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Cross Talk Between MicroRNA and Coding Cancer Genes

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Abstract

MicroRNAs (miRNAs) are a class of non-coding RNAs (ncRNAs) and post-transcriptional gene regulators shown to be involved in pathogenesis of all types of human cancers. Their aberrant expression as tumor suppressors can lead to cancerogenesis by inhibiting malignant potential, or when acting as oncogenes, by activating malignant potential. Differential expression of miRNA genes in tumorous tissues can occur due to several factors including positional effects when mapping to cancer-associated genomic regions, epigenetic mechanisms and malfunctioning of the miRNA processing machinery, all of which can contribute to a complex miRNA-mediated gene network misregulation. They may increase or decrease expression of protein-coding genes, can target 3'-UTR or other genic regions (5'-UTR, promoter, coding sequences), and can function in various subcellular compartments, developmental and metabolic processes. Because expanding research on miRNA-cancer associations has already produced large amounts of data, our main objective here was to summarize main findings and critically examine the intricate network connecting the miRNAs and coding genes in regulatory mechanisms, their function and phenotypic consequences for cancer. By examining such interactions we aimed to gain insights for development of new diagnostic markers as well as identify potential venues for more selective tumor therapy. To enable efficient examination of the main past and current miRNA discoveries, we developed a web based miRNA timeline tool that will be regularly updated (http://www.integratomics-time.com/miRNA_timeline). Further development of this tool will be directed at providing additional analyses to clarify complex network interactions between miRNAs, other classes of ncRNAs and protein coding genes and their involvement in development of diseases including cancer. This tool therefore provides curated relevant information about the miRNA basic research and therapeutic application all at hand on one site to help researchers and clinicians in making informed decision in regards to their miRNA cancer-related research or clinical practice.

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Keywords

microRNA (miRNA); cancer; oncogene; tumor suppressor; epigenetics; genetic variation; transcriptional regulation

Cancer develops through a complex multistep process involving structural and expression abnormalities of genes, including those encoding microRNAs (miRNAs) (1,2). MiRNAs are a class of non-protein coding RNAs that post-transcriptionally regulate expression of the target mRNAs. MiRNAs that have been associated with cancer are referred to as oncomiRs (3,4). The role of miRNAs in cancer was hinted early in the history of miRNA research by three important observations (5): (1) miRNAs are involved in cell proliferation and apoptosis (6,7), (2) miRNA genes are frequently located at fragile sites and cancer-associated genomic regions (CAGRs) (8), and (3) miRNA expression is deregulated in malignant tumors and tumor cell lines in comparison with normal tissues (1,9,10). However, the first direct evidence of their oncogenic activity was reported when MIR15A and MIR16-1 were found to be deleted or down-regulated in most chronic lymphocytic leukemia (CLL) patients (11). Recent analyses of miRNA genes, their targets, processing machinery, genetic polymorphisms, and epigenetic modifications revealed that miRNA-mediated regulation in gene regulatory networks involves a far more complex system than initially expected. We therefore aimed here to present the main miRNA related discoveries in an online timeline format which will be updated regularly with new discoveries (http://www.integratomics-time.com/miRNA_timeline). A summary of the timeline is presented in Table 1. We also presented an online list of the most extensive review articles sorted according to the topic of miRNA research in cancer; *e.g.* (1) general, (2) polymorphisms, (3) miRNA host genes, (4) transcription factors, (5) epigenetics, *etc.* (http://www.integratomics-time.com/miRNA_timeline/reviews). This review and web-based tool developed should enable efficient examination of past and current miRNA-cancer publications and enable critical exploration of interaction networks involved. Curated and regularly updated information at one site will be useful for researchers and clinicians to guide their miRNA basic research and therapeutic applications in clinical practice.

Interplay between microRNA and cancer genes

A predominant reason for cancerogenic cell transformation is a combined interaction of both tumor suppressors and oncogenes. MiRNAs can function as oncogenes, by activating malignant potential, or as tumor suppressors, by blocking the cell's malignant potential (3,12). Modulation of miRNA biogenesis pathway can also promote tumorigenesis through increased repression of tumor suppressors and/or through incomplete repression of oncogenes (Figure 1). MiRNAs can affect all major hallmarks of malignant cells: self-sufficiency in growth signals, evasion of apoptosis, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, and tissue invasion and metastasis (13,14). MiRNA deregulation can therefore contribute to oncogenesis in various ways. Two studies described a more direct relationship between a miRNA cluster *MIR17HG*, *MYC* and cancer oncogenic pathway, revealing a complex genetic circuit that regulates cell proliferation, growth, and apoptosis (15,16). Overexpression of the *MIR17HG* cluster was found to act with c-Myc expression to accelerate tumorigenesis in mice; therefore this cluster was suggested to be a potential non-coding oncogene (15). On the other hand, the *MIRLET7* family showed tumor suppressor activity by regulating the expression of a proto-oncogene, the RAS protein, which is a membrane-associated signaling protein that regulates cell growth (17). Several studies therefore support the view that miRNAs are a class of non-coding nucleic acids that can function as oncogenes or as tumor suppressors contributing to oncogenesis.

It was estimated that about one-third of human mRNAs are considered as miRNA targets (18). Vertebrate miRNAs target about 200 transcripts each and more than one miRNA might coordinately regulate a single target (19) thereby providing a basis for complex networks. Predicted targets for the differentially expressed miRNAs in human solid tumors have been shown to be significantly enriched for protein-coding tumor suppressors and oncogenes (20). The standard “dogma”, that miRNAs target the 3'-UTR of genes and downregulate the expression of protein-coding genes in cytoplasm, has been expanded with the following additional observations: (1) miRNAs can be localized in the nucleus (21), (2) miRNAs target other genic regions in addition to 3'-UTR (5'-UTR, promoter regions, coding regions) (22,23,24,25), and (3) miRNA upregulate translation (26,23) (Figure 2). It has been shown that human miRNA MIR369-3 targets AU-rich elements (AREs) in the target gene *TNF* to activate translation of proteins whose expression they normally repress during cell proliferation (26). It has also been shown that miRNAs can affect transcription at the promoter level: human MIR373 binds to the E-cadherin (*CDH1*) promoter and induces transcription (23). MiRNA-dependent mRNA repression also occurs through binding sites located in mRNA coding sequences (CDS), as shown for miRNAs with targets in developmentally regulated genes (24,25). In addition to miRNA-mediated gene silencing through base pairing with mRNA targets, miRNAs also interfere with the function of regulatory proteins (decoy activity) (27). In particular, MIR328 binds to poly-C binding protein 2 (PCBP2), alternatively known as heterogeneous ribonucleoprotein (hnRNP) E2, independently of the miRNA's seed region and prevents its interaction with the target mRNA (27). Downregulation of MIR328 in chronic myelogenous leukemia allowed PCBP2 inhibition of myeloid differentiation and, as a result, led to tumor progression (Eiring et al. 2010). Other ncRNAs, such as noncoding ultraconserved genes (UCGs), have been found to be consistently altered at the genomic level in a high percentage of leukemias and carcinomas and may interact with miRNAs in leukemias (28). The findings provide support for a model in which both coding and noncoding genes are involved and cooperate in human tumorigenesis. Recently, the role of coding and non-coding RNAs has been emphasized and grouped in an unifying theory of competing endogenous RNAs (ceRNAs) that can regulate one another through their ability to compete for miRNA binding (29). The ceRNA hypothesis suggests that long non-coding RNAs (lncRNAs) may elicit their biological activity through the ability to act as endogenous decoys for miRNAs and that such activity would in turn affect the distribution of miRNAs on their targets (29). CeRNAs may therefore be active partners in miRNAs regulation exerting effects on their expression levels, which may have important implications in pathological conditions, such as cancer.

The causes of dysregulated expression can be explained by analyzing the many layers of gene network regulation, including the miRNA gene location in cancer-associated genomic regions, epigenetic mechanisms, and alterations in the miRNA processing machinery (30,31) (Figure 3). Because each miRNA has numerous targets, inherited minor variations in miRNA expression may have important consequences for the expression of various protein-coding oncogenes and tumor suppressors involved in malignant transformation. Accumulation of additional somatic abnormalities in protein-coding genes or ncRNAs, including miRNAs, is necessary for the full development of the malignant phenotype (32). The expanding field of miRNA and cancer research therefore requires the consideration of interplay that connects the regulatory mechanisms and their function into an intricate network.

Single nucleotide polymorphisms in miRNA genes, their targets and processing machinery

Single nucleotide polymorphisms (SNPs) of miRNA precursors, their target sites, and miRNA processing machinery were reported to affect miRNA function and lead to

phenotypic effects (33). When referring to SNPs occurring in miRNA genes the term “miR-SNPs” is used, and “miR-TS-SNP” for SNPs located within miRNA target binding sites (34,35) (Figure 3). Even though many miRNA sequence variations observed in cancer alter the secondary structure with no demonstrated effects on miRNA processing (36), several recent reports show that SNPs located in miRNA genes are associated with cancer susceptibility (37,35,38). It was observed that miR-SNPs can affect function by modulating the miRNA precursor transcription, processing and maturation (39), or miRNA-mRNA interaction (17). Sequence variations in the mature miRNA, especially in the seed region (miR-seed-SNP), may have an effect on miRNA target recognition (40). Human miRNAs comprising miR-seed-SNPs have been shown to be frequently located within quantitative trait loci (QTL), chromosome fragile sites, and cancer susceptibility loci (40). Because of the miRNA-target interaction, the miR-SNPs (and/or miR-seed-SNPs) and miR-TS-SNPs function in the same manner to create or destroy miRNA binding sites. Chin *et al* (41) demonstrated a SNP that modified the MIRLET7 binding site in the v-Ki-ras 2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and was significantly associated with increased risk for non-small cell lung cancer (NSCLC). Even though miR-TS-SNPs were shown to influence susceptibility to tumorigenesis (42), additional association studies and follow-up functional experiments should still be applied to provide a clearer view on the interplay of these variations in disease development. In order for the miR-TS-SNP to be functional it must have a proven association with cancer, both miRNA and its predicted target must be expressed in the tissue, and allelic changes must result in different binding of miRNA and affect expression of the target gene (43). Nevertheless, we can conclude from available published studies that “miR-SNPs” provide an additional layer of functional variability of miRNAs in cancerogenesis and that ongoing studies using next generation sequencing technology and systems biology analyses are likely to provide additional evidence for miR-SNPs-cancer associations in the near future.

SNPs in miRNA-processing machinery may also have profound effects on the phenotype. SNPs that affect the proteins involved in miRNA biogenesis may have deleterious effects on the miRNAome and global repression of miRNA maturation was shown to lead to tumorigenesis (44). Several studies reported that genetic polymorphisms of the proteins involved in miRNA machinery affect cancer susceptibility (45,46,47). SNPs in the *GEMIN4* gene were significantly associated with altered renal cell carcinoma (45) and bladder cancer risk (46), SNP in the 3' UTR of *DICER1* was associated with an increased risk of premalignant oral lesions in individuals with leukoplakia and/or erythroplakia (47). The essential part in miRNA variation studies is identification of SNPs located within miRNA genes, their processing machinery, and targets, with bioinformatics tools such as Patrocles (48), miRNASNiPer (40), and PolymiRTS (49). These tools intercalate and cross-reference the data from dbSNP and as such aid in the search for miRNA-related polymorphisms. Although these tools provide useful information on existence of miR-SNPs and their possible effects on target regulation, we still need more experimental data to gain insights on which miR-SNP locations and types of nucleotide substitutions have the most profound effects on cancerogenesis. Such knowledge would have diagnostic power in predicting a person's risk for cancer development based on his/her high risk miR-SNP genotype. Additionally, in people carrying low risk miR-SNP alleles that have developed primary tumors, genotyping for miR-SNPs of tumor biopsies may reveal somatic mutations that generated high risk miR-SNPs potentially aiding in the correct diagnosis of the cancer type and in therapeutic decisions.

Transcription factor-miRNA regulatory network

MiRNAs are transcriptional and post-transcriptional gene regulators and, like protein-coding genes, are also regulated by transcription factors (TF), another class of gene regulators that

act at the transcriptional level. MiRNA genes are also linked with TFs in complex regulatory networks where they reciprocally regulate one another (50) (Figure 4a). It is estimated that up to 43% of human genes are under combined regulation at transcriptional and post-transcriptional level (51). Due to TF's and/or miRNA's involvement in cancer, the disruption of their co-regulation may be associated with oncogenesis. O'Donnell *et al* (16) found that the MIR17HG cluster is transactivated by *MYC*, an oncogene frequently dysregulated in cancer. Similarly, *TP53*, a tumor-suppressor gene whose pathway mutations have been discovered in many cancer types, was found to regulate expression of MIR34A (52). On the other hand, overexpression of MIR125B has been shown to reduce levels of TP53 protein and suppress apoptosis in human cancer cells, whereas knockdown of MIR125B elevates the level of TP53 (53).

Coordinated miRNA/TF regulation engage in a wider diversity of biological processes which can have a higher specificity than regulation within only one layer of regulation (54). MiRNAs and TFs have been found to cooperate in tuning gene expression, by which miRNAs were found to preferentially target genes with transcriptional regulation complexity (55,56). In regulatory networks, miRNAs and TFs can reciprocally regulate one another and form feedback loops, or form feed forward loops in which both TFs and miRNAs regulate their target genes (Figure 4b). An example of a complex network interaction in cancer was described by O'Donnell *et al* (16) who found that MIR20A modulates the translation of E2F transcription factor 1 (*E2F1*) mRNA by binding to its 3'-UTR. Moreover, E2F1 binds to the promoter of the MIR17HG cluster, activating its transcription (16). A miRNA/protein feedback circuitry (miRNA/TP53) has been found to be associated with pathogenesis and prognosis of CLL (57). In 13q deleted CLLs the MIR15A/16-1 cluster directly targets TP53 and its downstream effectors. In leukemic cell lines and primary B-CLL cells TP53 stimulates the transcription of both MIR15A/16-1 and MIR34B/34C clusters, and the MIR34B/34C cluster directly targeted the ZAP70 kinase. This mechanism provides a novel pathogenetic model for the association of 13q deletions with the indolent form of CLL that involves miRNAs, TP53 and ZAP70 (57). Complex patterns in miRNA-TF interplay have also been computationally analyzed and the generated databases (TransmiR and dPORE-miRNA), present valuable regulatory framework for future experimental analyses (58,59). Transcription factor -miRNA regulation is therefore not confined only to the "classical" action of transcription factors on miRNA promoters and their regulation but is extended to reciprocal and mutual interactions forming a complex regulatory network.

MiRNA and epigenetics in cancer

Several aspects of epigenetic regulation have been found to be associated with miRNAs: (1) they regulate target gene expression; regulation of gene expression mediated by miRNAs is frequently reported in cancer and presents one component of an interacting network of epigenetic mechanisms. (2) A subclass of miRNAs (epi-miRNAs) directly controls the epigenetic machinery through a regulatory loop by targeting its regulating enzymes. (3) MiRNA expression could be affected by CpG island hypermethylation-associated silencing in the promoter region or CpG de-methylation-associated activation of miRNA promoters (Figure 5).

Several epi-miRNAs have been shown to be directly connected to the epigenetic machinery by regulating the expression of its regulatory enzymes (60,61,62). The first epi-miRNAs were identified in lung cancer in a study where MIR29 family (*MIR29A*, *MIR29B*, and *MIR29C*) was shown to target and downregulate *de novo* DNA methyltransferases (DNMT3A and DNMT3B) (60). Additionally, MIR29B has been shown to induce global DNA hypomethylation and tumor suppressor gene re-expression in acute myeloid leukemia by targeting directly *DNMT3A* and *3B* and indirectly the DNMT (*DNMT1*) (63). This led to

demethylation of CpG islands in the promoter regions of tumor-suppressor genes, allowing their reactivation and a loss of the cell's tumorigenicity (60). It was also reported that MIR449A targets histone deacetylase 1 (*HDAC1*), a gene that is frequently overexpressed in many types of cancer, and consecutively induces growth arrest in prostate cancer (61). Also, MIR101 was shown to directly modulate the expression of enhancer of zeste homolog 2 (*EZH2*), a catalytic subunit of the polycomb repressive complex 2 (PRC2), which mediates epigenetic silencing of tumor-suppressor genes in cancer (62).

Expression of miRNA genes have also been found to be silenced in human tumors by epigenetic mechanisms, such as aberrant hypermethylation of CpG islands encompassing and/or in proximity of miRNA genes, or by histone acetylation (64). The first evidence that altered methylation status can deregulate the expression of a miRNA in cancer was reported by Saito *et al* (65). *MIR127*, embedded within a CpG island promoter, was silenced in several cancer cells, but strongly upregulated after treatment with a hypomethylating agent (DNMT inhibitor). A similar scenario was observed with MIR124A whose function can be restored by erasing DNA methylation and has functional consequences on cyclin D kinase 6 (*CDK6*) activity (66). On the other hand, Brueckner *et al* (67) observed that hypomethylation of MIRLET7A3 facilitates reactivation of the gene and elevates expression of MIRLET7A3 in human lung cancer cell lines, which resulted in enhanced tumor phenotypes. Compared with protein-coding genes, human oncomiRs were found to have an order of magnitude higher methylation frequency (64,68). Future studies of epigenetic regulation of miRNA expression coupled to downstream signaling pathways are likely to lead to development of novel drug targets in cancer therapy (68).

MiRNA expression profiles / signatures

Profiling of miRNAs is used to document their expression variability, and it was shown to be more accurate for cancer classification than by using sets of known protein-coding genes (32,69). In cancer, the loss of tumor-suppressor miRNAs enhances the expression of target oncogenes, whereas increased expression of oncogenic miRNAs can repress target tumor suppressor genes. Paired expression profiles of miRNAs and mRNAs can be used to identify functional miRNA-target relationships with high precision (70). The aberrant expression of miRNAs in cancer is characterized by abnormal levels of expression for mature and/or precursor miRNA transcripts in comparison to those in the corresponding normal tissue. Lu *et al* (69) observed a general down-regulation of miRNAs in tumor samples compared to normal tissue samples. It was also found that miRNA expression profiles could differentiate human cancers according to their developmental origin, with cancers of epithelial and hematopoietic origin having distinct miRNA profiles (69). The first evidence that miRNA expression could be altered in cancer came from the observation by Carlo Croce group that MIR15A/MIR16-1 gene cluster is located in a genomic region frequently deleted in CLL and that their expression is frequently downregulated or deleted in CLL (11). Afterwards, numerous studies examined aberrant miRNA expression signatures in cancer. A review analyzing 58 studies (71) revealed 70 differentially expressed miRNAs in cancers, which were reported in at least five studies. The causes of widespread alterations of miRNA expression in cancer cells include different factors, such as: the location of miRNAs at cancer-associated genomic regions (CAGR) (8), epigenetic regulation (72), and abnormalities in genes and proteins of the miRNA processing machinery (73). Since deregulated miRNA expression is an early event in tumorigenesis, measuring the levels of circulating miRNAs may also be useful for early cancer detection. Fluid-expressed miRNAs have been discussed as reliable cancer biomarkers and treatment response predictors as well as potential new patient selection criteria for clinical trials (74). Profiling of miRNA expression correlates with clinical and biological characteristics of tumors and has enabled the identification of signatures associated with diagnosis, staging, progression, prognosis,

and response to treatment of human tumors (75). MiRNA fingerprinting therefore represents an additional tool in the clinical oncology.

MiRNAs as diagnostic, prognostic and therapeutic targets in cancer

Based on their regulatory function, miRNAs are important players in the oncogenic signaling pathway, which is why they should be considered in cancer diagnosis and prognosis. As already mentioned, miRNA expression profiles contain much information that could explain developmental processes in cancer, and are disrupted by different mechanisms mentioned in the previous section on miRNA expression profiles / signatures (32). A general down-regulation of miRNAs was observed in tumor samples, compared to normal tissue samples. A unique miRNA signature is associated with prognostic factors and disease progression in CLL. Mutations in miRNA transcripts are common and may have functional importance (76).

Several studies have indicated various strategies for therapeutic usage of miRNAs in cancer. Four different strategies for potential therapies were proposed (77): (1) anti-miRNA oligonucleotides (AMOs), inhibitory molecules that block the interactions between miRNA and its target mRNA by competition (78). (2) MiRNA sponges, synthetic miRNAs that contain multiple binding sites for an endogenous miRNA and prevent the interaction between miRNA and its endogenous targets (79). Ebert *et al* (79) have also designed these sponges with complementary seed regions, which effectively repress an entire miRNA seed family. (3) MiRNA masking refers to a sequence with perfect complementarity to the binding site for an endogenous miRNA in the target gene, which can in turn form a duplex with the target mRNA with higher affinity and blocking the access of the miRNA (80). (4) Small molecule inhibitors against specific miRNAs refer to chemicals or reagents able to specifically inhibit miRNA synthesis. One such example is azobenzene, a specific and efficient inhibitor of *MIR21* biogenesis (81). Apart from aforementioned four therapeutic approaches proposed by Li et al. (77) others have proposed and demonstrated alternative strategies such as restoring activity of tumor-suppressive miRNAs to rescue its anti-tumor function, (82,83,84). Some studies also suggest that by selecting miRNAs that are highly expressed in normal tissues but lost in cancer cells can be used in the general strategy for restoring tumor suppressor miRNAs as therapy (85,86,87). Another approach to utilize miRNA in cancer therapy is in sensitizing tumors to chemotherapy (88). Due to ability of miRNAs to target signaling pathways that are frequently misregulated in cancers, studies have examined the potential of miRNAs or antagomirs to sensitize resistant cells to already known and successful cancer therapies (*e.g.*, tamoxine, gefitinib treatments, etc.). Several promising *in vitro* and mouse model studies have already shown efficacy of this approach guiding now the clinical development of miRNA-based therapies for sensitization to chemotherapy.

However, despite the advances made in the miRNA-mediated therapy, two major hurdles still remain: the first is to maintain target specificity, which is especially challenging and its effect needs to be evaluated on a proteome-wide scale to prevent unwanted gene alterations, due to partial complementary binding between miRNAs and protein-coding transcripts (77). The second hurdle is to achieve high therapeutic efficiency, which is linked with delivery efficiency. In addition to lipid- and polymer-based nanoparticles for systemic delivery, viral vectors may be also used; these approaches were found suitable for certain types of tumors and further investigations are needed for evaluation of these approaches in various tumor types (77).

Conclusions and future directions

The causes of differential expression of miRNA genes in tumorous tissues can be better understood by including multiple layers, such as their location in cancer-associated genomic regions, epigenetic mechanisms, and alterations in the miRNA processing machinery into a coordinately regulated network. The expanding field of miRNA and cancer research therefore requires the consideration of such an interplay that connects the regulatory mechanisms and their function into an intricate network. Future studies will further clarify complex interactions of short and long ncRNAs with protein coding genes and their involvement in shaping phenotypes and cancer development.

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Figure 1.
Tumorigenesis promoted by modulation of miRNA biogenesis pathway.

Figure 2.

Cross talk between miRNAs and target.

ARE: AU-rich elements; CDS: coding sequence; *CDH1*: E-cadherin; *DNMT*: DNA methyltransferase; *HDAC*: histone deacetylase; *Nanog*: Nanog homeobox; *Pou5f1*: POU class 5 homeobox 1 (synonym: *Oct4*); *PRC*: polycomb repressive complex; *Sox2*: sex determining region Y box 2; *TNFα*: tumor necrosis factor alpha; UCG: ultraconserved gene.

Figure 3.

MiRNA biogenesis and mechanisms of regulation. MiRNA expression and regulation can be affected by transcriptional deregulation, epigenetic modifications (DNA methylation and/or histone acetylation), polymorphisms (SNPs) present in miRNA genes, their processing machinery and targets.

miR-SNP: single nucleotide polymorphisms (SNPs) in the miRNA gene; miR-TS-SNP: SNPs in the miRNA target sites; Ac:acetyl groups; *empty circles*: unmethylated CpG sites; *filled circles*: methylated CpG sites

Figure 4.

TF-miRNA regulatory network. **(a)** Basic network motif in transcriptional and post-transcriptional gene regulation. **(b)** Schematic representation of examples of regulatory circuit, including MIR17HG – MYC – E2F1 pathway (left) and the MIR15A/16-1 cluster – TP53 – MIR34B/4C cluster – ZAP70 pathway (right).

Figure 5. Epigenetic concepts of miRNA regulatory network. DNMTs: DNA methyltransferases; HDACs: histone deacetylases; PRC: polycomb repressive complex; Ac:acetyl groups;*empty circles*: unmethylated CpG sites; *filled circles*: methylated CpG sites.

Table 1

A timeline of main miRNA discoveries.

year	discovery	species	associated with	reference
1960s	non-protein coding transcripts (activator RNAs) regulate gene activity	eukaryotes		(Britten and Davidson 1969)
1993	first miRNA (lin-4) discovered	<i>C. elegans</i>		(Lee, Feinbaum, and Ambros 1993)
2000	discovered microRNA (MIRLET7)	<i>C. elegans</i>		(Reinhart et al. 2000)
2000	RNAi "unit": 21–23 nt	<i>Drosophila</i>		(Zamore et al. 2000)
2001	a large class of small RNAs (named miRNAs) are co-expressed in clusters and have potential regulatory roles	<i>C. elegans</i> , invertebrates, vertebrates		(Lau et al. 2001) (Lagos-Quintana et al. 2001) (Lee and Ambros 2001)
2001	Dicer in miRNA biogenesis pathway	<i>C. elegans</i> , <i>Drosophila</i>		(Grishok et al. 2001) (Hutvagner et al. 2001)
2002	miRNAs discovered in plants	plants		(Reinhart et al. 2002) (Rhoades et al. 2002)
2002	miRNA alterations found in cancer cells (MIR15A and MIR16-1 deleted or downregulated in most chronic lymphocytic leukemias)	Human	cancer	(Calin et al. 2002)
2004	more than 50% of miRNA genes are located in cancer-associated genomic regions or fragile sites	human	cancer	(Calin et al. 2004)
2004	miRNA as diagnostic/prognostic biomarker	human	cancer	(Takamizawa et al. 2004)
2004	co-expression of miRNAs and their host genes	mouse		(Rodríguez et al. 2004)
2005	miRNA-target interaction relevant to cancer	<i>C.elegans</i> , human	cancer	(Johnson et al. 2005)
2005	altered expression of miRNAs affects tumor formation/growth <i>in vivo</i>	Human	cancer	(He et al. 2005)
2005	connection between miRNAs and the MYC oncogene	human, rat	cancer	(O'Donnell et al. 2005)
2005	inhibition of miRNA by antagomirs in mammals	mouse		(Krützfeldt et al. 2005)
2006	molecule of the year (MIR155 and MIRLET7A2)	human	cancer	(Yanaihara et al. 2006)
2006	epigenetic regulation (DNA methylation and histone deacetylase inhibition) of miRNAs	human	cancer	(Saito et al. 2006)
2007	miRNA target sites can also occur in 5'-UTR	<i>C. elegans</i>		(Lytle, Yario, and Steitz 2007)
2007	miRNAs deregulation in cancer metastasis	human	cancer	(Ma, Teruya-Feldstein, and Weinberg 2007)
2007	miRNAs can up-regulate mRNA expression and initiate the translation of proteins	human		(Vasudevan, Tong, and Steitz 2007)
2007	miRNAs can affect epigenetic changes and cause the reactivation of silenced tumor suppressor genes	human	cancer	(Fabbri et al. 2007)
2007	miRNAs can regulate ncRNAs from the category of long ultraconserved genes (UCGs)	human	cancer	(Calin et al. 2007)
2007	miRNAs carrying hexanucleotide terminal motifs are enriched in the nucleus			(Hwang, Wentzel, and Mendell 2007)
2008	miRNA (MIR373) targets promoter sequences and induces gene expression	human		(Place et al. 2008)
2008	miRNAs can transcriptionally silence gene expression	human		(Kim et al. 2008)
2008	functional single nucleotide polymorphism (SNP) in the miRNA seed region	human	cancer	(Shen et al. 2008)
2008	miRNA binding sites located within mRNA-coding sequence			(Tay et al. 2008)

year	discovery	species	associated with	reference
2009	proof of concept of miRNA delivery as cancer therapy	human, mouse	cancer	(Kota et al. 2009)
2010	miRNA as molecular decoys	human, mouse	cancer	(Eiring et al. 2010)
2010	miRNAs predominantly cause mRNA destabilization	human, mouse		(Guo et al. 2010)
2010	pseudogene PTEN saturates miRNA binding sites	human	cancer	(Poliseno et al. 2010)
2010	overexpression of a single miRNA is sufficient to cause cancer	mouse	cancer	(Medina, Nolde, and Slack 2010)
2011	competing endogenous RNA (ceRNA) communicate with and regulate other RNA transcripts by competing for shared miRNAs	human		(Salmena et al. 2011)