

Chapter 2

Epigenetics, MicroRNAs and Human Cancer

Jiazeng Xia, Xiaoqiang Guo, and Kaiyuan Deng

Contents

1	Brief Introduction of Epigenetics and Common Epigenetic Mechanisms.....	30
1.1	DNA Methylation	30
1.2	Histone Modifications.....	31
1.2.1	Histone Acetylation	32
1.2.2	Histone Methylation.....	33
1.2.3	Other Histone Modifications.....	35
2	The Role of Epigenetic Changes in Cancer	35
3	Epigenetic Regulation of MicroRNAs in Cancer.....	37
3.1	MiRNA.....	37
3.2	Mechanisms of miRNA Deregulation in Human Cancer	38
3.3	Epigenetic Regulation of miRNAs in Cancers.....	40
4	MicroRNAs Can Regulate the Epigenetic Effectors in Cancer	45
5	Clinical Utility of Epigenetically Silenced MicroRNAs in Cancer	49
6	Concluding Remarks.....	50
	References.....	51

Abstract Classical genetics alone cannot explain how cancer occurs pretty well, and the proposal of concept of epigenetics provides a partial explanation about the cause of cancers. DNA methylation and histone modifications are the best-known epigenetic marks. MicroRNAs (miRNAs), a class of endogenous, single-stranded, non-coding small RNA with 18–22 nucleotides in length, play a critical role in initiation, progression, metastasis and invasion of cancers. It is widely recognized that deregulation of miRNAs is a hallmark of cancer. The expression of miRNAs can be regulated by several mechanisms, including epigenetic changes. Furthermore, it has been discovered that a subgroup of miRNAs, which are known as epi-miRNAs,

J. Xia (✉) • X. Guo • K. Deng

Department of General Surgery and Center of Translational Medicine, Nanjing Medical University Affiliated Wuxi Second Hospital, 214002 Wuxi, Jiangsu, China

e-mail: jiazengxia@yahoo.com

can regulate the expression of effectors of the epigenetic mechanisms by directly or indirectly targeting these epigenetic-modifying enzymes and molecules. This chapter will focus on how epigenetic changes regulate the miRNAs expression as well as how epi-miRNAs affect the epigenome, and how to translate these findings into clinical application.

Keywords Epigenetics • DNA methylation • Histone modifications • MicroRNA • Human cancer

1 Brief Introduction of Epigenetics and Common Epigenetic Mechanisms

The human genome contains two types of genetic information: one is genetic information in the traditional sense; the other is the epigenetic information. In 1942, the term “epigenetics” was proposed for the first time by Waddington in a paper entitled “The epigenotype Endeavour”, and it was defined as “the causal interactions between genes and their products which bring the phenotype into being” (Waddington 2012). “Epigenetics” is derived from “epigenesis” and can be split into two parts, which are “epi” and “genetics”. “Epi” is a Greek prefix that means “above” or beyond, hence the word indicated that epigenetic events were needed to be studied above or beyond genetics. However, the word “epigenetics” was rarely mentioned in the following three decades. Even if it was mentioned, the meaning of it was not the same as the definition proposed by Waddington. In 1980s, some scholars began to use the word “epigenetics” in the same meaning as Waddington’s definition. By the early 1990s, epigenetics entered into the fast development period. With a deeper knowledge of the phenomenon of epigenetics, the meaning of “epigenetics” has been constantly evolved. Nowadays, the meaning of “epigenetics” is different from the definition given by Waddington. At present, the widely accepted definition of “epigenetic” is that there are mitotically and potential meiotically heritable alternations in gene expression without any concomitant changes in original DNA sequence (Taby and Issa 2010). In other words, the phenotype changes while genotype does not change. The epigenetics mainly includes three characteristics: heritability, reversibility and no underlying DNA sequence alteration. There are several epigenetic modifications that can influence DNA, RNA or protein expression, the most common of which are DNA methylation and histone modifications.

1.1 DNA Methylation

DNA methylation is the epigenetic change which is studied earliest and most completely. It occurs when a methyl group from active methylene compounds, such as S-adenosylmethionine (SAM), is transferred into C5 position of cytosine ring in CpG dinucleotide under the catalysis of DNA methyltransferases (DNMTs). There

are two kinds of distribution of CpG dinucleotides in human genome: genome-wide dispersed distribution and local concentration distribution. Approximately 80 % of CpG dinucleotides are genome-wide dispersed distribution and are usually located in repetitive DNA sequences, such as LINE and Alu sequences, and the other CpG dinucleotides are mainly concentrated in CpG islands (CGI). CGI, a 1–2 kb genomic region with CpG dinucleotides cluster in mammals, is mainly located in promoter region of approximately 50 % of human genes, but sometimes it can also be found in the first exon or 5' untranslated region of genes (Dunn 2003). In a healthy cell, the genome-wide scattered distribution of CpG dinucleotides are always heavily methylated, whereas the CpG dinucleotides in CGI are protected from methylation. Aberrant DNA methylation of CGI has been shown to play an important role in gene silencing, genomic imprinting, inactivation of X chromosome in women and carcinogenesis (Bird 2002). In addition, DNA methylation of CGI in promoter region can regulate expression of genes through repression of transcription factors, such as E2F, binding to corresponding locus of DNA sequences and recruitment of methyl-CpG binding domain proteins (MBDs), including MBD1–4 and MeCP2, which can recruit enzymatic machinery to establish silent chromatin (Campanero et al. 2000; Bogdanovic and Veenstra 2009).

The addition of a methyl group to C5 position of cytosine is catalyzed by a family of enzymes, DNMTs. The active DNMTs mainly include DNMT1, DNMT3a and DNMT3b. DNMT1, as a maintenance methyltransferase, plays a critical role in maintaining the methylation patterns via replication of methylation patterns during S phase of mitosis (Leonhardt et al. 1992). Nevertheless, DNMT3a and DNMT3b act as *de novo* methyltransferases that methylate the unmethylated genomic regions (Okano et al. 1999). Hence, DNMT1 can unite with DNMT3a/3b to establish and maintain the DNA methylation patterns. DNMT2 is also the member of DNMT family, but its catalytic activity is very weak. DNA methyltransferase 3-like (DNMT3L) is a member of DNMT3 family, and it has been found to enhance the catalytic activity of DNMT3a/3b through binding to their catalytic domains (Gowher et al. 2005). DNA methylation is a reversible process, and DNMT inhibitors can be used to reduce the level of methylation. Among these inhibitors, 5-aza-2'-deoxycytidine (5-aza-dC) may be the most commonly used one. The 5-aza-dC is a cytidine analog, and inhibits the function of DNMTs by covalently bonded with them.

1.2 Histone Modifications

In eukaryotic cells, the basic unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around two copies of each of the core histones H2A, H2B, H3 and H4. Moreover, H1, known as a linker histone, plays a role in linking nucleosome to each other. In the early 1960s, Allfrey first reported the acetylation and methylation of histones and supposed that the two histone modifications might play role in regulation of transcription (Allfrey et al. 1964). Through studies for almost half century, we have known that there are many other post-translational histone

modifications. Moreover, in addition to the effect on transcription, histone modifications have also been found to be associated with various other fundamental biological processes, such as DNA repair, DNA replication and chromosome condensation (Kouzarides 2007). C-terminal globular domain of histones is involved in interactions between histones and organization of the DNA wrapped around the histones (Peterson and Laniel 2004), whereas the N-terminal tails can undergo a variety of post-translational modifications, including acetylation, methylation, phosphorylation, sumoylation, ubiquitination, ADP ribosylation, etc. By these chemical modifications, histone proteins can store epigenetic information, which is defined as “histone code”, and induce the switch versus heterochromatin or euchromatin (Iorio et al. 2010). So far, the most studied histone modifications should be methylation and acetylation of special residues on histones H3 and H4. Moreover, several enzymes have been found to play the role in activation or repression of transcription through transferring or removing methyl group and acetyl group to histones, including histone methyl-transferases (HMTs), histone de-methylases (HDMs), histone acetyl-transferases (HATs) and histone de-acetylases (HDACs).

1.2.1 Histone Acetylation

Since the first report by Allfery et al. (1964), histone acetylation has been paid more and more attention. It has been found that the lysine's positive charge can be neutralized by combined with acetyl groups with negative charge, which may decrease the affinity between Histone octamer and DNA (Hong et al. 1993). Hence, histone acetylation can lead to relaxation of nucleosome structure, allowing that the transcription factors and cofactors bind to DNA binding sites, which active the gene transcription. As we known, histone acetylation that occurs at lysine residues in N-terminal tails is a dynamic process, and it is reversible. Histone acetylation is regulated by two enzymatic families, HATs and HDACs, which play the opposite effect. HATs can add the acetyl group to ϵ -amino group of lysine residues with the help of acetyl-CoA cofactor, and induces activation of transcription. According to the acting position and function, HATs can be divided into two types: the cytoplasmic type-B HATs which acetylate free histone and the nuclear type-A HATs which acetylate nucleosome histones and play a major role in transcriptional regulation. In addition to acetylate histones, HATs have also been found to acetylate non-histone proteins, such as p53 (Glozak et al. 2005). Compared with type-B HATs, type-A HATs are more diversified. Type-A HATs can be divided into three major families based on the sequence homology and structure: general control non-derepressible 5 (GCN5)-related N-acetyl-transferase (GNAT), MYST family and p300/CREB-binding protein (CBP) (Hodawadekar and Marmorstein 2007). These enzymes have been found to exist in multisubunit complexes, in which the specific functions of catalytic subunits are regulated by the other non-catalytic subunits (Lee and Workman 2007). Different HAT complexes that are made up of unique substrates generally have different residences and distinctive features, whereas several complexes which share many same subunits can also have specific functions. For

example, SAGA complex and NuA3 complex have overlapped substrates, but the former gives priority to modify H3K9 and the latter preferentially modifies H3K14 (John et al. 2000).

Nevertheless, HDACs are a family of enzymes that reverse the acetylation of histone proteins through removing the acetyl group from lysine residues, which can induce euchromosome into heterochromosome, resulting in repression of transcription. Based on the sequence homology as well as structural and functional differences, HDACs can be divided into four classes: Classes I, II, III and IV (Brandl et al. 2009). Class I HDACs that include HDAC 1–3 and 8 are homologous to yeast *scRpd3* and are widely expressed, whereas Class II HDACs that include HDAC 4–7 and HDAC 9–10 are most closely related to yeast *scHda1*. Moreover, according to domain organization, Class II HDACs can be further subdivided into Class IIa HDACs which include HDAC 4, 5, 7 and 9 and Class IIb HDACs which include HDAC 6 and 10, and they are expressed in a cell-special manner. Class IV HDACs only have one member, HDAC11, and its function is poorly understood. Unlike the above mentioned three classes that require a zinc metal ion to play a catalytic role, the Class III HDACs, including sirtuin 1–7, that are homologous to yeast *scSir2* have deacetylase activity depending on a specific cofactor, NAD (nicotinamide adenine dinucleotide)⁺. Similar to HATs, HDACs can target histone proteins as well as non-histone proteins, and play catalytic activity by existing in multisubunit complexes, such as Sin3, NCoR/SMR, NuRD and CoREST (Hayakawa and Nakayama 2011).

1.2.2 Histone Methylation

Histone methylation is another common histone modification. Different from histone acetylation, histone methylation does not neutralize the positive charge of histones, and it can not only occur on lysine residues, but also on the arginine and histidine residues. Lysine residues can be mono-, di-, or tri-methylated, and arginine residues can be mono-methylated, symmetrically or asymmetrically di-methylated. Histidine residues have also been found to be mono-methylated, whereas this type of methylation is seldom studied. The histone methylation which occurs on the lysine residues may be the most widely studied one, and the methylated sites of lysine mainly include H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79, and H4K20 (Greer and Shi 2012). The addition of a methyl group from SAM to ϵ -amino group of lysine residues is catalyzed by a family of enzymes, histone lysine methyltransferases (HKMTs). Since the first HKMT, SUV39H1, which can specifically methylate H3K9 was reported in human (Rea et al. 2000), a number of HKMTs have been found. HKMTs can be divided into two types: SET domain-containing HKMTs and non-SET domain HKMTs. Almost all of the HKMTs contain the SET domain which harbours the methyltransferase activity, whereas DOT1-like protein (DOT1L) as the unique H3K79 methyltransferase does not contain the SET domain. The reason for the structural difference of DOT1L is not sufficiently clear, but DOT1L-mediated H3K79 methylation has been found to be involved in regulation of gene expression, regulation of cell cycle and DNA damage response, etc. (Nguyen and Zhang 2011).

Calmodulin methyltransferase is another non-SET domain HKMT, and it has been reported to trimethylate Lys-115 (Magnani et al. 2010). HKMTs can specifically target lysine residues and modify the certain lysines to certain degree of methylation. For example, human HKMT SET7/9 targets H3K4 and can exclusively mono-methylate the target (Xiao et al. 2003). Arginine methylation is also an important type of histone methylation, and the methylated sites of arginine mainly include H3 arginine 2 (H3R2), H3R8, H3R17, H3R26 and H4R3 (Greer and Shi 2012). Arginine methylation has been found to play a critical role in many cellular processes, such as transcriptional regulation, DNA repair, RNA process, cellular proliferation and signal transduction (Wolf 2009). The addition of a methyl group from SAM to arginine's guanidiny group is catalyzed by a family of enzymes, protein arginine methyltransferases (PRMTs). So far, 11 human PRMTs, PRMT1-11, have been identified. PRMTs can be divided into type I, type II or type III. Type I and type II PRMTs can mono- and di-methylate the arginine's ω -guanidino nitrogen atoms in human, whereas the type III PRMTs enzymes mono-methylate the arginine's δ -guanidino nitrogen atom and are only found in yeast. In humans, both type I PRMTs, including PRMT1, PRMT3, PRMT4/CARM1, PRMT6 and PRMT8, and type II PRMTs, including PRMT5, PRMT7 and PRMT9, can catalyze the production of ω - N^G -monomethylarginine (MMA), but type I PRMTs further produce asymmetric ω - N^G , N^G -dimethylarginine (ADMA) and type II PRMTs further produce symmetric ω - N^G , N'^G -dimethylarginine (SDMA). Nevertheless, the type of the remaining three PRMTs, PRMT2, 10 and 11, is still not confirmed.

Histone methylation is not identified as a dynamic and reversible modification until the identification of the first HDM, lysine-specific histone demethylase 1 (LSD1), in 2004 (Shi et al. 2004). There are two families of HDMs that have been found: LSD family with two members, LSD1 and LSD2, and JMJC demethylases which contain JmjC domain. Due to the fact that LSD family needs a free electron pair at the methylated lysines to initiate demethylation, LSD1 and LSD2 are only able to demethylate the mono- or di-methyllysine. It has been found that LSD1/2 catalyzes demethylation through the amine oxidation reaction which is flavin adenine dinucleotide (FAD)-dependent. Since JmjC domain-containing histone demethylase 1 (JHDM1) that can specifically target H3K36 was discovered in 2006 (Tsukada et al. 2006), another family of HDMs, JMJC family, has been identified. The demethylation reaction catalyzed by JMJC family is an iron-dependent and α -ketoglutarate-dependent dioxygenase reaction. Unlike LSD family that needs protonated nitrogen for demethylation reaction and, hence, JMJC family can also remove methyl groups from tri-methylated substrates. The first identified JmjC domain proteins that can reverse the tri-methylation status of histone Lysines were JMJD2 family of proteins, which are able to catalyze demethylation of tri-methylated H3K9 and H3K36 (Whetstine et al. 2006). To date, the members of JMJC family have been found to target Lys4, 9, 27 and 36 on H3 and Lys20 on H4 (Iwase et al. 2007; Agger et al. 2007; Whetstine et al. 2006; Tsukada et al. 2006; Liu et al. 2010). Nevertheless, the histone arginine demethylases are still poorly understood.

1.2.3 Other Histone Modifications

There are also other post-translational modifications that can target histone proteins, including phosphorylation, ubiquitination and ADP ribosylation. Histone phosphorylation can target serine, threonine and tyrosine residues, and it is regulated by two enzymes, kinases and phosphatases, which have opposite effects. Kinases can result in phosphorylation of histones, whereas phosphatases are involved in dephosphorylation. Similar to histone acetylation, phosphorylation of specific sites can neutralize the positive charge of histone, which may influence the stability of chromatin structure. Histone phosphorylation can occur on four nucleosome core histones as well as histone H1, and it usually interplays with other post-translational modifications, called crosstalk, which is correlated with a number of cellular events, such as chromosome condensation, DNA repair, transcriptional regulation and cell cycle progression (Banerjee and Chakravarti 2011). It has been found that histones can also be modified by ubiquitin, a 76-amino-acid protein. Histone ubiquitination occurs on highly conserved lysine residues, and is catalyzed by three kinds of enzymes, ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3) (Pickart 2001). Histones can be mono-ubiquitinated and poly-ubiquitinated, and all types of histone proteins have been found to be modified by ubiquitin. Among these ubiquitinated histones, mono-ubiquitinated H2A and H2B may be the most studied. Histone ubiquitination is also a dynamic and reversible modification, and it is mainly removed from histone lysines by a class of cysteine proteases, namely deubiquitinating enzymes (DUBs). ADP-ribosylation of histones is also a reversible post-translational modification, and it is associated with cellular process, such as DNA repair, cell cycle regulation, replication or transcription. ADP-ribosylation of histones occurs when an ADP-ribosyl group from NAD⁺ is transferred to specific amino acid residues of histone tails or to histone-bound ADP-ribose under the catalysis of ADP-ribosyltransferases (ARTs), which lead to mono- ADP-ribosylation and poly-ADP-ribosylation, respectively. Histone ADP-ribosylation can be degraded by two families of enzymes: ADP-ribosylhydrolases (ARHs) and poly-ADP-ribose-glycohydrolases (PARGs). So far, only one RARG and three ARHs, ARH1-3, have been identified in human (Oka et al. 2006; Koch-Nolte et al. 2008; Mortusewicz et al. 2011).

2 The Role of Epigenetic Changes in Cancer

It is well known that tumorigenesis is a multistep process, which is involved in genetic and epigenetic alterations. Growing evidence has indicated that epigenetic modifications play critical roles in carcinogenesis. In cancers, aberrant methylation is classified into hypermethylation and hypomethylation. DNA hypomethylation is mostly genomic hypomethylation which often occurs in DNA-repetitive regions. Nevertheless, there is also gene-specific hypomethylation which usually occurs in promoter-associated CGI. Global hypomethylation results in genome instability, loss of imprinting and

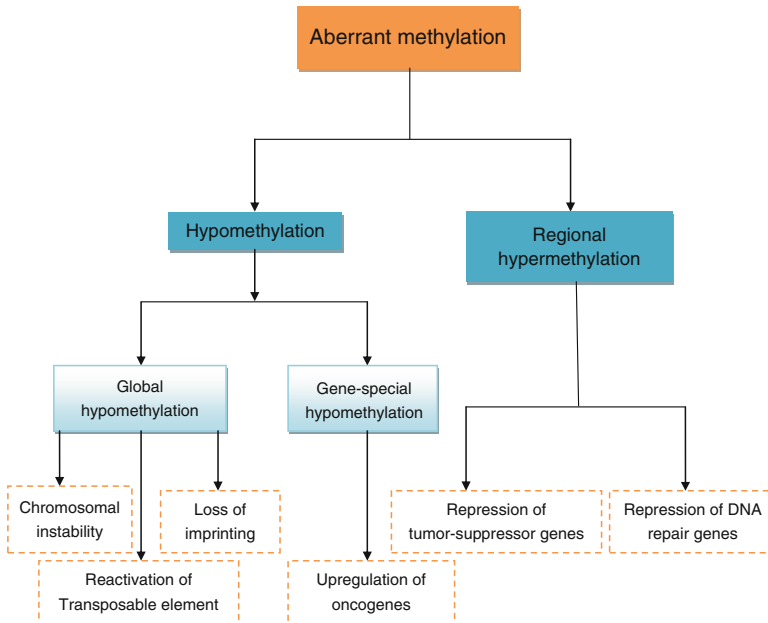


Fig. 2.1 Two types of aberrant methylation and their functional outcomes

reactivation of transposable elements (Dunn 2003; Eden et al. 2003), and gene-specific hypomethylation is associated with aberrant expression of oncogenes (Kwon et al. 2011; Tsai et al. 2010). However, the aberrant hypermethylation in CGI leads to transcription-silencing of tumor suppressor genes or DNA repair genes, such as tumor suppressor gene P16 and DNA repair gene MLH1 (Hossain et al. 2012), which contributes to tumor formation (Fig. 2.1). Moreover, based on the two-hit hypothesis, hypermethylation of tumor suppressor genes may act as the second hit after genes mutation, which is the first hit (Knudson 2001). In addition to aberrant DNA methylation, cancer cells also suffer from disruption of histone post-translational modifications. Understanding the critical roles of histone modifications in fundamental biological processes, such as transcription regulation, DNA repair, DNA replication and chromosome condensation, it is not surprising that aberrant histone modifications can be linked to carcinogenesis. Nevertheless, compared with DNA methylation, the information regarding histone modifications in cancers is limited, and only a fraction of modified residues in histone tails have been identified to be associated with carcinogenesis so far. In addition, a few histone modifiers, such as HDACs, HMTs, HDMs and HMT Polycomb group protein, EZH2, have been found to present abnormal expression in cancer, which may alter the levels and patterns of histone modifications and turn out to be deregulation of chromatin-based processes, finally resulting in development and progression of cancer. Furthermore, there is an epigenetic regulatory crosstalk between histone modifications and DNA methylation, which is associated with transcription regulation and abnormal silencing of genes in cancer (Vaissiere

et al. 2008). With the development of research on critical role of microRNAs (miRNAs) in tumorigenesis, the complicated network between epigenetic regulation and gene expression has become more complex.

3 Epigenetic Regulation of MicroRNAs in Cancer

3.1 *MiRNA*

Noncoding RNAs also belong to category of epigenetics. Noncoding RNAs (ncRNAs) can be divided into small (under 200 nucleotides) or large ncRNAs. Moreover, small ncRNAs can be further subdivided into microRNAs (miRNAs), endogenous small interfering RNAs (endo-siRNAs), PIWI-interacting RNAs (piRNAs) and small nucleolar RNAs (snoRNAs). Owing to the critical role of miRNAs in the process of life, this class of small ncRNAs has been attracting more and more attention from researchers. MiRNAs is a class of endogenous, single-stranded, small ncRNAs with ~22 nucleotides (nts) in length, which remain highly conservative in phylogeny. Since the first report in 1993 and truly recognized in early 2000 (Lee et al. 1993; Reinhart et al. 2000), miRNAs has been one of the most popular and fastest growing research areas in molecular biology. So far, there have been more than 1,000 miRNAs identified in human, which regulate approximately 30 % of human protein-coding genes (Filipowicz et al. 2008). MiRNAs can modulate gene expression at post-transcriptional level through base pairing to mRNAs. In mammals, miRNAs mainly sequence-specifically bind to 3'-untranslated region (3'UTR) of target mRNAs. The perfect complementarity between miRNA and target mRNA can trigger RNA interference (RNAi), which results in mRNA cleavage. However, partial complementarity between them leads to translational inhibition of target mRNAs. Recently, some studies showed that miRNAs could bind to 5'-untranslated region (5'UTR) and protein-coding region of several mRNAs and, however, their functions require to be further studied (Lal et al. 2009; Lee et al. 2009).

Due to the characteristic that miRNAs can bind to 3'UTR of target mRNAs by partial complementarity, single miRNA may target a number of mRNAs and, in contrast, one mRNA may be the targets of many miRNAs. Therefore, abnormal expression of miRNAs may affect the normal expression of numerous genes and ultimately deregulate the control of biological processes, resulting in development and progression of cancer. Moreover, genome-wide studies have indicated that approximately 50 % of miRNAs are located at genomic regions of loss of heterozygosity or amplification, fragile sites of chromosomes or other cancer-associated regions (Calin et al. 2004b), which further confirms the critical role of miRNAs in carcinogenesis. Since deregulation of two miRNAs, miR-15 and miR-16, was first reported in chronic lymphocytic leukemia (CLL) (Calin et al. 2002), a number of genome-wide profiling studies have identified signatures with deregulated miRNAs in a variety of cancers. Further study on these deregulated miRNAs indicated that they have dual role in carcinogenesis as new oncogenes or tumor suppressor genes.

For instance, miR-21 is universal over-expressed in cancers, and it acts as oncogene through inhibiting the expression of targets genes which can regulate cellular proliferation, differentiation, and apoptosis as tumor suppressor proteins, such as phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4) (Frankel et al. 2008; Meng et al. 2007). However, the miR-34 family, including miR-34a and miR-34b/c, targeted by p53 is universal downregulated in a variety of cancers, and the miR-34 members can play the role of tumor suppressor genes by regulating the expression of their targets. For example, the expression of miR-34a and miR-34c have been identified to be significantly down-regulated in breast cancers with lymph node metastasis, and reactivation of miR-34a/c can inhibit the metastasis and invasion of breast cancer through directly binding to 3'UTR of Fos-related antigen 1 (Fra-1) oncogene and repressing its expression (Yang et al. 2012). Interestingly, a few miRNAs can function as tumor suppressor genes in some cancers, while they can act as oncogenes in other cancers. For instance, miR-25 has been found to be down-regulated in human colon cancer, and it could inhibit growth and migration of colon cancer cells via repression of a direct target, Smad7 (Li et al. 2013). Nevertheless, another report found that miR-25 presented over-expression in esophageal squamous cell carcinoma (ESCC), and it might induce migration and invasion of ESCC through directly targeting 3'UTR of E-cadherin (CDH1) and consequently inhibiting the expression of CDH1 (Xu et al. 2012). It demonstrates that deregulation of some miRNAs may be tissue specific.

Increased data has demonstrated that miRNAs play critical roles in cellular processes associated with differentiation, proliferation, apoptosis, metastasis and invasion, and aberrant expression of which may be associated with development, progression and prognosis of cancers. Hence, it is necessary to investigate the mechanisms which lead to aberrant miRNAs expression in cancer, and a large number of studies have been conducted to explore the regulatory mechanisms for miRNAs.

3.2 Mechanisms of miRNA Deregulation in Human Cancer

Recent high-throughput studies have shown that the expression of miRNAs is deregulated in most cancer types. Some studies suggest that miRNA expression may be widely down-regulated in human tumors relative to normal tissues, whereas other studies demonstrate a tumor-specific mixed pattern of down-regulation and up-regulation of miRNA genes. Even in the same cancer type, some studies have also shown that miRNA expression signatures are associated not only with specific tumor subtypes but with clinical outcomes as well. However, the underlying mechanisms of miRNA deregulation in human cancer are still not thoroughly understood. Increasing evidence indicates that transcriptional deregulations, epigenetic alterations, mutations, DNA copy number abnormalities and defects in the miRNA biogenesis machinery might be the possible mechanisms, these mechanisms may each contribute, either alone or more likely together to miRNA deregulation in human cancer (Deng et al. 2008). Furthermore, expression of miRNAs can also be affected by other miRNAs, thus creating a complex level of reciprocal interaction and regulation (Iorio and Croce 2012) (Fig. 2.2).

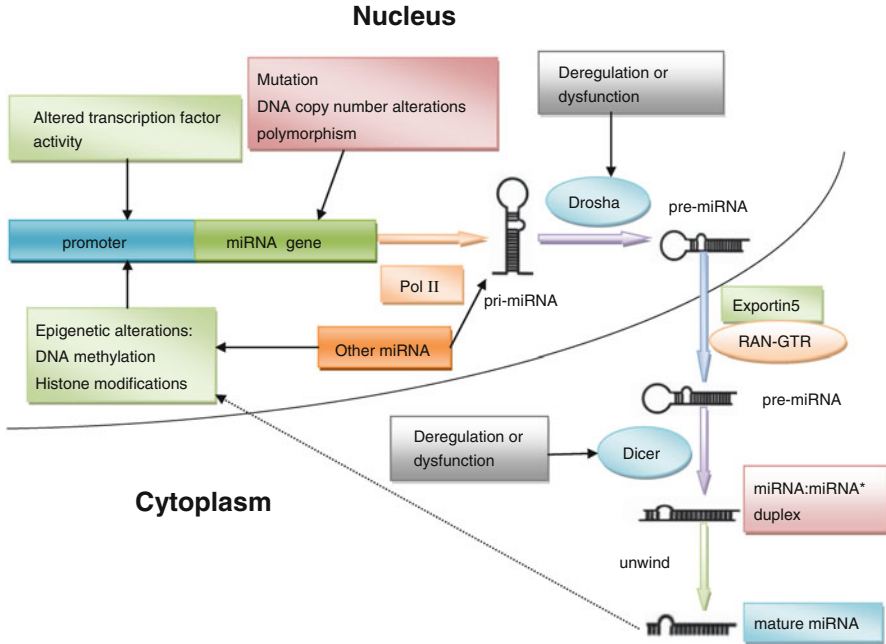


Fig. 2.2 Mechanisms of microRNA deregulation in human cancers

Transcriptional regulation is one of the important mechanisms in the control of miRNAs expression, which is based on the fact that most miRNA genes are derived from primary miRNA transcripts (pri-miRNAs) produced by Pol II and contain a 5' cap and a poly (A) tail. For example, miR-34a family has been shown to be directly induced by the tumor suppressor p53, and p53 inactivation is thought to decrease miR-34 expression in human cancers (He et al. 2007b). C-Myc oncogene and transcriptional factor HIF are amplified and over-expressed in several types of human cancers, which might contribute to up-regulated miR-17-92 expression and miR-210 expression in cancer, respectively (Chang et al. 2008; Camps et al. 2008). Taken together, the increasing evidence shows that deregulation of miRNAs can be a result of increased or decreased transcription due to an altered transcription factor activity.

Unlike protein-coding genes, at present information on the mutation and polymorphism of miRNAs in cancer is just emerging, but the studies are increasing. For example, inherited mutations in the primary transcripts of miR-15a and miR-16-1 were found to be responsible for the reduced expression of the two miRNAs *in vitro* and *in vivo* in CLL (Calin et al. 2004a). In solid human tumors one sequence variation in a miRNA precursor and 15 variations in primary miRNAs were also identified, but no functional consequence was observed as the results of these aberrations (Diederichs and Haber 2006). Other studies suggested that polymorphism of miRNAs might be associated with hepatocellular carcinoma (HCC) or breast cancer. These data showed that genetic structural genetic alterations contribute to the deregulation of miRNAs as it does to other protein-coding genes.

In addition, DNA copy number alterations of miRNAs are frequently found in cancer and may account in part for the miRNA gene deregulation. The first evidence was that miR-16-1 and miR-15a at 13q14 were deleted in more than 50 % of the CLL patients, with concurrent reduced expression in ~65 % patients (Calin et al. 2002). A recent study has also shown that in advanced ovarian tumors genomic copy number loss may account for the down-regulation of approximately 15 % of miRNAs (Zhang et al. 2008). Moreover, some key proteins in the miRNAs biogenesis pathway may be dysfunction or deregulated in cancer, thus may further enhance tumorigenesis. For example, conditional deletion of Dicer1 enhanced tumor development in a *K-Ras*-induced mouse model of lung cancer (Kumar et al. 2007). However, this mechanism might be tissue or cancer specific, because another study has shown that in ovarian cancer, Droscha and Dicer are not altered in expression levels of either mRNA or protein, therefore, functional assays might have important implications to examine the activities of these key proteins (Zhang et al. 2008). Furthermore, similar to protein-coding genes, miRNAs expression can also be modulated by epigenetic mechanisms, such as DNA methylation and histone acetylation.

3.3 *Epigenetic Regulation of miRNAs in Cancers*

The first evidence regarding deregulation of miRNAs due to epigenetic mechanisms was reported by Scott et al. (2006). Using miRNA array analysis, they found that the expression of 27 miRNAs in SKBr3 cell line altered rapidly by treatment of this cell line with an HDAC inhibitor, LAQ824, suggesting the relationship between epigenetic factors and miRNAs expression. Shortly thereafter, Saito et al. (2006) reported that 17 of 313 miRNAs in T24 bladder cancer cells were up-regulated after treatment of the cells with a DNMT inhibitor, 5-aza-dC, and a HDAC inhibitor, 4-phenylbutyric acid (4-PBA). Among these up-regulated miRNAs, miR-127, as a member of a miRNA cluster which also includes miR-136 and miR-431-433, is expressed in normal tissues, but it is down-regulated or silenced in T24 cells and many primary tumor tissues, such as bladder, prostate and colon cancers. MiR-127 is located in a CGI, and its re-expression is induced by DNA de-methylation, histone H3 acetylation and trimethylation of H3K4 after treated with two epigenetic drugs. However, either 5-aza-dC or 4-PBA used alone had no function, which indicated that combined effects of histone modification and DNA methylation are necessary to regulate miR-127 expression. Moreover, the human oncogene B-cell lymphoma 6 (BCL6) may be a potential target of miR-127, and re-expression of miR-127 led to down-regulation of BCL6, indicating that miR-127 can function as a tumor suppressor gene. Thus, pharmacological unmasking by DNMT and/or HDAC inhibitors is often used to identify the epigenetically silenced miRNAs in various cancers. In addition, genetic unmasking, such as knockout of DNMTs, is also a common method. Moreover, with the development of technology, methylation status of miRNAs in cancer cells can be identified more intuitively by genome-wide DNA methylation analysis.

Recently, a large number of studies on epigenetic silencing of miRNAs have been reported in various cancers, and the relationship between aberrant DNA methylation and deregulated miRNAs may be most frequently and deeply studied. CGI hypermethylation is identified as an epigenetic aberration resulting in silencing of miRNAs which act as tumor suppressor genes. As previously mentioned, the miR-34 family is targeted by transcriptional factor p53, and this family has been found to epigenetically inactivate due to CGI hypermethylation in various cancers. MiR-34a is located on chromosomal locus 1p36 and, however, miR-34b and miR-34c as dicistronic cluster in one transcription unit, BC021736, locate on chromosome 11q23. Inactivation of both miR-34a and miR-34b/c due to abnormal promoter-associated CGI methylation has been identified in multiple cancer cell lines and primary tumors (Vogt et al. 2011). Re-expression of miR-34 family members can induce cell cycle arrest at G1 phase, apoptosis and senescence, and inhibit migration or invasion (Hermeking 2010). MiR-34 family members play the above-mentioned potential roles by down-regulation of candidate target genes as, for instance, Bcl2, E2F transcription factor-3 (E2F3), c-MYC, hepatocyte growth factor receptor (MET), cyclin E2 (CCNE2), CD44 and cyclin-dependent kinase 4/6 (CDK4/6) (Yamakuchi et al. 2008; Bommer et al. 2007; Lujambio et al. 2008; He et al. 2007a; Liu et al. 2011). Moreover, it has been found that DNA methylation of miR-34b/c is related to *H. pylori* infection in normal individuals, and the methylation levels of miR-34b/c in non-cancerous gastric mucosae of patients with multiple GC are higher than that of patients with single GC, suggesting that methylation of miR-34b/c was involved in epigenetic field defect (Suzuki et al. 2010). Similar to miR-34b/c, *H. pylori* infection induces promoter methylation of miR-124 family (miR-124-1, miR-124-2, and miR-124-3), and methylation level of miR-124 family is significantly higher in non-cancerous gastric mucosae of patients with GC than that in normal mucosae of healthy individuals, indicating that methylation of miR-124 family is also associated with epigenetic field defect (Ando et al. 2009). Epigenetic loss of miR-124 was first reported in colorectal line HCT-116 deficient in both DNMT3b and DNMT1, and its inactivation contributed to activation of CDK6, an oncogenic factor, and tumor suppressor gene retinoblastoma (Rb) phosphorylation (Lujambio et al. 2007). Epigenetic inactivation of miR-124 due to promoter hypermethylation has also been reported in many other malignancies, including acute lymphoblastic leukemia (ALL), hepatocellular cancer (HCC), clear cell renal cell carcinoma, cervical cancer and pancreatic cancer (Wang et al. 2013b; Wilting et al. 2010; Agirre et al. 2009; Gebauer et al. 2013; Furuta et al. 2010). Furthermore, miR-124 plays the role of tumor suppressor gene not only through directly targeting CDK6, but also through repression of other potential targets, such as SMYD3, VIM, IQGAP1 and Rac1 (Furuta et al. 2010; Wang et al. 2013b). Methylation of miR-181c has also been reported to be associated with formation of epigenetic field defect in non-cancerous tissues corresponded to GC samples, and it acts as tumor suppressor gene via repressing target genes, NOTCH4 and KRAS (Hashimoto et al. 2010).

Epithelial-mesenchymal transition (EMT) is considered as an important step in metastasis and invasion of cancer, and the miR-200 family members, including miR-200a, miR-200b, miR-200c, miR-141, and miR-429, and miR-205 can play critical

role in regulation of EMT through down-regulation of two direct targets, zinc finger E-box binding homeobox 1 (ZEB1) and ZEB2, which can lead to repression of CDH1 expression (Gregory et al. 2008). The epigenetic silencing of miR-200c/141 cluster due to aberrant methylation was discovered in human breast and prostate cancer cell lines (Vrba et al. 2010). Inactivation of miR-200c by promoter-associated hypermethylation has also been identified in non-small cell lung cancer (NSCLC), which may be involved in formation of aggressive and chemoresistant phenotype (Ceppi et al. 2010). Thereafter, the whole miR-200 family was reported epigenetic repression by hypermethylation of CGI in colon, lung and breast cancer (Davalos et al. 2012). In invasive bladder cancer, coordinated inactivation of miR-200 family and miR-205 is correlated with promoter hypermethylation and repressive histone marks (Wiklund et al. 2011). In addition, silencing of miR-200b, miR-200c, and miR-205 by DNA methylation was identified in carcinogen-treated lung epithelial cells and contributed to EMT induction, which could be involved in initiation of lung cancer (Tellez et al. 2011). In a recent study, loss of miR-200 family expression due to both DNA methylation and histone modifications was discovered to have the capability to promote transition from a non-stem to a stem-like phenotype, which might occur during progression of breast cancer (Lim et al. 2013).

In human, miR-9 family has three members, miR-9-1, miR-9-2 and miR-9-3, which locate on chromosomes 1, 5 and 15, respectively. Epigenetic repression of miR-9-1 due to aberrant promoter hypermethylation was first reported in breast cancer (Lehmann et al. 2008). Shortly thereafter, CGI hypermethylation of miR-9-1 was identified in pancreatic cancer using methylated CGI amplification microarrays (Omura et al. 2008). The promoter-associated CGI hypermethylation-mediated silencing of all three miR-9 family members was observed in metastatic cancer cell lines, indicating that epigenetic inactivation of miR-9 family can induce metastasis formation (Lujambio et al. 2008). Consistent with this finding, the methylation of both miR-9-1 and miR-9-3 plays a role in metastatic recurrence of clear cell renal cell carcinoma (Hildebrandt et al. 2010). Reactivation of miR-9 family members shows tumor suppressor features through down-regulation of some target genes, such as fibroblast growth factor receptor 1 (FGFR1), CDK6 and caudal-related homeobox 2 (CDX2) (Rodriguez-Otero et al. 2011; Rotkrue et al. 2011). However, a study showed that miR-9 is over-expressed in breast cancer, which leads to down-regulation of CDH1, and induces the formation of invasive phenotype (Wang et al. 2013a). These findings indicate that miR-9 may play a dual role. In a recent research, frequent hypermethylation and concomitant inactivation of miR-9-2, miR-9-3, miR-124 family, miR-129-2, miR-596 and miR-1247 was observed in human HCC, which can be reversed through DNMT1 knockdown or DNMT inhibition, and united hypermethylation of three or more miRNAs can be viewed as a new diagnostic and prognostic marker of HCC with high specificity (Anwar et al. 2013). Similar to miR-9 family, specific CGI hypermethylation-mediated silencing of miR-148a was also observed in metastatic cancer cell lines, which mediated the activation of metastatic gene transforming growth factor-beta-induced factor-2 (TGIF2), and reintroduction of miR-148a could repress motility, growth and metastasis of cancer cells (Lujambio et al. 2008). In addition, the presence of hypermethylation

of miR-148 in promoter-associated CGI has also been observed in breast, lung, colon, head and neck cancers and melanomas, and is implicated in lymph node metastasis (Lujambio et al. 2008).

MiR-335 located at chromosome 7q32.2 was reported to be silenced by epigenetic promoter hypermethylation, and it can inhibit metastasis and migration of breast cancer by inhibition of its targets, SRY-related high-mobility group box 4 (SOX4) and extracellular matrix component tenascin C (Tavazoie et al. 2008; Png et al. 2011). Over-expression of SOX4 has also been found to be associated with epigenetic inactivation of miR-192-2 due to aberrant DNA methylation in endometrial cancer, gastric cancer and HCC (Chen et al. 2013; Huang et al. 2009; Shen et al. 2010). In addition, the high methylation level of miR-129-2 has been identified in ESCC and colorectal cancer (Chen et al. 2012; Bandres et al. 2009).

There are still many other miRNAs that are silenced by CGI hypermethylation in human cancers, including miR-137, which targets CDK6, LSD1 and cell division cycle 42 (CDC42), and is aberrantly methylated in multiple cancers, such as colon cancer, gastric cancer and lung cancer (Zhu et al. 2013; Balaguer et al. 2010; Chen et al. 2011); miR-375, which targets 3-phosphoinositide-dependent protein kinase-1 (PDK1) and insulin-like growth factor 1 receptor (IGF1R), and undergoes methylation-associated silencing in oesophageal cancer, cervical cancer and human melanoma (Mazar et al. 2011; Li et al. 2011; Kong et al. 2012; Wilting et al. 2013); and miR-203, which targets ATP-binding cassette, subfamily E, member 1 (ABCE1), CDK6 and ABL1, and is epigenetically silenced in Gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), HCC, cervical cancer and hematological malignancies (Wilting et al. 2013; Furuta et al. 2010; Craig et al. 2011; Chim et al. 2011).

MiRNAs can be hosted in intronic regions of coding genes, and the connection between miRNAs and their host genes should be elucidated. Intronic miRNAs can co-express with the host genes, and they are subjected to the same regulation by epigenetic changes. One example is miR-342, which is encoded in an intron of its host gene *Ena/Vasp-like (EVL)* (Grady et al. 2008). The EVL promoter-associated CGI hypermethylation occurs in the early stages of colorectal cancer, which leads to silencing of both the protein and miR-342. In addition, methylation of EVL/miR-342 is significantly more common in non-cancerous colorectal mucosae of patients with colorectal cancer than that in normal mucosae of healthy individuals, indicating that methylation of EVL/miR-342 is involved in epigenetic field defect. MiR-126 is hosted in an intron of the *EGFL7*, and both are down-regulated in cancer cell lines as well as in primary prostate and bladder cancers (Saito et al. 2009a). Interestingly, both mature miRNA-126 and one of the *EGFL7* transcripts, which owns a CGI promoter, are concomitantly up-regulated through epigenetic therapy using DNA methylation and histone deacetylation inhibitors, indicating that epigenetic changes of host genes can affect the expression of intronic miRNAs. MiR-152 as a tumor suppressor gene is frequently silenced in endometrial cancer cell lines and primary samples, and it is located within intron 1 of its host gene, coatomer protein complex, subunit zeta 2 (*COPZ2*), which is also inactivated in endometrial cancer (Tsuruta et al. 2011). The silencing of both miR-152 and its host

gene is caused by DNA hypermethylation, which can be reversed by treatment with 5-aza-dCyd. Moreover, DNMT1, E2F3, MET, and Rictor have been identified as the potential targets of miR-152, illustrating how miR-152 plays its role in endometrial carcinogenesis. A recent study has shown that the expression of miR-335 and its host gene MEST can be repressed by DNA hypermethylation in HCC, which may be involved in distant metastasis (Dohi et al. 2013).

In contrast, some miRNAs, which act as oncogenes, are up-regulated in cancers due to DNA hypomethylation. For instance, the CGI of human let-7a-3 gene, which is located at 22q13.31, is always heavily methylated in normal human tissues, whereas it is hypomethylated in lung cancer tissues, leading to epigenetic activation of let-7a-3 (Brueckner et al. 2007). Over-expression of let-7a-3 in lung cancer cells contributes to formation of tumor phenotypes, and plays its oncogenic role through affecting the expression of several target genes, which are associated with cell proliferation and cell adhesion processes. The expression of miR-21, miR-205, and miR-203 are up-regulated in epithelial ovarian cancer (EOC) compared with the normal tissues, and their expression levels can significantly elevated by treatment of OVCAR3 cells with demethylation drug 5-aza-dC, indicating that their over-expression may be due to DNA hypomethylation (Iorio et al. 2007). Another study showed that expression of miR-200a and miR-200b is significantly up-modulated in pancreatic cancer compared with the normal tissues, which is due to CGI hypomethylation (Li et al. 2010a). In addition, miR-196b functions as an oncogene in primary GC, and its elevated expression is induced by abnormal CGI hypomethylation statue in promoter regions (Tsai et al. 2010). Furthermore, miR-191 is highly expressed in HCC due to aberrant hypomethylation, and it exerts the role in inducing EMT by directly targeting and repressing the expression of tissue inhibitor of metalloproteinase 3 (TIMP3) (He et al. 2011).

Silencing of tumor suppressor miRNAs and over-expression of oncogenic miRNAs due to DNA hypermethylation and hypomethylation, respectively, could play critical role in initiation and progression of various cancers. Interestingly, let-7a-3 has been described to present over-expression and aberrant hypomethylation in lung cancer cells (Brueckner et al. 2007), whereas it has also been found to be silenced by hypermethylation in EOC (Lu et al. 2007). In addition, miR-203, miR-200a and miR-200b are down-regulated in many types of cancer due to hypermethylation and, however, miR-203 is identified to be up-regulated in EOC, and miR-200a/b is up-modulated in pancreatic cancer due to CGI hypomethylation (Iorio et al. 2007; Li et al. 2010a). These data indicates that epigenetic regulation of some miRNAs may be tissue specific, which is likely to be associated with their expression of tissue specificity.

DNA methylation is not the only epigenetic change that affects the normal expression of miRNAs. Similar to protein-coding genes, epigenetic regulation of miRNAs is also closely correlated with histone modification. The first evidence regarding deregulation of miRNAs due to histone modification was reported by Scott et al. (2006). Using miRNA microarray analysis, they discovered that the expression of 27 miRNAs altered rapidly by treatment of SKBr3 breast cancer cell line with HDAC inhibitor. Another study showed that miR-127 was silenced in human T24 breast cancer cells, and DNA demethylation, histone H3 acetylation and tri-methylation of H3K4 by 5-Aza-dC and PBA treatment can lead to its re-activation (Saito et al. 2006).

Similarly, re-expression of miR-512-5p may be associated with DNA demethylation, histone H3 acetylation and di-methylation of H3K4 by epigenetic treatment with 5-aca-dC and PBA, and its activation leads to inhibition of Mcl-1 and induces apoptosis of gastric cancer cells (Saito et al. 2009b). Furthermore, a study showed that expression of miRNAs is positively associated with tri-methylation of H3K4 and negatively associated with tri-methylation of H3K27 in promoter regions of human miRNAs based on the data of miRNA expression microarrays and chromatin immunoprecipitation (ChIP)-on-chip (Ke et al. 2009). In ALL, the CGIs of 13 miRNAs exist two abnormal histone modifications, increased di-methylation of H3K9 and decreased tri-methylation of H3K4, which may be involved in silencing of these miRNAs (Roman-Gomez et al. 2009). Accumulated tri-methylation of H3K27 has been identified to inhibit the transcriptional expression of miR-22 in ALL, and its expression can be up-regulated after treatment with trichostatin A (TSA), a HDAC inhibitor (Li et al. 2010b). In addition, analysis of CHIP revealed that microbial stimulus promotes NFkappaB p50-C/EBPbeta silencer complex binding to promoter region of let-7i, which leads to histone H3 deacetylation and silencing of miRNA let-7i (O'Hara et al. 2010). More recently, a study showed that accumulation of repressive histone marks, including tri-methylation of H3K9 and H3K27, is an important epigenetic change, which results in silencing of miR-125b1 in breast cancer (Soto-Reyes et al. 2012). Taking together, histone H3 acetylation and di-/tri-methylation of H3K4 can promote transcriptional activation of miRNAs, whereas tri-methylation of H3K27 and di/tri-methylation of H3K9 can lead to epigenetic inactivation of miRNAs. Moreover, aberrant histone modifications often coexist with DNA methylation in epigenetically deregulated miRNAs in cancer.

Interestingly, it has been founded that some oncoproteins can bind to promoter regions of miRNAs and recruit epigenetic effectors, leading to deregulation of miRNAs. For example, Fazi et al. (2007) reported that epigenetic silencing of miR-223 can be induced by AML1/ETO oncoprotein, the product of AML-associated t(8;21) translocation, through binding to pre-miR-223 region and recruiting epigenetic effectors, such as DNMTs, MeCP2 and HDAC. However, there is interaction between epigenetic machinery and miRNAs. MiRNAs can not only be regulated by epigenetic machinery, but also regulate the components of epigenetic machinery, which is associated with methylation or acetylation.

4 MicroRNAs Can Regulate the Epigenetic Effectors in Cancer

It has been discovered that a subgroup of miRNAs can regulate the expression of effectors of the epigenetic mechanisms by directly or indirectly targeting these epigenetic-modifying enzymes and molecules, such as DNMTs, HATs, HMTs, HDACs, Retinoblastoma-Like 2 (RBL2), enhancer of zeste homolog 2 (EZH2) and Polycomb Repressive Complex (PRC). These miRNAs, called “epi-miRNAs”, have been widely investigated in the past few years because their deregulation may be

Table 2.1 Epi-miRNAs in human cancers

microRNA	Targets	Tissue type	Reference(s)
miR-29a	DNMT3a, DNMT3b	Lung cancer	Fabbri et al. (2007)
miR-29b	DNMT3a/3b, sp1	Lung cancer, AML	Fabbri et al. (2007) and Garzon et al. (2009)
miR-148a	DNMT3b, DNMT1	Cervical cancer, Gastric cancer, Cholangiocarcinoma	Duursma et al. (2008), Braconi et al. (2010), and Zhu et al. (2012)
miR-152	DNMT1	Cholangiocarcinoma NiS-transformed cells	Braconi et al. (2010) and Ji et al. (2013)
miR-301	DNMT1	Cholangiocarcinoma	Braconi et al. (2010)
miR-143	DNMT-3a	Colorectal cancer	Ng et al. (2009)
miR-342	DNMT1	Colorectal cancer	Wang et al. (2011)
miR-185	DNMT1	Hepatocellular carcinoma	Zhang et al. (2011)
miR-290 cluster	RBL-2	Mouse ES cells	Benetti et al. (2008) and Sinkkonen et al. (2008)
miR-34a	SIRT1	Colon cancer	Yamakuchi et al. (2008)
miR-449	SIRT1, HDAC1	Gastric cancer, Prostate cancer	Bou Kheir et al. (2011) and Noonan et al. (2009)
miR-200 family	SIRT1	Breast cancer	Eades et al. (2011)
miR-1	HDAC4	Skeletal muscle tissue	Chen et al. (2006)
miR-140	DNMT1	Hepatocarcinoma	Takata et al. (2013)
	HDAC4	Mouse cartilage tissue	Tuddenham et al. (2006)
miR-101	EZH2	Prostate cancer	Friedman et al. (2009) and Varambally et al. (2008)
miR-K12- 4-5p	RBL-2	KSHV	Lu et al. (2010)

Abbreviation: *DNMT* DNA methyltransferase, *RBL2* Retinoblastoma-Like 2, *SIRT1* sirtuin 1, *HDAC* histone de-acetylases, *EZH2* enhancer of zeste homolog 2, *AML* acute myeloid leukemia, *KSHV* Kaposi's sarcoma-associated herpesvirus

closely correlated with human carcinogenesis (Table 2.1). Recent studies have suggested that epi-miRNAs may occupy an important position in tumor mediation by modulating various cellular processes like cell proliferation, apoptosis, cellular movement and metastasis.

The existence of epi-miRNAs was first discovered in lung cancer, where miR-29 family has been proved to directly target DNMT3a and DNMT3b through some interesting complementarities to the 3'UTR of DNMT3 (Fabbri et al. 2007). In addition, Garzon et al. (2009) have demonstrated that miR-29b is also able to indirectly suppress DNMT1 in AML cells by directly targeting the transactivator Sp1. Studies have shown that expression of miR-29s is inversely correlated with the expression levels of both DNMT3a and DNMT3b in lung cancer and AML. The enforced expression of miR-29s can induce disruption of *de novo* DNA methylation and contribute to promoter-associated CGI demethylation of epigenetically silenced tumor suppressor genes, such as *WHOX*, *FHIT*, and *p15INK4B*, due to promoter hypermethylation, which ultimately inhibits tumorigenesis by inducing reactivation

of these tumor suppressor genes. These findings indicate a role of miR-29s in tumor suppression and provide a basic principle for the development of miRNA-based approaches for cancer therapy.

After that, miRNAs have drawn more attention than ever and emerged as a new category of tumor inhibitors or regulators of signal transduction. A few reports have revealed that miR-148a can regulate DNMT3b expression through an unusual binding site within the coding region instead of the 3'UTR of DNMT3b mRNA (Duursma et al. 2008). Studies also suggest that DNMT1 is directly modulated by miR-148a, along with miR-152 and miR-301, in cholangiocarcinoma (Braconi et al. 2010). The expression of these miRNAs is down-regulated and emerges tumor suppressive abilities in cancer cells. Zhu et al. found that silencing of miR-148a in gastric cancer was related to aberrant methylation in promoter region, which could contribute to activation of DNMT1, and this may in turn lead to inactivation of miR-148a through promoting DNA methylation (Zhu et al. 2012). Additionally, the expression of miR-152 in nickel sulfide (NiS)-transformed cells can directly suppress DNMT1 by targeting the 3' untranslated regions of its transcript, which demonstrates a significant interaction between miR-152 and DNMT1 via a double-negative feedback mechanism involved in NiS-induced malignant transformation (Ji et al. 2013). Another miRNA with tumor suppressive abilities linked to DNMT3a is miR-143. Studies indicate a direct and specific crosstalk between miR-143 and DNMT3a 3'UTR, thus ectopic miR143 expression can reduce DNMT3a expression and repress cell proliferation in colorectal cancer (Ng et al. 2009). MiR-342 has also been found to inhibit colorectal cancer cell proliferation. MiR-342 presents decreased expression in colorectal cancer samples and cells, and its reactivation can directly inhibiting the expression of DNMT1, which reactivating several tumor suppressor genes, such as ADAM metallopeptidase domain 23 (ADAM23) and Ras association domain family member 1A (RASSF1A), through promoter demethylation (Wang et al. 2011). Furthermore, miR-185 can also modulate methylation levels of several gene promoters by targeting DNMT1 in human glioma (Zhang et al. 2011). More recently, miR-140 was found to be down-regulated in HCC due to deficiency of DDX20, and reactivation of its expression can increase metallothionein expression through directly targeting DNMT1, leading to decreased NF- κ B activity resulting in inhibition of hepatocarcinogenesis (Takata et al. 2013).

In addition, a study was performed with Dicer-deficient mouse embryonic stem cells. In this study the level of miR-290 cluster was identified to be able to indirectly regulate the expression of DNMT3a and DNMT3b by directly silencing RBL2, a suppressor of DNMT3 genes (Benetti et al. 2008; Sinkkonen et al. 2008). The experiments have shown that the expression of miR-290 cluster is dramatically down-regulated in Dicer1-null embryonic stem cells with respect to wild-type controls, which results in decreased DNMT3 expression and DNA-methylation defects in these cells via loss of RBL2 silencing. Decreased DNMT expression leads to a remarkable hypomethylation of the genome, which impairs the embryonic stem cells differentiation program and promotes telomere elongation and telomere recombination. Furthermore, re-expression of the miR-290 cluster is able to rescue the down-regulation of DNMT3 expression and restore the normal methylation pattern via simultaneously silencing the expression of

RBL2. Altogether, these results demonstrate an important role of the miR-290 cluster in the regulatory of DNMT3 enzymes and global DNA methylation, which in turn mediate the appearance of telomeric phenotypes.

Over-expression of HDACs has been observed in a broad range of cancer types, which is a significant mechanism to promote proliferation and reduce apoptosis of cancer cells by repressing some important growth suppressive genes. HDACs play a key role in epigenetic modifications of cancers and are also under regulation of epi-miRNAs. It has been found that miR-34a can regulate the pathway that mediates cellular aging and limits longevity in various cancers including colon cancer, by interacting with the expression of mammalian sirtuin 1 (SIRT1) and p53 (Yamakuchi et al. 2008). SIRT1, a homologue of yeast gene silent information regulator 2 (Sir2), is a class III HDAC with an enzymatic activity dependent on NAD⁺. P53 is an important tumor suppressor, which can affect cell proliferation, cell apoptosis, DNA repair and angiogenesis by modulating a variety of physical responses to many cancer-related stress signals. SIRT1 is frequently up-regulated in human cancers and inactivates apoptosis of cancer cells by inducing deacetylation of p53. Studies demonstrate that miR-34a directly inhibits SIRT1 expression by binding to the 3' UTR region of SIRT1, which indirectly results in an increase of acetylated p53 and in turn prevent cell cycle arrest and induce apoptosis. Thereby, miR-34a functions as a tumor inhibitor, in part, through a SIRT1-p53 pathway.

A recent study using gastric cancer cells in a mouse model elucidated that SIRT1 was directly regulated by miR-449 (Bou Kheir et al. 2011). Researchers have found that miR-449 is part of the miR-34 family and may act as a tumor suppressor, which is down-regulated in gastric cancer and prostate cancer (Bou Kheir et al. 2011; Noonan et al. 2009). Restoration of miR-449 expression contributes to decreased expression of SIRT1. Moreover, re-introduction of miR-449 into cancer cell lines inhibits cell proliferation and induces cell cycle arrest, apoptosis and senescent-like phenotype by targeting various cell cycle regulators concomitant with the activation of p53 pathway. Interestingly, miR-449 was recently found to be epigenetically modulated and possessed a key position in a negative feedback loop in which E2F1 activated the transcription of miR-449 that in turn exerted tumor suppressive ability by directly targeting CDC25A and CDK6 (Yang et al. 2009).

A crosstalk between miR-200 family and SIRT1 has also been reported in recent studies. The miR-200 family is an important regulator of EMT, which is a common embryological process linked to various pathologies including cancer metastasis and tumorigenicity. The miR-200 family is down-regulated and acts as an inhibitor in renal, prostate, breast, bladder, pancreatic, and gastric cancers. Up-regulation of SIRT1 is observed in breast cancer tissues, which is associated with decreased expression of miR200a. Additionally, re-introduction of miR-200a or knockdown of SIRT1 inhibits transformation of normal mammary epithelial cells and prevents cancer metastasis, elucidating that miR-200a may be a potential tumor suppressor involved in breast cancer metastasis (Eades et al. 2011). Several other miRNAs have also been associated with the modulation of HDACs, such as miR-1, involved in myogenesis and related disease, and miR-140, reduced in various cancer types and suppressed cell proliferation in colon cancer cells, both of which directly target HDAC4 (Chen et al. 2006; Tuddenham et al. 2006).

EZH2 is the catalytic subunit of the PRC2 and mediates heterochromatin formation by trimethylating histone H3K27, contributing to aberrant silencing of several tumor suppressor genes in cancer. As recently shown in both prostate and bladder cancers, the expression of miR-101 was down-regulated during cancer progression and inversely correlated with up-regulation of EZH2 (Friedman et al. 2009; Varambally et al. 2008). Further studies showed that transfection of miR-101 resulted in a stable EZH2 knockdown and decreased levels of trimethylation of H3K27, which significantly suppressed proliferation, migration, clonogenicity, and tumorigenicity of cancer cells, suggesting a role as tumor suppressor for miR-101.

A regulatory loop where miRNAs can also be used by virus to modulate the epigenetic mechanism of the host cell has been revealed recently. Researchers found the Kaposi's sarcoma-associated herpesvirus (KSHV) miR-K12-4-5p can directly target RBL2 3' UTR and decrease RBL2 protein levels, which indirectly up-regulated mRNA levels of DNMT1, -3a, and -3b, thus regulating global epigenetic reprogramming (Lu et al. 2010).

Besides, a subtle and fascinating regulatory mechanism of methylation pattern has been reported: it seems that activation of epigenetic silencing by DNA methylation is dependent on the ratio of miRNA and its target RNA (Khraiwesh et al. 2010). In most *Physcomitrella patens* mutants, the loss of DICER-LIKE 1b gene leads to a maturation of normal miRNAs, which in turn suppresses the disruption of target RNAs and causes accumulation of miRNAs. As a result, target RNA duplexes, hypermethylation of the genes encoding target RNA and ultimate gene silencing.

5 Clinical Utility of Epigenetically Silenced MicroRNAs in Cancer

With the help of microarray technology, we can detect thousands of miRNAs expression at the same time and establish miRNAs expression profiles of various cancers. From a clinical point of view, the miRNAs expression profile has potential to be a useful tool for diagnosis, prognosis judgment and prediction of treatment response. The discovery of circulating miRNAs in human blood serum and plasma has been frequently reported recently. Moreover, the expression levels of these circulating miRNAs are stable, reproducible and consistent among individuals of the same species, and they can be detected more easily. At present, circulating miRNAs profiles have been successfully evaluated in a number of solid cancers as novel early diagnostic markers. In addition to aberrant expression levels of miRNAs, the abnormal epigenetic marks of miRNAs can also be useful diagnostic and prognostic biomarkers. For instance, by analyzing bone marrow samples from 353 ALL patients, at least one of 13 methylated miRNA genes could be found in 65 % of all ALL cases, which had a significantly higher relapse and mortality rate, indicating that miRNA gene methylation can be a critical prognostic factor of ALL (Agirre et al. 2012).

Given that epigenetically inactivated miRNAs lead to initiation and progression of cancer, reactivation of these miRNAs may have great potential to treat cancer.

It has been widely proved that restoration of epigenetically silenced miRNA by epigenetic agents can result in down-regulation of oncogenic target genes and inhibition of development and progression of cancers. DNMT inhibitors can be used to reduce the level of methylation. Among these inhibitors, 5-aza-dC and 5-azacytidine have been approved by US Food and Drug Administration (FDA) to treat acute myeloid leukemia and myelodysplastic syndromes, respectively (Rodriguez-Paredes and Esteller 2011). In addition, current data reveal that miRNA mimics instead of miRNAs can effectively make up the loss of miRNAs expression and may be a potential therapeutic strategy. Moreover, HDAC inhibitors have also been approved by FAD to treat certain types of lymphomas. However, we cannot ignore that these epigenetic drugs are nonselective, and their side effects are still poorly understood.

6 Concluding Remarks

Cancer is associated with accumulation of epigenetic and genetic alterations. The epigenetic changes can result in silencing of tumor suppressor genes and up-regulation of oncogenes. Owing to that miRNAs can target a large number of mRNAs involved in control of various biological processes, they can also play the role of tumor suppressor genes or oncogenes (Babashah and Soleimani 2011; Babashah et al. 2012). The aberrant expression of miRNAs is the common pathogenesis of human cancers and, hence, it is of great significance in treatment of cancers to study the regulation mechanism of miRNAs. Currently, experimental evidence indicates that deregulation of miRNAs is closely associated with epigenetic changes, including abnormal DNA methylation and histone modifications. Moreover, with the development of the detection technology, it is possible for us to detect a wide range of epigenetic deregulation of miRNAs in a cancer, which can be used as new diagnostic and prognostic marker of the cancer. In addition, a group of miRNAs, which are known as epi-miRNAs, have been discovered to be able to regulate the expression of epigenetic effectors, such as DNMTs, HDACs and polycomb genes, which indirectly leads to epigenetic changes and in turn affects the expression of miRNAs. Hence, there is interaction between miRNAs and epigenetic machinery.

The relationship between epigenetics and miRNAs has been widely studied, but the understanding of it is still far from perfect. Further studies should be focused on the regulation system involving epigenetic machinery and miRNAs and how to translate research results into clinical treatment of cancers. In addition, epigenetic alteration is a reversible process, and treatment with epigenetic drugs, such as DNMT or HDAC inhibitors, can lead to re-expression of the silenced miRNAs and restore their normal function, which may achieve the purpose of cancer therapy. Hence, future studies also need to be conducted to develop the miRNA- and epi-miRNA-based treatment. It can be predicted that, with the continuous deepening research, the diagnosis and treatment of cancer will make great progress.

References

- Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J et al (2007) UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* 449(7163):731–734
- Agirre X, Vilas-Zornoza A, Jimenez-Velasco A, Martin-Subero JJ, Cordeu L, Garate 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
- Agirre X, Martinez-Climent JA, Odero MD, Prosper F (2012) Epigenetic regulation of miRNA genes in acute leukemia. *Leukemia* 26(3):395–403
- Allfrey VG, Faulkner R, Mirsky AE (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A* 51:786–794
- Ando T, Yoshida T, Enomoto S, Asada K, Tatematsu M, Ichinose M et al (2009) DNA methylation of microRNA genes in gastric mucosae of gastric cancer patients: its possible involvement in the formation of epigenetic field defect. *Int J Cancer* 124(10):2367–2374
- Anwar SL, Albat C, Krech T, Hasemeier B, Schipper E, Schweitzer N et al (2013) Concordant hypermethylation of intergenic microRNA genes in human hepatocellular carcinoma as new diagnostic and prognostic marker. *Int J Cancer* 133(3):660–670
- Babashah S, Soleimani M (2011) The oncogenic and tumour suppressive roles of microRNAs in cancer and apoptosis. *Eur J Cancer* 47(8):1127–1137
- Babashah S, Sadeghizadeh M, Tavirani MR, Farivar S, Soleimani M (2012) Aberrant microRNA expression and its implications in the pathogenesis of leukemias. *Cell Oncol (Dordr)* 35(5):317–334
- Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR et al (2010) Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. *Cancer Res* 70(16):6609–6618
- 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
- Banerjee T, Chakravarti D (2011) A peek into the complex realm of histone phosphorylation. *Mol Cell Biol* 31(24):4858–4873
- Benetti R, Gonzalo S, Jaco I, Munoz 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
- Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16(1):6–21
- Bogdanovic O, Veenstra GJ (2009) DNA methylation and methyl-CpG binding proteins: developmental requirements and function. *Chromosoma* 118(5):549–565
- Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE et al (2007) p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr Biol* 17(15):1298–1307
- Bou Kheir T, Futoma-Kazmierczak E, Jacobsen A, Krogh A, Bardram L, Hother C et al (2011) miR-449 inhibits cell proliferation and is down-regulated in gastric cancer. *Mol Cancer* 10:29
- 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
- Brandl A, Heinzl T, Kramer OH (2009) Histone deacetylases: salesmen and customers in the post-translational modification market. *Biol Cell* 101(4):193–205
- 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
- Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E et al (2002) Frequent deletions and down-regulation of microRNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 99(24):15524–15529
- Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD et al (2004a) MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci U S A* 101(32):11755–11760

- Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S et al (2004b) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A* 101(9):2999–3004
- Campanero MR, Armstrong MI, Flemington EK (2000) CpG methylation as a mechanism for the regulation of E2F activity. *Proc Natl Acad Sci U S A* 97(12):6481–6486
- Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H et al (2008) hsa-miR-210 Is induced by hypoxia and is an independent prognostic factor in breast cancer. *Clin Cancer Res* 14(5):1340–1348
- Ceppi P, Mudduluru G, Kumarswamy R, Rapa I, Scagliotti GV, Papotti M et al (2010) Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in non-small cell lung cancer. *Mol Cancer Res* 8(9):1207–1216
- Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM et al (2008) Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet* 40(1):43–50
- 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
- Chen Q, Chen X, Zhang M, Fan Q, Luo S, Cao X (2011) miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. *Dig Dis Sci* 56(7):2009–2016
- Chen X, Hu H, Guan X, Xiong G, Wang Y, Wang K et al (2012) CpG island methylation status of miRNAs in esophageal squamous cell carcinoma. *Int J Cancer* 130(7):1607–1613
- Chen X, Zhang L, Zhang T, Hao M, Zhang X, Zhang J et al (2013) Methylation-mediated repression of microRNA 129–2 enhances oncogenic SOX4 expression in HCC. *Liver Int* 33(3):476–486
- 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
- Craig VJ, Cogliatti SB, Rehrauer H, Wundisch T, Muller A (2011) Epigenetic silencing of microRNA-203 dysregulates ABL1 expression and drives Helicobacter-associated gastric lymphomagenesis. *Cancer Res* 71(10):3616–3624
- Davalos V, Moutinho C, Villanueva A, Boque R, Silva P, Carneiro F et al (2012) Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. *Oncogene* 31(16):2062–2074
- Deng S, Calin GA, Croce CM, Coukos G, Zhang L (2008) Mechanisms of microRNA deregulation in human cancer. *Cell Cycle* 7(17):2643–2646
- Diederichs S, Haber DA (2006) Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing. *Cancer Res* 66(12):6097–6104
- Dohi O, Yasui K, Gen Y, Takada H, Endo M, Tsuji K et al (2013) Epigenetic silencing of miR-335 and its host gene MEST in hepatocellular carcinoma. *Int J Oncol* 42(2):411–418
- Dunn BK (2003) Hypomethylation: one side of a larger picture. *Ann N Y Acad Sci* 983:28–42
- Duursma AM, Kedde M, Schrier M, le Sage C, Agami R (2008) miR-148 targets human DNMT3b protein coding region. *RNA* 14(5):872–877
- Eades G, Yao Y, Yang M, Zhang Y, Chumsri S, Zhou Q (2011) miR-200a regulates SIRT1 expression and epithelial to mesenchymal transition (EMT)-like transformation in mammary epithelial cells. *J Biol Chem* 286(29):25992–26002
- Eden A, Gaudet F, Waghmare A, Jaenisch R (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 300(5618):455
- Fabbri M, Garzon R, Cimmino A, Liu Z, Zanasi 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 U S A* 104(40):15805–15810
- 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
- Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 9(2):102–114
- Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH (2008) Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J Biol Chem* 283(2):1026–1033

- 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
- Furuta M, Kozaki KI, Tanaka S, Arai S, Imoto I, Inazawa J (2010) miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. *Carcinogenesis* 31(5):766–776
- Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E 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
- Gebauer K, Peters I, Dubrowskaja N, Hennenlotter J, Abbas M, Scherer R et al (2013) Hsa-miR-124-3 CpG island methylation is associated with advanced tumours and disease recurrence of patients with clear cell renal cell carcinoma. *Br J Cancer* 108(1):131–138
- Glozak MA, Sengupta N, Zhang X, Seto E (2005) Acetylation and deacetylation of non-histone proteins. *Gene* 363:15–23
- Gowher H, Liebert K, Hermann A, Xu G, Jeltsch A (2005) Mechanism of stimulation of catalytic activity of Dnmt3A and Dnmt3B DNA-(cytosine-C5)-methyltransferases by Dnmt3L. *J Biol Chem* 280(14):13341–13348
- 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
- Greer EL, Shi Y (2012) Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* 13(5):343–357
- Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G et al (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 10(5):593–601
- Hashimoto Y, Akiyama Y, Otsubo T, Shimada S, Yuasa Y (2010) Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis. *Carcinogenesis* 31(5):777–784
- Hayakawa T, Nakayama J (2011) Physiological roles of class I HDAC complex and histone demethylase. *J Biomed Biotechnol* 2011:129383
- He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y et al (2007a) A microRNA component of the p53 tumour suppressor network. *Nature* 447(7148):1130–1134
- He L, He X, Lowe SW, Hannon GJ (2007b) microRNAs join the p53 network—another piece in the tumour-suppression puzzle. *Nat Rev Cancer* 7(11):819–822
- 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
- Hermeking H (2010) The miR-34 family in cancer and apoptosis. *Cell Death Differ* 17(2):193–199
- Hildebrandt MA, Gu J, Lin J, Ye Y, Tan W, Tamboli P et al (2010) Hsa-miR-9 methylation status is associated with cancer development and metastatic recurrence in patients with clear cell renal cell carcinoma. *Oncogene* 29(42):5724–5728
- Hodawadekar SC, Marmorstein R (2007) Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. *Oncogene* 26(37):5528–5540
- Hong L, Schroth GP, Matthews HR, Yau P, Bradbury EM (1993) Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 “tail” to DNA. *J Biol Chem* 268(1):305–314
- Hossain MB, Vahter M, Concha G, Broberg K (2012) Environmental arsenic exposure and DNA methylation of the tumor suppressor gene p16 and the DNA repair gene MLH1: effect of arsenic metabolism and genotype. *Metallomics* 4(11):1167–1175
- Huang YW, Liu JC, Deatherage DE, Luo J, Mutch DG, Goodfellow PJ et al (2009) Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 oncogene in endometrial cancer. *Cancer Res* 69(23):9038–9046
- Iorio MV, Croce CM (2012) Causes and consequences of microRNA dysregulation. *Cancer J* 18(3):215–222
- Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P et al (2007) MicroRNA signatures in human ovarian cancer. *Cancer Res* 67(18):8699–8707

- Iorio MV, Piovan C, Croce CM (2010) Interplay between microRNAs and the epigenetic machinery: an intricate network. *Biochim Biophys Acta* 1799(10–12):694–701
- Iwase S, Lan F, Bayliss P, de la Torre-Ubieta L, Huarte M, Qi HH, Whetstine JR et al (2007) The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell* 128(6):1077–1088
- 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
- John S, Howe L, Tafrov ST, Grant PA, Sternglanz R, Workman JL (2000) The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. *Genes Dev* 14(10):1196–1208
- Ke XS, Qu Y, Rostad K, Li WC, Lin B, Halvorsen OJ et al (2009) Genome-wide profiling of histone h3 lysine 4 and lysine 27 trimethylation reveals an epigenetic signature in prostate carcinogenesis. *PLoS One* 4(3):e4687
- Khraiwesh B, Arif MA, Seumel GI, Ossowski S, Weigel D, Reski R et al (2010) Transcriptional control of gene expression by microRNAs. *Cell* 140(1):111–122
- Knudson AG (2001) Two genetic hits (more or less) to cancer. *Nat Rev Cancer* 1(2):157–162
- Koch-Nolte F, Kernstock S, Mueller-Dieckmann C, Weiss MS, Haag F (2008) Mammalian ADP-ribosyltransferases and ADP-ribosylhydrolases. *Front Biosci* 13:6716–6729
- Kong KL, Kwong DL, Chan TH, Law SY, Chen L, Li Y et al (2012) MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor. *Gut* 61(1):33–42
- Kouzarides T (2007) Chromatin modifications and their function. *Cell* 128(4):693–705
- Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T (2007) Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* 39(5):673–677
- Kwon OH, Park JL, Kim M, Kim JH, Lee HC, Kim HJ et al (2011) Aberrant up-regulation of LAMB3 and LAMC2 by promoter demethylation in gastric cancer. *Biochem Biophys Res Commun* 406(4):539–545
- Lal A, Navarro F, Maher CA, Maliszewski LE, Yan N, O'Day E et al (2009) miR-24 Inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to “seedless” 3'UTR microRNA recognition elements. *Mol Cell* 35(5):610–625
- Lee KK, Workman JL (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol* 8(4):284–295
- Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75(5):843–854
- Lee I, Ajay SS, Yook JI, Kim HS, Hong SH, Kim NH et al (2009) New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res* 19(7):1175–1183
- 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
- Leonhardt H, Page AW, Weier HU, Bestor TH (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell* 71(5):865–873
- Li A, Omura N, Hong SM, Vincent A, Walter K, Griffith M et al (2010a) Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. *Cancer Res* 70(13):5226–5237
- Li X, Liu J, Zhou R, Huang S, Huang S, Chen XM (2010b) Gene silencing of MIR22 in acute lymphoblastic leukaemia involves histone modifications independent of promoter DNA methylation. *Br J Haematol* 148(1):69–79
- Li X, Lin R, Li J (2011) Epigenetic silencing of microRNA-375 regulates PDK1 expression in esophageal cancer. *Dig Dis Sci* 56(10):2849–2856
- Li Q, Zou C, Zou C, Huang H, Jin J, Han Z et al (2013) MicroRNA-25 functions as a potential tumor suppressor in colon cancer by targeting Smad7. *Cancer Lett* 335(1):168–174
- Lim Y, Wright JA, Attema JL, Gregory PA, Bert AG, Smith E et al (2013) Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem cell-like state. *J Cell Sci* 126(Pt 10):2256–2266

- Liu W, Tanasa B, Tyurina OV, Zhou TY, Gassmann R, Liu WT et al (2010) PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. *Nature* 466(7305):508–512
- Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H et al (2011) The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med* 17(2):211–215
- Lu L, Katsaros D, de la Longrais IA, Sochirca O, Yu H (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
- Lu F, Stedman W, Yousef M, Renne R, Lieberman PM (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
- 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
- Lujambio A, Calin GA, Villanueva A, Ropero S, Sanchez-Cespedes M, Blanco D et al (2008) A microRNA DNA methylation signature for human cancer metastasis. *Proc Natl Acad Sci U S A* 105(36):13556–13561
- Magnani R, Dirk LM, Trievel RC, Houtz RL (2010) Calmodulin methyltransferase is an evolutionarily conserved enzyme that trimethylates Lys-115 in calmodulin. *Nat Commun* 1:43
- Mazar J, DeBlasio D, Govindarajan SS, Zhang S, Perera RJ (2011) Epigenetic regulation of microRNA-375 and its role in melanoma development in humans. *FEBS Lett* 585(15):2467–2476
- Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133(2):647–658
- Mortusewicz O, Fouquerel E, Ame JC, Leonhardt H, Schreiber V (2011) PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms. *Nucleic Acids Res* 39(12):5045–5056
- Ng EK, Tsang WP, Ng SS, Jin HC, Yu J, Li JJ et al (2009) MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *Br J Cancer* 101(4):699–706
- Nguyen AT, Zhang Y (2011) The diverse functions of Dot1 and H3K79 methylation. *Genes Dev* 25(13):1345–1358
- 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
- O'Hara SP, Splinter PL, Gajdos GB, Trussoni CE, Fernandez-Zapico ME, Chen XM et al (2010) NFkappaB p50-CCAAT/enhancer-binding protein beta (C/EBPbeta)-mediated transcriptional repression of microRNA let-7i following microbial infection. *J Biol Chem* 285(1):216–225
- Oka S, Kato J, Moss J (2006) Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. *J Biol Chem* 281(2):705–713
- Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99(3):247–257
- Omura N, Li CP, Li A, Hong SM, Walter K, Jimeno A et al (2008) Genome-wide profiling of methylated promoters in pancreatic adenocarcinoma. *Cancer Biol Ther* 7(7):1146–1156
- Peterson CL, Laniel MA (2004) Histones and histone modifications. *Curr Biol* 14(14):R546–R551
- Pickart CM (2001) Mechanisms underlying ubiquitination. *Annu Rev Biochem* 70:503–533
- Png KJ, Yoshida M, Zhang XH, Shu W, Lee H, Rimner A et al (2011) MicroRNA-335 inhibits tumor reinitiation and is silenced through genetic and epigenetic mechanisms in human breast cancer. *Genes Dev* 25(3):226–231
- Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M et al (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406(6796):593–599
- Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE et al (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 403(6772):901–906
- Rodriguez-Otero P, Roman-Gomez J, Vilas-Zornoza A, Jose-Eneriz ES, Martin-Palanco V, Rifon 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

- Rodriguez-Paredes M, Esteller M (2011) Cancer epigenetics reaches mainstream oncology. *Nat Med* 17(3):330–339
- Roman-Gomez J, Agirre X, Jimenez-Velasco A, Arqueros V, Vilas-Zornoza A, Rodriguez-Otero P et al (2009) Epigenetic regulation of microRNAs in acute lymphoblastic leukemia. *J Clin Oncol* 27(8):1316–1322
- Rotkrue P, Akiyama Y, Hashimoto Y, Otsubo T, Yuasa Y (2011) MiR-9 downregulates CDX2 expression in gastric cancer cells. *Int J Cancer* 129(11):2611–2620
- Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA et al (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 9(6):435–443
- Saito Y, Friedman JM, Chihara Y, Egger G, Chuang JC, Liang G (2009a) Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells. *Biochem Biophys Res Commun* 379(3):726–731
- Saito Y, Suzuki H, Tsugawa H, Nakagawa I, Matsuzaki J, Kanai Y et al (2009b) Chromatin remodeling at Alu repeats by epigenetic treatment activates silenced microRNA-512-5p with down-regulation of Mcl-1 in human gastric cancer cells. *Oncogene* 28(30):2738–2744
- 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
- Shen R, Pan S, Qi S, Lin X, Cheng S (2010) Epigenetic repression of microRNA-129-2 leads to over-expression of SOX4 in gastric cancer. *Biochem Biophys Res Commun* 394(4):1047–1052
- Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA et al (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119(7):941–953
- 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
- Soto-Reyes E, Gonzalez-Barrios R, Cisneros-Soberanis F, Herrera-Goepfert R, Perez V, Cantu D et al (2012) Disruption of CTCF at the miR-125b1 locus in gynecological cancers. *BMC Cancer* 12:40
- Suzuki H, Yamamoto E, Nojima M, Kai M, Yamano HO, Yoshikawa K et al (2010) Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. *Carcinogenesis* 31(12):2066–2073
- Taby R, Issa JP (2010) Cancer epigenetics. *CA Cancer J Clin* 60(6):376–392
- Takata A, Otsuka M, Yoshikawa T, Kishikawa T, Hikiba Y, Obi S et al (2013) MicroRNA-140 acts as a liver tumor suppressor by controlling NF-kappaB activity by directly targeting DNA methyltransferase 1 (Dnmt1) expression. *Hepatology* 57(1):162–170
- Tavazoie SF, Alarcon 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
- Tellez CS, Juri DE, Do K, Bernauer AM, Thomas CL, Damiani LA et al (2011) EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. *Cancer Res* 71(8):3087–3097
- Tsai KW, Hu LY, Wu CW, Li SC, Lai CH, Kao HW et al (2010) Epigenetic regulation of miR-196b expression in gastric cancer. *Genes Chromosomes Cancer* 49(11):969–980
- Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P et al (2006) Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 439(7078):811–816
- Tsuruta T, Kozaki K, Uesugi A, Furuta M, Hirasawa A, Imoto I et al (2011) miR-152 is a tumor suppressor microRNA that is silenced by DNA hypermethylation in endometrial cancer. *Cancer Res* 71(20):6450–6462
- 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
- Vaissiere T, Sawan C, Herceg Z (2008) Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat Res* 659(1–2):40–48
- 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

- Vogt M, Munding J, Gruner M, Liffers ST, Verdoodt B, Hauk J et al (2011) Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. *Virchows Arch* 458(3):313–322
- Vrba L, Jensen TJ, Garbe JC, Heimark RL, Cress AE, Dickinson S et al (2010) Role for DNA methylation in the regulation of miR-200c and miR-141 expression in normal and cancer cells. *PLoS One* 5(1):e8697
- Waddington CH (2012) The epigenotype. 1942. *Int J Epidemiol* 41(1):10–13
- 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
- Wang J, Zhao H, Tang D, Wu J, Yao G, Zhang Q (2013a) Overexpressions of microRNA-9 and microRNA-200c in human breast cancers are associated with lymph node metastasis. *Cancer Biother Radiopharm* 28(4):283–288
- Wang P, Chen L, Zhang J, Chen H, Fan J, Wang K et al (2013b) Methylation-mediated silencing of the miR-124 genes facilitates pancreatic cancer progression and metastasis by targeting Rac1. *Oncogene*. doi:[10.1038/onc.2012.598](https://doi.org/10.1038/onc.2012.598). [Epub ahead of print]
- Whetstone JR, Nottke A, Lan F, Huarte M, Smolnikov S, Chen Z et al (2006) Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell* 125(3):467–481
- Wiklund ED, Bramsen JB, Hulf T, Dyrskjot L, Ramanathan R, Hansen TB et al (2011) Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. *Int J Cancer* 128(6):1327–1334
- Wilting SM, van Boerdonk RA, Henken FE, Meijer CJ, Diosdado B, Meijer GA et al (2010) Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer. *Mol Cancer* 9:167
- Wilting SM, Verlaet W, Jaspers A, Makazaji NA, Agami R, Meijer CJ et al (2013) Methylation-mediated transcriptional repression of microRNAs during cervical carcinogenesis. *Epigenetics* 8(2):220–228
- Wolf SS (2009) The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans. *Cell Mol Life Sci* 66(13):2109–2121
- Xiao B, Jing C, Wilson JR, Walker PA, Vasisht N, Kelly G et al (2003) Structure and catalytic mechanism of the human histone methyltransferase SET7/9. *Nature* 421(6923):652–656
- Xu X, Chen Z, Zhao X, Wang J, Ding D, Wang Z et al (2012) MicroRNA-25 promotes cell migration and invasion in esophageal squamous cell carcinoma. *Biochem Biophys Res Commun* 421(4):640–645
- Yamakuchi M, Ferlito M, Lowenstein CJ (2008) miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci U S A* 105(36):13421–13426
- Yang X, Feng M, Jiang X, Wu Z, Li Z, Aau M et al (2009) miR-449a and miR-449b are direct transcriptional targets of E2F1 and negatively regulate pRb-E2F1 activity through a feedback loop by targeting CDK6 and CDC25A. *Genes Dev* 23(20):2388–2393
- Yang S, Li Y, Gao J, Zhang T, Li S, Luo A et al (2012) MicroRNA-34 suppresses breast cancer invasion and metastasis by directly targeting Fra-1. *Oncogene* 32(36):4294–4303
- Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N et al (2008) Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. *Proc Natl Acad Sci U S A* 105(19):7004–7009
- Zhang Z, Tang H, Wang Z, Zhang B, Liu W, Lu H et al (2011) MiR-185 targets the DNA methyltransferases 1 and regulates global DNA methylation in human glioma. *Mol Cancer* 10:124
- Zhu A, Xia J, Zuo J, Jin S, Zhou H, Yao L et al (2012) MicroRNA-148a is silenced by hypermethylation and interacts with DNA methyltransferase 1 in gastric cancer. *Med Oncol* 29(4):2701–2709
- Zhu X, Li Y, Shen H, Li H, Long L, Hui L et al (2013) miR-137 inhibits the proliferation of lung cancer cells by targeting Cdc42 and Cdk6. *FEBS Lett* 587(1):73–81

MicroRNAs: Key Regulators of Oncogenesis

Babashah, S. (Ed.)

2014, XXVI, 433 p. 26 illus., 21 illus. in color., Hardcover

ISBN: 978-3-319-03724-0