'UTR transcript and inhibition of miR-214 rescues this process in differentiating C2C12 cells. Ectopic expression of miR-214 reduces EZH2 expression, increases myogenin expression, and promotes muscle differentiation [70]. EZH2 is also highly expressed in nasopharyngeal carcinoma (NPC) patients and correlates with a higher risk of relapse [71]. Depletion of EZH2 is associated with decreased cell proliferation, induced apoptosis in C666-1 cells and delayed tumor growth in SCID mice. In this model three miRNAs (namely miR-26a, miR-98, and 101), whose expression is consistently downregulated in human NPC specimens when compared to normal nasopharyngeal epithelial tissue samples, have been shown to directly target EZH2 [71]. Recently, there has been an extensive series of studies unraveling a central role of miR-101 in the regulation of EZH2 in several types of cancer. In hepatoma tissues, it was shown that miR-101 and miR-29c are downregulated, but their

Table 2.4 Regulation of EZH2 by microRNAs

miRNA	Target	Cell type	Year of discovery	References
miR-101	EZH2	Prostate cancer	2008, 2010	[24, 32]
		Bladder transitional cell carcinoma	2009	[27]
		Glioblastoma	2010	[30]
		Gastric cancer	2010	[31]
		Hepatocellular carcinoma	2010	[33]
		Nasopharyngeal carcinoma	2010	[29]
		Angiogenesis	2011	[34]
miR-26a	EZH2	NSCLC	2011	[35]
		Muscle differentiation	2008	[25, 26]
		Burkitt lymphoma		
		Nasopharyngeal carcinoma	2010	[29]
miR-214	EZH2	Skeletal muscle differentiation	2009	[28]
miR-98	EZH2	Nasopharyngeal carcinoma	2010	[29]
miR-29c	EZH2	Hepatocellular carcinoma	2010	[33]

expression can be restored (leading to reduced levels of EZH2, EED and H3K27me3 proteins) after treatment with TPA (12-O-tetradecanoylphorbol 13-acetate), which is Protein Kinase C (PKC) and ERK pathway dependent in HepG2 cells [72]. Also, Smiths et al. have established a pro-angiogenic effect of miRNA-101 working together with EZH2 and VEGF during the process of angiogenesis [73]. The group analyzed the expression of miR-101 in endothelial cells derived from glioma patients and found to it be low. VEGF downregulates the expression levels of miR-101 resulting in increased protein expression of EZH2, induces elongation of endothelial cells leading to a pro-angiogenic response. Transfection with pre-miR-101, or EZH2 siRNA, or treatments with DZNep, a small inhibitor of EZH2 methyltransferase activity, reverses this process in HBMVECs controls, providing a network between VEGF/miR-101/EZH2 proteins towards pro-angiogenic response in endothelial cells [73].

Overall, an increasing number of studies has identified a central role for miRNAs as modulators of key effectors of the epigenetic machinery, revealing a more complex layer of reciprocal regulation between “traditional” epigenetic effectors (such as DNMTs, HDACs, PcG) and ncRNAs. The series of studies showing miRNAs regulating PcGs is listed in Table 2.4.

2.4 Conclusion

MiRNAs play a central and pivotal role in the regulation of gene expression. The series of studies covered in this chapter clearly indicate that while these small ncRNAs are kept under a rigorous epigenetic control in several different types of tumors, they can actually also affect the expression of other epigenetically

regulated PCGs by targeting key effectors of the epigenetic machinery. Therefore, miRNAs interpose their action between DNMTs, HDACs, PcGs and their epigenetic target PCG. Intriguingly, the world of ncRNAs is being more and more extensively studied and is being populated by an increasing number of biologic transcripts. Among them, the transcribed ultraconserved regions (T-UCRs) also play an important role in human carcinogenesis [5]. Noteworthy, it has been shown that miRNAs can regulate the expression of T-UCRs, suggesting an additional layer of complexity in gene expression regulation, involving two different groups of ncRNAs [5]. Moreover, it was demonstrated that certain miRNAs directly target transcription factors regulating the expression of other miRNAs. By doing this, it has been observed that one miRNA ultimately affects the expression levels of another miRNA [6]. This increasing complexity of interactions should not scare. Indeed, it can be safely stated that cancer is probably the most complex genetic disease. A better comprehension of such a complexity, while a little bit disorienting at first, it is actually the necessary background to fully understand the whole picture of the epigenetic regulation in human malignancies. Such a knowledge represents the necessary platform to build new treatments based on the biologic rationale provided by these discoveries and ultimately to offer new therapeutic options to cancer patients.

References

1. Ambros V (2003) MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 113(6):673–676
2. Croce CM (2009) Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 10(10):704–714
3. Fabbri M, Calin GA (2010) Epigenetics and miRNAs in human cancer. *Adv Genet* 70:87–99
4. Fabbri M, Calore F, Paone A, Galli R, Calin GA (2013) Epigenetic regulation of miRNAs in cancer. *Adv Exp Med Biol* 754:137–148
5. Calin GA, Liu C, Ferracin M, Hyslop T, Spizzo R, Sevignani C et al (2007) Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. *Cancer Cell* 12(3):215–229
6. Fabbri M, Bottoni A, Shimizu M, Spizzo R, Nicoloso MS, Rossi S et al (2011) Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *J Am Med Assoc* 305(1):59–67
7. Scott GK, Mattie MD, Berger CE, Benz SC, Benz CC (2006) Rapid alteration of microRNA levels by histone deacetylase inhibition. *Cancer Res* 66(3):1277–1281
8. Lehmann U, Hasemeier B, Christgen M, Muller M, Romermann D, Langer F et al (2008) Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. *J Pathol* 214(1):17–24
9. Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD et al (2008) Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 451(7175):147–152
10. Xu Q, Jiang Y, Yin Y, Li Q, He J, Jing Y, Qi YT, Xu Q, Li W, Lu B, Peiper SS, Jiang BH, Liu LZ (2013) A regulatory circuit of miR-148a-152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. *J Mol Cell Biol* 5(1):3–13. doi:10.1093/jmcb/mjs049
11. Chang S, Sharan SK (2012) Epigenetic control of an oncogenic microRNA, miR-155, by BRCA1. *Oncotarget* 3(1):5–6

12. Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, Setien F et al (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res* 67(4):1424–1429
13. Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y et al (2008) Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. *Cancer Res* 68(11):4123–4132
14. Grady WM, Parkin RK, Mitchell PS, Lee JH, Kim YH, Tsuchiya KD et al (2008) Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. *Oncogene* 27(27):3880–3888
15. Bandres E, Agirre X, Bitarte N, Ramirez N, Zarate R, Roman-Gomez J et al (2009) Epigenetic regulation of microRNA expression in colorectal cancer. *Int J Cancer* 125(11):2737–2743
16. Yan HCA, Lee BH, Ting AH (2011) Identification and functional analysis of epigenetically silenced microRNAs in colorectal cancer cells. *PLoS One* 6(6):e20628. doi:[10.1371/journal.pone.0020628](https://doi.org/10.1371/journal.pone.0020628)
17. Chen WS, Leung CM, Pan HW, Hu LY, Li SC, Ho MR et al (2012) Silencing of miR-1-1 and miR-133a-2 cluster expression by DNA hypermethylation in colorectal cancer. *Oncol Rep* 28(3):1069–1076
18. Vinci S, Gelmini S, Mancini I, Malentacchi F, Pazzagli M, Beltrami C et al (2013) Genetic and epigenetic factors in regulation of microRNA in colorectal cancers. *Methods* 59(1):138–146. doi:[10.1016/j.ymeth.2012.09.002](https://doi.org/10.1016/j.ymeth.2012.09.002)
19. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M et al (2007) The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. *Cancer Res* 67(4):1419–1423
20. Lu L, Katsaros D, de la Longrais IA, Sochirca O, Yu H et al (2007) Hypermethylation of let-7a-3 in epithelial ovarian cancer is associated with low insulin-like growth factor-II expression and favorable prognosis. *Cancer Res* 67(21):10117–10122
21. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M et al (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9(3):189–198
22. Incoronato M, Urso L, Portela A, Laukkanen MO, Soini Y, Quintavalle C et al (2011) Epigenetic regulation of miR-212 expression in lung cancer. *PLoS One* 6(11):e27722
23. Incoronato M, Garofalo M, Urso L, Romano G, Quintavalle C, Zanca C et al (2010) miR-212 increases tumor necrosis factor-related apoptosis-inducing ligand sensitivity in non-small cell lung cancer by targeting the antiapoptotic protein PED. *Cancer Res* 70(9):3638–3646
24. Datta J, Kutay H, Nasser MW, Nuovo GJ, Wang B, Majumder S et al (2008) Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res* 68(13):5049–5058
25. Yuan JH, Yang F, Chen BF, Lu Z, Huo XS, Zhou WP et al (2011) The histone deacetylase 4/SP1/microrna-200a regulatory network contributes to aberrant histone acetylation in hepatocellular carcinoma. *Hepatology* 54(6):2025–2035
26. He Y, Cui Y, Wang W, Gu J, Guo S, Ma K et al (2011) Hypomethylation of the hsa-miR-191 locus causes high expression of hsa-mir-191 and promotes the epithelial-to-mesenchymal transition in hepatocellular carcinoma. *Neoplasia* 13(9):841–853
27. Wang Y, Toh HC, Chow P, Chung AY, Meyers DJ, Cole PA et al (2012) MicroRNA-224 is up-regulated in hepatocellular carcinoma through epigenetic mechanisms. *FASEB J* 26(7):3032–3041
28. Liu RF, Xu X, Huang J, Fei QL, Chen F, Li YD et al (2013) Down-regulation of miR-517a and miR-517c promotes proliferation of hepatocellular carcinoma cells via targeting Pyk2. *Cancer Lett* 329(2):164–173. doi:[10.1016/j.canlet.2012.10.027](https://doi.org/10.1016/j.canlet.2012.10.027)
29. Mazar J, DeBlasio D, Govindarajan SS, Zhang S, Perera RJ et al (2011) Epigenetic regulation of microRNA-375 and its role in melanoma development in humans. *FEBS Lett* 585(15):2467–2476
30. Liu S, Howell PM, Riker AI (2013) Up-regulation of miR-182 expression after epigenetic modulation of human melanoma cells. *Ann Surg Oncol* 20(5):1745–1752. doi:[10.1245/s10434-012-2467-3](https://doi.org/10.1245/s10434-012-2467-3) [Epub ahead of print]

31. Asangani IA, Harms PW, Dodson L, Pandhi M, Kunju LP, Maher CA et al (2012) Genetic and epigenetic loss of microRNA-31 leads to feed-forward expression of EZH2 in melanoma. *Oncotarget* 3(9):1011–1025
32. Agirre X, Vilas-Zornoza A, Jiménez-Velasco A, Martín-Subero JI, Cordeu L, Gárate L et al (2009) Epigenetic silencing of the tumor suppressor microRNA Hsa-miR-124a regulates CDK6 expression and confers a poor prognosis in acute lymphoblastic leukemia. *Cancer Res* 69(10):4443–4453
33. Roman-Gomez J, Agirre X, Jiménez-Velasco A, Arqueros V, Vilas-Zornoza A, Rodríguez-Otero P et al (2009) Epigenetic regulation of microRNAs in acute lymphoblastic leukemia. *J Clin Oncol* 27(8):1316–1322
34. Rodríguez-Otero P, Román-Gómez J, Vilas-Zornoza A, José-Eneriz ES, Martín-Palanco V, Rifón J et al (2011) Deregulation of FGFR1 and CDK6 oncogenic pathways in acute lymphoblastic leukaemia harbouring epigenetic modifications of the MIR9 family. *Br J Haematol* 155(1):73–83
35. Fazi F, Racanicchi S, Zardo G, Starnes LM, Mancini M, Travaglini L et al (2007) Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell* 12(5):457–466
36. Chim CS, Wong KY, Leung CY, Chung LP, Hui PK, Chan SY et al (2011) Epigenetic inactivation of the hsa-miR-203 in haematological malignancies. *J Cell Mol Med* 15(12):2760–2767
37. Chim CS, Wong KY, Qi Y, Loong F, Lam WL, Wong LG et al (2010) Epigenetic inactivation of the miR-34a in hematological malignancies. *Carcinogenesis* 31(4):745–750
38. Wong KY, So CC, Loong F, Chung LP, Lam WW, Liang R et al (2011) Epigenetic inactivation of the miR-124-1 in haematological malignancies. *PLoS One* 6(4):e19027
39. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S et al (2008) The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol* 10(2):202–210
40. Ma L, Teruya-Feldstein J, Weinberg RA (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449(7163):682–688
41. Lujambio A, Calin GA, Villanueva A, Ropero S, Sánchez-Céspedes M, Blanco D et al (2008) A microRNA DNA methylation signature for human cancer metastasis. *Proc Natl Acad Sci USA* 105(36):13556–13561
42. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E et al (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* 104(40):15805–15810
43. Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F et al (2008) MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* 111(6):3183–3189
44. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, Schwind S, Pang J et al (2009) MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* 113(25):6411–6418
45. Duursma AM, Kedde M, Schrier M, le Sage C, Agami R et al (2008) miR-148 targets human DNMT3b protein coding region. *RNA* 14(5):872–877
46. Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, Schoeftner S et al (2008) A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nat Struct Mol Biol* 15(3):268–279
47. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG et al (2008) MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat Struct Mol Biol* 15(3):259–267
48. Huang J, Wang Y, Guo Y, Sun S et al (2010) Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology* 52(1):60–70
49. Houbaviy HB, Murray MF, Sharp PA (2003) Embryonic stem cell-specific MicroRNAs. *Dev Cell* 5(2):351–358

50. Liu L, Bailey SM, Okuka M, Muñoz P, Li C, Zhou L et al (2007) Telomere lengthening early in development. *Nat Cell Biol* 9(12):1436–1441
51. Braconi C, Huang N, Patel T (2010) MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* 51(3):881–890
52. Meng F, Wehbe-Janek H, Henson R, Smith H, Patel T et al (2008) Epigenetic regulation of microRNA-370 by interleukin-6 in malignant human cholangiocytes. *Oncogene* 27(3):378–386
53. Das S, Foley N, Bryan K, Watters KM, Bray I, Murphy DM et al (2010) MicroRNA mediates DNA demethylation events triggered by retinoic acid during neuroblastoma cell differentiation. *Cancer Res* 70(20):7874–7881
54. Lu F, Stedman W, Yousef M, Renne R, Lieberman PM et al (2010) Epigenetic regulation of Kaposi's sarcoma-associated herpesvirus latency by virus-encoded microRNAs that target Rta and the cellular Rbl2-DNMT pathway. *J Virol* 84(6):2697–2706
55. Wang H, Wu J, Meng X, Ying X, Zuo Y, Liu R et al (2011) MicroRNA-342 inhibits colorectal cancer cell proliferation and invasion by directly targeting DNA methyltransferase 1. *Carcinogenesis* 32(7):1033–1042
56. Ji W, Yang L, Yuan J, Yang L, Zhang M, Qi D et al (2013) MicroRNA-152 targets DNA methyltransferase 1 in NiS-transformed cells via a feedback mechanism. *Carcinogenesis* 34(2):446–453. doi:[10.1093/carcin/bgs343](https://doi.org/10.1093/carcin/bgs343)
57. Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I et al (2006) The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett* 580(17):4214–4217
58. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM et al (2006) The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38(2):228–233
59. Noonan EJ, Place RF, Pookot D, Basak S, Whitson JM, Hirata H et al (2009) miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene* 28(14):1714–1724
60. Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI et al (2009) Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. *Oncogene* 28(46):4065–4074
61. Roccaro AM, Sacco A, Jia X, Azab AK, Maiso P, Ngo HT et al (2010) microRNA-dependent modulation of histone acetylation in Waldenstrom macroglobulinemia. *Blood* 116(9):1506–1514
62. Jeon HS, Lee SY, Lee EJ, Yun SC, Cha EJ, Choi E et al (2012) Combining microRNA-449a/b with a HDAC inhibitor has a synergistic effect on growth arrest in lung cancer. *Lung Cancer* 76(2):171–176
63. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B et al (2008) Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science* 322(5908):1695–1699
64. Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W et al (2009) The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. *Cancer Res* 69(6):2623–2629
65. Smits M, Nilsson J, Mir SE, van der Stoop PM, Hulleman E, Niers JM et al (2010) miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. *Oncotarget* 1(8):710–720
66. Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, Xia YJ et al (2010) MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. *Eur J Cancer* 46(12):2295–2303
67. Zhang JG, Guo JF, Liu DL, Liu Q, Wang JJ et al (2011) MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2. *J Thorac Oncol* 6(4):671–678
68. Cao P, Deng Z, Wan M, Huang W, Cramer SD, Xu J et al (2010) MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1 alpha/HIF-1 beta. *Mol Cancer* 9:108. doi:<http://dx.doi.org/10.1016/j.canlet.2012.12.006>

69. Sander S, Bullinger L, Klapproth K, Fiedler K, Kestler HA, Barth TF et al (2008) MYC stimulates EZH2 expression by repression of its negative regulator miR-26a. *Blood* 112(10):4202–4212
70. Juan AH, Kumar RM, Marx JG, Young RA, Sartorelli V et al (2009) Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. *Mol Cell* 36(1):61–74
71. Alajez NM, Shi W, Hui AB, Bruce J, Lenarduzzi M, Ito E et al (2010) Enhancer of zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. *Cell Death Dis* 1:e85. doi:[10.1038/cddis.2010.64](https://doi.org/10.1038/cddis.2010.64)
72. Chiang CW, Huang Y, Leong KW, Chen LC, Chen HC, Chen SJ et al (2010) PKC α mediated induction of miR-101 in human hepatoma HepG2 cells. *J Biomed Sci* 17:35
73. Smits M, Mir SE, Nilsson RJ, van der Stoop PM, Niers JM, Marquez VE et al (2011) Down-regulation of miR-101 in endothelial cells promotes blood vessel formation through reduced repression of EZH2. *PLoS One* 6(1):e16282

Epigenetics and Cancer

Sarkar, F.H. (Ed.)

2013, X, 287 p. 36 illus., 28 illus. in color.,

ISBN: 978-94-007-6612-9