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MicroRNAs in cancer

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Abstract

During the last few years, studies on microRNA (miRNA) and cancer have burst onto the scene. Profiling of the miRNome (global miRNA expression levels) has become prevalent and abundant miRNome data are currently available from various cancers. The pattern of miRNA expression can be correlated with cancer type, stage, and other clinical variables, so that miRNA profiling can be used as a tool for cancer diagnosis and prognosis. miRNA expression analyses also suggested oncogenic (or tumor suppressive) roles of miRNAs. miRNAs play roles in almost all aspects of cancer biology such as proliferation, apoptosis, invasion/metastasis, and angiogenesis. Given that many miRNAs are deregulated in cancers but have not yet been further studied, it is expected that more miRNAs will emerge as players in the etiology and progression of cancer. miRNAs will be also discussed as a tool for cancer therapy.

SYNOPSIS—During the last decade, a major discovery in biology was the discovery of small RNAs, including miRNA (microRNA) and siRNA (small interfering RNA), as highlighted by the 2002 December issue of *Science* magazine (1). Since RNA interference (RNAi) phenomenon was discovered in nematodes (2), siRNA has provided a technical breakthrough for short term genetics in mammalian systems. The big impact of small RNAs was well celebrated by the 2006 Nobel prize awarded to the two scientists who discovered RNAi.

On the other side, miRNAs shed new insight on the post-transcriptional regulation of gene expression. miRNAs were also first discovered in worms (3,4), and later in a number of animals, plants, and viruses. During the last couple of years, the miRNA field has been expanding with many recent publications implicating miRNAs in diverse cellular processes.

Cancer is a major cause of death in the United States (“Cancer Facts & Figures 2007” from American Cancer Society; http://www.cancer.org/docroot/stt/stt_0.asp). Cancer is a complex genetic disease caused by the accumulation of mutations that lead to deregulation of gene expression and uncontrolled cell proliferation. Given the wide impact of miRNAs on gene expression, it is not surprising that a number of miRNAs have been implicated in cancer. In this review, the links between miRNA and cancer will be comprehensively described and discussed.

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CONCLUDING REMARKS

The discovery of miRNAs has provided a new layer in the regulation of gene expression. In addition to transcriptional regulation, post-transcriptional repression by miRNAs might act like “rheostats” for fine-tuning of gene expression. miRNAs at end stages of differentiation may contribute to keeping cells differentiated by suppressing a large number of genes simultaneously.

In recent years, there has been an explosion of publications on miRNAs. Many of these have emphasized the role of miRNAs in cancer biology. miRNAs play a role in almost all aspects of cancer biology such as proliferation, apoptosis, invasion/metastasis, and angiogenesis. New technical developments are allowing the description of a variety of cancers miRNomes. Therefore, more miRNAs are expected to be identified as oncogenes or tumor suppressors. Characterization of individual miRNA pathways will be greatly facilitated by an improvement of target prediction algorithm.

miRNA expression is associated with clinical variables of cancers, so that miRNAs can be readily used as tools for cancer diagnosis and prognosis. For therapeutic benefit, the methods to manipulate miRNAs in vivo should become more robust.

Keywords

microRNA; cancer; tumorigenesis; oncogene; tumor suppressor; microRNA expression profile; diagnosis and prognosis; therapy

INTRODUCTION

MicroRNAs (miRNAs) are small non-coding regulatory RNAs with sizes of 17-25 nucleotides (miRBase; <http://microrna.sanger.ac.uk/>). The definition of miRNAs is based on their generation by the action of Dicer, an RNase that processes hairpin structured precursors (called pre-miRNA) into mature miRNAs (5). miRNAs post-transcriptionally repress gene expression by recognizing complementary target sites in the 3'untranslated region (UTR) of target mRNAs.

Since the first miRNA was reported by Ambros and Ruvkun (3,4) in 1993, it took eight years for the miRNA era to begin in earnest when three groups identified tens of small RNAs from *C. elegans*, *Drosophila*, and human (6-8). During the last seven years, the number of miRNAs as well as related publications have expanded enormously.

Genes, sequences, and genomic organization

Currently, more than 5,000 miRNAs from over 50 organisms are registered in the database (miRBase Release 10.0: August 2007). 533 human miRNAs are known now but this number may rise, as up to 1000 miRNAs are predicted to exist from bioinformatic studies (9,10). miRNAs are named as miR- plus numbers, bearing a few exceptions. miRNAs of similar sequence are usually distinguished by an additional letter (a, b, c...) following the miRNA number (e.g. miR-125b). A miRNA of identical mature sequence may appear at several genomic loci with different precursor sequences. In this case, the different miRNA genes are distinguished by the addition of another number (1, 2, 3...) at the end (e.g. miR-125b-1).

In humans, approximately one third of miRNAs are organized in clusters. A given cluster is likely to be a single transcriptional unit, suggesting a coordinated regulation of miRNAs in the cluster. In silico analysis revealed that more than half of the clusters contain two or more miRNAs of similar sequence (11). However, it is very rare that miRNAs of an identical mature sequence are duplicated in a cluster. This genomic organization confers simultaneous expression of similar miRNAs, possibly leading to combinatorial diversity and synergy in the biological effects. However, it is worth noting that all the miRNAs from a single transcriptional cluster are not expressed at equal levels, suggesting that miRNAs are also regulated post-transcriptionally.

A significant portion of miRNAs are located in the intronic region of protein-coding or non-coding transcription units (12), while a minor subset of miRNAs are mapped to repetitive sequences such as LINEs (long interspersed nuclear elements) (13).

Expression

Many miRNAs exhibit characteristic expression pattern. Some miRNAs are differentially expressed in developmental stages, like the two founding members, *lin-4* and *let-7* in *C. elegans*. For this reason, they were once called stRNA (small temporal RNA) because they are expressed in specific temporal phases of development and regulate the developmental timing. Many miRNAs are expressed in a tissue-specific manner. In the few cases where a forward genetic approach in worms or flies led to identification of a miRNA, the function of the miRNA can be inferred from the phenotype of the mutant. In mammalian cells, however, a miRNA

expression profile is usually the first clue for its possible role. Analogous to mRNA expression, miRNA expression is determined by intrinsic cellular factors as well as diverse environmental variables.

Expression of a miRNA can be measured by molecular biology techniques, such as Northern blot, RNase protection assay or primer extension assay. The small size of miRNAs initially hampered PCR-based methods. However, since adaptor mediated qRT-PCR (quantitative real time-PCR) protocols were developed (reviewed in (14)), PCR-based techniques have now become very popular due to their high sensitivity. Microarray techniques (reviewed in (15)) are widely used to comprehensively assay the entire miRNome (global miRNA expression profile) in tissues or cell lines (Table 1). Besides microarray or qRT-PCR, miRNomes have been obtained by in situ hybridization (16) or SAGE (serial analysis of gene expression) adapted for small RNAs (17). The SAGE approach has been stimulated by recent innovations in ultra-high-throughput sequencing that provide a powerful tool for various genomics studies. Overall, these technical improvements are expected to greatly widen the repertoire of miRNAs in a variety of biological systems.

Biogenesis

Biogenesis of a miRNA begins with the synthesis of a long transcript called a pri-miRNA (Fig 1). In general, pri-miRNAs are transcribed by RNA polymerase II and retain mRNA features such as 5' cap structure and 3' poly(A) tail (18,19). However, other pathways generate in a minor set of miRNAs especially from genomic repeats. For example, RNA polymerase III is responsible for transcription of miRNAs in Alu repeats (20).

In the nucleus, pri-miRNA is processed to pre-miRNA by RNase III enzyme Drosha and its interacting partner DGCR8 (21-23). DGCR8 recognizes the stem and the flanking single stranded RNA (ssRNA) and serves as a ruler for Drosha to cut the stem approximately 11 nucleotides away from the stem-ssRNA junction to release the hairpin-shaped pre-miRNA (24). A subset of miRNAs (miRtrons) bypass the Drosha requirement, by taking an alternative pathway where pre-miRNAs are derived as a by-product of a splicing event (25-27). The pre-miRNA is exported by exportin-5 to the cytoplasm (28,29) and converted to mature duplex miRNA by another RNase III enzyme, Dicer (30). The two strands of the duplex are separated by a RNA helicase (31) during the specific assembly of a miRNA particle (miRNP). Like the strand selection of an siRNA (32,33), the strand whose 5' end forms the more unstable duplex with its partner seems to preferentially survive as the miRNA in the miRNP (24,32,33). However, detailed molecular events during the miRNP assembly and strand selection are not yet clear.

Mechanisms of action

miRNAs post-transcriptionally suppress the target mRNA expression, mostly through interaction with the 3'UTR (Fig 1). However, the exact mechanism for miRNAs on their targets is still controversial (reviewed in (34)). Unlike perfect sequence complementarity between siRNA and mRNA, there are mismatches and bulges in most miRNA target sites. Comparison between siRNA and miRNA in earlier papers suggested that siRNA destabilizes mRNA whereas miRNA inhibits mRNA translation without affecting the mRNA level. Therefore, the degree of complementarity between short RNA and target was thought to be a major determinant distinguishing the two mechanisms.

Although the translational repression mechanism still holds true for many miRNAs, it has been also demonstrated in a number of publications that a miRNA can decrease the level of a target mRNA despite imperfect sequence complementarity between the miRNA and the target (35-38). mRNA degradation by a miRNA, which is distinguished from siRNA-mediated

mRNA cleavage, can be explained by RNA processing bodies (P-bodies), sites for RNA decay (39,40). Plausibly, miRNAs inhibit translation of target mRNAs which are then sequestered to P-bodies and subject to degradation. This model fits well for both mechanisms, though miRNAs have also been proposed to lead to the degradation of the target mRNAs without sequestration to P-bodies (41,42). In some cases where mRNA translation is inhibited by a miRNA but mRNA level stays similar, sequestration to P-bodies might be inefficient. Nonetheless, it is an open possibility that different mechanisms apply to individual miRNA-mRNA interactions.

Target prediction and identification

Prediction of miRNA target is important, given that miRNAs exert their function by regulating the target mRNAs. The specificity of miRNA-mRNA interaction is mainly conferred by the first eight nucleotides of a miRNA (called seed sequence) (43). Besides seed pairing, the likelihood that a predicted target is a bona fide target is influenced by other factors such as the number of target sites, context of surrounding sequence in mRNA (44), and occlusion of target sites by RNA-binding proteins (45). Currently, several computational algorithms (reviewed in (46)) are available to predict the target mRNA(s) but they are far from perfect. The gold standard is experimental demonstration that a luciferase reporter fused to the 3'UTR of the predicted target is repressed by overexpression of the miRNA and that this repression is abrogated by point mutation in the target sequence(s) in the 3'UTR. Many targets are predicted by in silico analyses but all of them are not confirmed as real targets in this biological assay.

The in silico predictions have been complemented by experimental screenings for targets of miRNAs. As miRNAs destabilize mRNAs, miRNA depletion by a knockdown of miRNA processing machinery is expected to result in upregulation of target mRNA, which can be assayed by microarray analysis. This approach identified HMGA2 as a target of *let-7* (38). Microarrays can also identify mRNAs that are decreased upon transfection of a miRNA and screening these mRNAs for computationally predicted sites targeted by the miRNA yields some bona fide targets (37). A proteomics approach to identify proteins decreased by miRNAs led to the identification of tropomyosin 1 (TPM1) as a miR-21 target (47). Another screen employed a library of miRNA-expressing vectors and a sensor plasmid containing GFP fused with the 3'UTR of the gene of interest. p27(Kip1) was found to be targeted by miR-221 and -222 by this approach (48). Another approach is to isolate miRNA-target mRNA complex by immunoprecipitation of a RISC component followed by microarray hybridization of the precipitated mRNAs (49).

Although a growing number of miRNA-target pairs are being identified (Table 4), the fraction of validated pairs are still small, given that 30% of mRNA have been predicted to be miRNA targets (50,51). New target identification will be facilitated by the accumulation of validated miRNA-target pairs which can be utilized to develop a better prediction algorithm. In addition, experimental tools for high-throughput target screening need to be improved.

Biological roles

miRNAs play important roles in cell fate determination, proliferation, and cell death. Besides these vital processes, miRNAs are implicated in diverse cellular activities, such as immune response (reviewed in (52,53)), insulin secretion (54), neurotransmitter synthesis (55), circadian rhythm (56), viral replication (57), etc. This list will undoubtedly expand as experimental data accumulate.

miRNA transfection results in up- and down-regulation of a number of mRNAs. In several cases, a set of genes belonging to a particular cellular pathway are enriched in the list of genes whose expression was altered. (35,58-62). The length of miRNA seed sequence is comparable

to that of the consensus sequence of transcription factor binding elements. Thus, although miRNAs are expected to directly regulate a large set of genes simultaneously, random chance is unlikely to explain the enrichment of genes in a particular pathway in the list of putative targets.

MIRNA AND CANCER

Profiling of miRNA expression in cancers

miRNAs are aberrantly expressed in a variety of cancers. The first example was miR-15a and -16-1 which are clustered at chromosome 13q14, a frequently deleted region in B cell chronic lymphocytic leukemia (CLL) and other cancers (63). Concomitantly, reduction of these two miRNAs was observed in the cancer samples relative to the normal tissues.

miRNome analyses have become easier by microarray techniques (Table 1). Many miRNAs are found to be up- or down-regulated in the cancer samples relative to the normal tissue counterparts (Table 2). It is beyond the capacity of this review to describe all the miRNAs that are changed in cancers from profiling data. Instead, in Table 1 we have summarized the literature reporting miRNomes in tumors. If a change in miRNA expression has been corroborated by a conventional method (Northern, RNase protection assay, or qRT-PCR), it was included in Table 2.

In addition to the distinction of tumors from normal tissue, miRNA expression is characteristic for a cancer type, stage, and other clinical variables. The first systematic analyses of hundreds of cancer samples and normal tissues (64) successfully classified various cancers based on the miRNome. It was surprising that the miRNome was better at predicting cancer type and stage than the mRNA expression profile and therefore was proposed as a useful tool for cancer diagnosis or prognosis. For example, clustering of CLL samples according to the miRNome revealed miRNA signatures that correlated with overexpression of ZAP-70, a predictor of early disease progression (65). The utility of miRNA in diagnosis will be discussed later.

Although some miRNAs are increased, most miRNAs are repressed in cancers relative to normal tissue counterparts (64,66-69). In agreement with these observations, global depletion of miRNAs by knockdown of the miRNA processing machinery stimulated cell transformation and tumorigenesis in vivo (70). This implies that the miRNA alteration is not simply an end result of tumorigenesis but actively contributes to cancer development. Despite the general reduction of miRNAs in cancers, there are several miRNAs that are up-regulated, some of which undoubtedly play oncogenic roles.

Mechanism for miRNA deregulation in cancers

As documented in the previous section, many miRNAs are deregulated in cancers. Aberrant expression of miRNAs can arise through a number of different mechanisms (Fig. 2).

1. Genomic abnormality—As exemplified by miR-15a and -16-1, chromosomal abnormality is one reason for miRNA deregulation in cancers. Tumorigenesis is often accompanied by chromosomal aberration such as deletion, amplification, translocation, etc. In silico analysis revealed that a significant fraction of miRNAs are mapped to these cancer-associated genomic regions or fragile sites in human (71) and mouse (72). In many cases, miRNA levels are correlated with changes in copy number of the genomic loci, as demonstrated by comparison between array CGH (comparative genomic hybridization) data and miRNA expression data (66,73-78).

2. Epigenetic factors—Epigenetic factors could also affect miRNA expression. In many cancers, hypermethylation of CpG islands in promoter regions results in heritable

transcriptional silencing of tumor suppressor genes. Gene silencing by DNA methylation is closely related with histone modification. In silico analyses indicated CpG islands near dozens of miRNAs (79). In addition, some miRNAs were up-regulated upon exposure of cells to the demethylating agent 5-aza-2'-deoxycytidine (79), upon mutation of DNMTs (DNA methyltransferases) (80), or upon HDAC (histone deacetylase) inhibitor treatment (81). These studies identified some miRNAs which are repressed by CpG hypermethylation in cancers relative to normal tissue. Representative examples are miR-9-1 in breast cancer (79) and miR-124a in colorectal tumors (80). In case of miR-124a, hypermethylation is tumor-type specific, as no methylation was seen in neuroblastoma. Epigenetic silencing of a miRNA may be a reflection of tissue specificity. For example, miR-124a is normally highly expressed in neuronal tissues, so that its epigenetic repression in colorectal tumors is not surprising.

miRNAs may counteract CpG methylation. For example, miR-29 directly targets DNA methyltransferases Dnmt3A and -3B. In agreement with this, ectopic expression of miR-29 resulted in a global reduction of DNA methylation, subsequently leading to a de-repression of some tumor suppressor genes which had been silenced by promoter methylation in cancer cells (82).

3. Transcriptional regulation—Transcription factors may induce miRNAs by activating the transcription of pri-miRNAs. This mechanism is well documented in several cases where tissue specific miRNAs are turned on by transcription factors during differentiation. Given the wide impact of transcription factors in fundamental cellular processes, it is obvious that many oncogenes or tumor suppressors are transcription factors. Many miRNA-transcription factor relationships have been discovered in cancers, as listed in Table 3. Among them, p53, c-Myc, and E2F will be elaborated later.

4. Regulation at miRNA processing steps—In addition to the transcription rate of pri-miRNA, the steady state level of a mature miRNA is determined by the processing efficiency of its precursors and by its stability. miRNAs often exhibit a discrepancy in expression of the mature form relative to that of a precursor (83-88). Although miRNAs in a genomic cluster are usually expressed from a common pri-miRNA, the levels of individual miRNAs in the cluster are not necessarily coordinated (89,90). A time course experiment after induction of pri-miR-21 revealed a delayed kinetics in accumulation of mature miR-21 (91). Collectively, these observations indicate that miRNA processing and stability are important factors that determine miRNA expression level.

This mechanism has been underscored in cancers by a comprehensive analyses of expression data (92). miRNAs are generally reduced in cancers relative to the normal tissues. In cases where a miRNA resides within a gene, host genes can be regarded as the pri-miRNA. Comparison of microarray data between mRNA and miRNA revealed that the miRNA reduction in cancers is poorly correlated with a reduction of the host gene expression. Aberrant expression of miRNAs during tumorigenesis is presumably often due to alterations at post-transcriptional steps.

The expression levels of Dicer or Drosha were altered in a number of cancers (70,93-96). Drosha up-regulation is seen in more than half of cervical SCC (squamous cell carcinoma) specimens and is likely due to the copy number gain at chr5p where the *Drosha* gene is located (95). Hierarchical clustering of miRNA expression data successfully classified cervical SCC samples into two groups according to Drosha overexpression. It is noteworthy that some miRNAs were reduced upon Drosha overexpression, indicating that individual miRNAs respond differentially to an elevation of the miRNA processing machinery. Interestingly, Drosha was reported to interact with an oncogenic fusion protein derived from a chromosomal

translocation in some leukemias (97). This interaction affected pri-miRNA selection of Drosha and, as a result, miRNA expression pattern.

Consequence of aberrant miRNA expression in cancers

miRNAs regulate the expression of their target mRNA, so that over- or under-expression of miRNAs is expected to respectively result in down- or up-regulation of the protein product of target mRNAs. It is easy to implicate a miRNA in a cancer if a direct target of a miRNA is an oncogene or a tumor suppressor. Since *let-7* miRNA was shown to directly regulate *RAS* oncogene (98), a number of other miRNA-target pairs have been studied. However, the number of experimentally validated pairs is still small relative to the number of miRNAs shown to be aberrantly expressed in tumors or the number of in silico predicted pairs, mostly because current target prediction algorithms are not very accurate. A current list of miRNA-target pairs implicated in various cancers is in Table 4, and important examples will be discussed later.

If global gene expression profiles are compared between cancers and normal tissues, many miRNAs and mRNAs are found to be deregulated. Therefore, it is very plausible that tumorigenesis or at least the progression of a cancer results from changes in the entire miRNome, rather than from the change of a single miRNA which regulates an oncogenic (or tumor suppressive) target gene.

Some miRNAs appear to be deregulated in cancers much more frequently than other miRNAs (Table 2). These miRNAs may play key roles during tumorigenesis. For example, the miR-17-92 cluster and miR-155 have been experimentally demonstrated to be bona fide oncogenes, as their ectopic expression accelerated tumor development. These two miRNAs will be described in detail in separate sections.

Aberrant action of miRNAs without alteration of miRNA expression level

The function of protein-coding genes are rendered abnormal by point mutations which transform proto-oncogenes to oncogenes or abrogate functions of tumor suppressor genes. In theory, the same mechanism of activation/inactivation may apply to miRNAs. However, variation in mature miRNA sequence seems to be a very rare event (72,99-101), presumably due to the small size. In addition, miRNAs are thought to be more tolerant of point mutations or of single nucleotide insertion/deletion mutation than protein coding genes.

In contrast, sequence variation in miRNA target sites may occur and play a role in cancer. In silico analysis of EST (expressed sequence tag) and SNP (single nucleotide polymorphism) databases indicated different allele frequencies of miRNA binding sites in cancers versus normal tissues (102). In a few experimental results, sequence polymorphisms in miRNA target sites were shown to affect miRNA-mRNA interaction and implicated in disease phenotypes (100,103-105) (Fig 2).

With regard to this mechanism, an interesting example is *let-7* and its direct target oncogene HMGA2 (38,106). Chromosomal rearrangements at the HMGA2 locus in several tumors separate the ORF from the 3'UTR containing *let-7* target sites. As a result, HMGA2 escapes from suppression by *let-7*, is overexpressed, and promotes tumorigenesis (Fig 2).

An alternative splicing event may result in a different 3' UTR that displays different miRNA target sites, as exemplified in the targeting of tropomyosin-related kinase C (*trkC*) by miR-9, -125a, and -125b. One mRNA isoform encodes a truncated ORF that is functionally dominant negative to the intact protein. In this isoform, the 3'UTR contains the target sites of these miRNAs. In contrast, the target sites are absent in another isoform encoding the intact ORF. Only the former isoform was repressed by the miRNAs (107). Although the stop codon is usually located in the last exon, generation of different 3'UTRs by alternative splicing has been

known to occur in a small but significant fraction of genes (108). Thus, variation of 3'UTR and of attendant miRNA target sites is expected to be a mechanism for oncogene activation or tumor suppressor inactivation (Fig 2).

Recently, Steitz and colleagues reported that miRNAs activate the translation of the target mRNA in cells arrested at G0/G1 stage (109). Besides aberrant miRNA expression, the switch from repression to activation should be considered in studying miRNA role in differentiation or tumorigenesis, as the same miRNA may exert an opposite effect between resting cells in a tissue and proliferating cancer cells.

Viruses and miRNAs in tumorigenesis

Several viruses regulate tumorigenesis by expressing viral oncogenes or activating cellular oncogenes through integration of viral DNA into genomic loci. Both mechanisms are applicable to oncogenic miRNAs. For example, the Epstein Barr virus (EBV)-encoded miRNAs (110) directly target a viral oncogene LMP1 whose overexpression is deleterious to host cells. EBV miRNAs are thought to enhance EBV mediated cellular transformation by adjusting LMP1 level to a sub-lethal dose (111). In another case a viral miRNA repressed a host tumor suppressor gene miRNAs from Kaposi sarcoma-associated herpesvirus (KSHV) directly targets an anti-angiogenic factor thrombospondin-1 (112). Thus viral miRNAs act as viral oncogenes.

Viral integration near miRNA loci may lead to aberrant expression of miRNAs. Indeed, miR-155 (BIC is the pri-miRNA) was originally described as a frequent integration site in virally induced lymphomas (113). Viral integration sites are often mapped to miRNA loci (114), such as the miR-17-92 cluster (115), miR-106a-363 cluster (116), miR-29a-29b-1 cluster (117), and miR-106a cluster (118).

Various cancer types

Comprehensive miRNA profiles have been reported with clinical specimens from various cancers. The data are summarized in Table 1. Consistent with the notion that miRNAs are expressed in a tissue specific manner, miRNAs are different among cancers from various tissue origins. There are also a couple of miRNAs that appear to be frequently deregulated in many cancers (Table 2), suggesting that these miRNAs regulate fundamental processes such as cell proliferation or apoptosis.

Oncogenic or tumor suppressive miRNAs

1. miR-17-92 cluster—The miR-17-92 cluster, located at chr13q31.3 in humans, is composed of six miRNAs (mir-17, -18a, -19a, -20a, -19b-1, and -92a-1). There exists a similar cluster at chrX called miR-106a-363 cluster (mir-106a, -18b, -20b, -19b-2, -92a-2, and -363). The miR-17-92 cluster contains the first miRNAs demonstrated to be oncogenic. The development of B-cell lymphoma was significantly accelerated by the forced expression of the miR-17-92 cluster in transgenic mice overexpressing *c-myc* oncogene (119).

Consistent with its oncogenic role, the miR-17-92 cluster is up-regulated in a variety of cancers including lymphomas (74,75,119,120), lung cancers (77,121), and others (122). There appear to be two mechanisms for up-regulation of this cluster in cancers (Fig 3). One is the amplification of chr13q31 locus in several lymphomas and other cancers (74,77,78,120). The other is transcriptional activation of the pri-miRNA. An oncogenic transcription factor c-Myc binds the genomic locus upstream of the miR-17-92 cluster and activates its expression (123). In neuroblastoma cells, MycN, a protein highly homologous to c-Myc, appears to activate this cluster instead of c-Myc (124). E2Fs also activate this cluster (125,126) (Table 3).

Given that E2Fs are direct targets of miR-17 and -20a, the miR-17-92 cluster constitute a very complex regulatory network with c-Myc and E2Fs. E2F1 and c-Myc are known to activate each other to form a positive feedback loop (Fig 3). All of E2Fs (E2F1, 2, and 3) are capable of activating miR-17-92 cluster and are subject to repression by miR-17 and -20a from this cluster (Table 4). However, degrees of activation and repression are variable between individual pairs of miRNA and E2F family member. In general, E2Fs are thought to be pro-proliferative, but E2F1 is also pro-apoptotic. Although this network is too complicated to describe in a straightforward manner, the miR-17-92 cluster is clearly responsible for fine-tuning the regulatory network. Upon a proliferative cue, c-Myc and E2Fs turn on the miR-17-92 cluster. This cluster represses E2Fs, thereby preventing the uncontrolled amplification of the positive feedback loop between E2Fs and c-Myc. In addition, repression of E2F1 by the miRNAs may help minimize the pro-apoptotic potential of E2F1. This network is depicted in Fig 3.

2. miR-21—miR-21 is up-regulated in almost all kinds of cancers (Table 2). It has been shown that miR-21 is transcriptionally activated by Stat3 (signal transducer and activator of transcription 3) in the IL-6 signaling pathway (91) (Table 3). miR-21's role is well characterized in invasion and metastasis, which will be further discussed later.

3. miR-155—Like the miR-17-92 cluster, the oncogenic potential of miR-155 was demonstrated by transgenic mice expressing miR-155 in B-cells (127). The primary transcript for miR-155 is the BIC gene, which was originally known as a common viral integration site in lymphomas in chicken. High expression of miR-155 (BIC) is reported in various B-cell malignancies (128-130) (Table 2). miR-155 seems to be regulated at multiple levels. Besides viral integration, miR-155 (BIC) appears to be regulated by NF- κ B through the B cell-receptor (BcR)-mediated signaling pathway (85) or through the Toll-like receptor (TLR) activated signaling pathway (131). However, a discrepancy between the level of miR-155 and BIC suggests that miR-155 may also be regulated during processing steps (129). For example, the induction of BIC does not lead to up-regulation of miR-155 in several Burkitt lymphoma cell lines. In contrast, miR-155 is concordantly increased upon the induction of BIC in Raji, an EBV latency type III positive Burkitt lymphoma cell line (85).

4. *let-7*—*let-7* is one of the earliest discovered miRNAs. In human, there are 12 paralogous *let-7*s. Whereas *let-7* is almost absent during embryonic stages or tissues, high expression of *let-7* is seen in most differentiated tissues. The reduction of *let-7* in cancers is reminiscent of *let-7* expression during development in that it is most decreased in less differentiated, advanced stages of cancer cells with mesenchymal characters (132).

Probably, *let-7* is more abundant than any other miRNAs, so that reduction of *let-7* may exhibit a prominent effect on cell physiology compared to other miRNAs. To support this hypothesis, enhanced cellular transformation by global miRNA depletion was largely recapitulated by inhibition of *let-7* alone (70). Well known oncogenes such as RAS, c-Myc, and HMGA2 are validated as direct targets of *let-7* (Table 4). Hence, *let-7* is an important tumor suppressor.

5. miR-34s (miR-34a and miR-34b/-34c cluster)—miR-34s acquired recent notoriety because they are induced by p53 (Fig 4). Consistent with p53 being a tumor suppressor, miR-34s were down-regulated in several tumors such as non-small cell lung cancers (NSCLCs) (60) and pancreatic cancers (61) (Table 2). However, the reduction of miR-34s is not always correlated with p53 loss, suggesting a p53 independent mechanism of miR-34 reduction in some cancers. In fact, miR-34a is located in 1p36, a locus frequently deleted in a number of cancers (76). p53 regulation and miR-34s roles will be elaborated later.

Although miR-34s are thought to be tumor suppressors, they were found to be up-regulated in several cancers including renal cell carcinoma (133), colon cancers (58), and hepatocellular carcinoma (134) (Table 2). The role of high miR-34 and p53 status awaits investigation in these tumors.

Aspects of cancer biology regulated by miRNAs

1. Cell cycle—Cell cycle regulators often act as oncogenes or tumor suppressors. The best characterized example is a cell cycle inhibitor p27(Kip1). p27(Kip1) is a tumor suppressor, as indicated by low levels in some cancers. In addition, p27(Kip1) mutation predisposes cells to tumorigenesis upon exposure to carcinogens. p27(Kip1) binds to Cdk2-cyclin E and prevents G1 to S transition. p27(Kip1) is a direct target of miR-221 and -222 in glioblastomas (48, 135) and prostate cancer cells (136). In these cancer cells, p27(Kip1) is anti-correlated with these two miRNAs. Targeting p27(Kip1) is responsible for the pro-proliferative role of these miRNAs, as artificial knockdown of p27(Kip1) mirrored the phenotype of these miRNAs. miR-221 and -222 are overexpressed in other cancers (see Table 2), implying their role in a wide range of cancers.

Besides p27(Kip1), miRNAs regulate other cell cycle proteins including Cdk6, Cdc25A, Ccnd2 (cyclinD2) (59), Cdk4 (62), a Rb family protein (89), and p180 of DNA polymerase α (37). A number of miRNAs have been shown to perturb normal cell cycle when overexpressed or inhibited. (37,48,62,70,137-139) (Table 5). Oscillation of a miRNA during the normal cell cycle has not yet been reported.

2. Programmed cell death—Apoptosis is an active process controlled by a gene expression program that varies depending on the biological context. Since a balance between proliferation and apoptosis is essential for tissue homeostasis and proper differentiation, aberrant apoptosis may give rise to tumors. miRNAs participate in tumorigenesis by directly targeting anti-apoptotic genes. Representative examples are the repression of anti-apoptotic genes Mcl-1 and Bcl-2 by miR-29b (84) and miR-34s (60), -15a, and -16 (140), respectively (Table 4). The loss of these miRNAs due to mutation of p53 or deletion of chr13q14 leads to an increase in the anti-apoptotic gene expression and persistence of tumor cells that would have been normally removed by apoptosis. It is very likely that miRNAs may target other genes in the apoptotic pathway, since transfection or expression of a number of miRNAs is associated with apoptosis (69,76,121) (Table 5).

3. p53—p53, a sequence-specific transcription factor, is called the guardian of the genome owing to its critical role in regulation of the cell cycle and apoptosis in the face of genomic damage. Genotoxic stress and oncogene activation activate p53 to modulate the transcription of several target genes. p53 is the most extensively studied tumor suppressor and its importance is underscored by mutation of p53 in almost 50% of human cancers.

miRNA profiling after p53 induction indicated miR-34a, b, and c (miR-34s, collectively) as the most up-regulated miRNAs (61,62,137,141) (Fig 4). These miRNAs are induced after genotoxic stress in a p53-dependent manner in vitro and in vivo (62,141). miR-34b and -34c are clustered at chr11, while miR-34a is located in a separate genomic locus. Both pri-miRNAs are directly activated by p53. miR-34s seem to be a critical downstream effector of p53, since ectopic expression of miR-34s recapitulated the phenotype of p53 activation. miR-34s promote cell cycle arrest, apoptosis and senescence. (58,60-62,137,141) (Table 5). These effects are explained by the repression of several direct targets of miR-34s, such as Bcl2 (60), Cdk4, and hepatocyte growth factor receptor (MET) (62) (Table 4).

Besides miR-34s, other miRNAs might be important in the p53 pathway. miR-30c, -103, -26a, -107, and -182 were induced clearly, albeit less robustly, upon DNA damage in a p53 dependent

manner (61). miR-26a expression was also shown to be dependent on p53 (142). In another approach, searching for p53 binding elements in DNA sequence near miRNAs identified miR-129 as a good candidate for regulation by p53 (143).

4. Invasion and metastasis—Features of malignant tumors, distinct from benign tumors, are invasion and metastasis. Malignant tumors are fatal, mostly due to their capacity to invade neighboring tissues and metastasize through the bloodstream to distant organs. An effect of miRNAs on invasion and migration has been reported (Table 5). Ectopic expression of miR-125 impaired cell motility and invasion in a breast cancer cell line (144). Reduction of global miRNA expression enhanced migration of cells (70).

These sporadic in vitro observations were followed by more detailed studies on miR-10b and miR-21. miR-21 is one of the most frequently up-regulated miRNAs in cancers (Table 2). miR-21 promotes cell motility and invasion by directly targeting PTEN, a tumor suppressor known to inhibit cell invasion by blocking the expression of several MMPs (matrix metalloprotease) (134). Recently, another pathway was reported in colorectal cancers where miR-21 promotes invasion, intravasation, and metastasis by down-regulating Pcd4 (145)

miR-10b is the other miRNA implicated in metastasis. In metastatic breast cancer cells, miR-10b is up-regulated presumably as a result of transcriptional activation by Twist. Ectopic expression of miR-10b promoted invasion, intravasation, and metastasis in otherwise non-invasive or non-metastatic breast cancer cell lines. miR-10b directly targets Homeobox D10 whose reduction induces the expression of a well characterized pro-metastatic gene, RhoC (146).

5. Angiogenesis—Recruiting blood vasculature is crucial to the survival of neoplastic cells. So far, the miR-17-92 cluster has been characterized in this process. The stimulation of neovascularization by c-Myc involves a down-regulation of anti-angiogenic factor Tsp-1 (thrombospondin-1). c-Myc represses Tsp-1 and a related protein CTGF (connective tissue growth factor) by activating miR-17-92 cluster. Tsp-1 and CTGF seem to be direct targets of miR-19 and -18 in this cluster, respectively. Ectopic expression of the miR-17-92 cluster is sufficient for promoting angiogenesis (147). A recent observation suggested that other miRNAs, miR-378 and -27a, may play a role in angiogenesis (139,148) (Table 5). Viral miRNAs may also play a role in angiogenesis, since Tsp-1 was shown to be a direct target of KSHV miRNAs (112).

6. Others—The microenvironment inside a solid tumor is usually hypoxic. Tumor cells under hypoxia tend to be resistant to therapies and to be of poor prognosis. Up-regulation of some miRNAs under hypoxia (149,150) (Table 3) suggests that these miRNAs could influence the phenotype of hypoxic tumor cells. Cancers are predisposed by other external factors, such as genotoxic stress, folate deficient diet, and exposure to arsenic. There are a few reports of miRNA profiles under these conditions (151,152), but whether miRNA changes are responsible for the cancer predisposition remains to be investigated.

Clinical applications

1. Diagnostic tools—miRNAs may be used as diagnostic or prognostic tools, since miRNA expression profiles reflect tumor origin, stage, and other pathological variables. Practically, miRNAs can be accurate molecular markers because miRNAs are relatively stable and resistant to RNase degradation probably owing to the small size (35,153,154). It has been shown that miRNAs can be isolated and quantitated from formalin-fixed paraffin-embedded (FFPE) specimens. qRT-PCR and microarray data were reliably and reproducibly obtained from FFPE

samples which had been routinely processed and stored for 10 years. The data from FFPE samples were consistent with that from frozen samples (155,156).

The development of qRT-PCR methods improved the sensitivity of miRNA detection down to a few nanograms of total RNA (68,157,158). This amount can easily be obtained by fine needle aspiration biopsies (FNABs) and in fact, there is a report of successful miRNA measurement by qRT-PCR on FNAB samples (159).

miRNA markers that could be used for cancer diagnosis are becoming available. For example, miR-196a is high in pancreatic ductal adenocarcinoma (PDAC) but low in normal tissues and chronic pancreatitis. miR-217 exhibits the opposite expression pattern (Table 2). Thus, the ratio of miR-196a/-217 calculated by qRT-PCR measurement of the two miRNAs from tiny amount of total RNA, indicates whether the sample contains PDAC (160). Once reliable indicator miRNAs are chosen, we expect them to yield easy and accurate tools for cancer diagnosis.

2. Cancer therapeutic tool—A number of miRNAs affect the growth of cancer cells in vitro and in vivo when overexpressed or inhibited. Therefore, cancer cell growth can be controlled by manipulating miRNAs. Overexpression or inhibition of miRNAs can be achieved in several ways. Synthetic miRNA mimics include siRNA-like oligoribonucleotide duplex (161) or chemically modified oligoribonucleotide (162). Conversely, miRNAs can be inhibited by variously modified antisense oligonucleotides such as 2'-O-methyl antisense oligonucleotide, antagomirs and so on (reviewed in (163)). As the first successful tool for knockdown of a miRNA in vivo, antagomirs are of special interest (164). Antagomirs seem to be delivered to all tissues after tail-vein injections into mice, except brain. The therapeutic value of an antagomir would be greatly elevated by technical improvements for selective tumor-specific or tissue-specific delivery.

Synthetic oligonucleotides are effective in vivo for at most a couple of weeks, as demonstrated by an experiment with cancer cells engrafted in mice (165) or with tail-vein injection to mice (166). To circumvent this limitation, miRNAs can be stably expressed through transcription of hairpin RNA from plasmid vector (reviewed in (167)). Recently, artificial overexpression of a miRNA target sequence was shown to inhibit the miRNA function, presumably by titrating the miRNA away from endogenous targets (168,169). Thus it should be possible to apply such competitive inhibitors for the long term sequestration of a miRNA.

Non-specific side effects are as important as effectiveness and duration of miRNA expression (or inhibition). In some examples, it has been argued that miRNA mimics or inhibitors are specific enough to distinguish between similar miRNAs (170,171). However, crossreactivity between miRNAs of similar sequence is likely to be unavoidable at high doses of the antagonists or agonists. Another possible side effect is that high expression of miRNA mimics may interfere with endogenous miRNA action by saturating the cellular machinery for miRNA processing or action. This may result in a change in expression of other miRNAs leading to a deleterious effect in the cells. Indeed, a fatal side effect was reported to occur as a result of saturation of miRNA pathway (172). To minimize undesirable side effects, the expression or knock-down of a miRNA should be improved so that it is more accurate and controllable. An alternate approach to improve specificity is to target the pre-miRNAs with antisense or siRNA strategies (173,174).

Some chemical compounds alter expression of a group of miRNAs (81). Thus it may be possible to screen for drugs that could shift the miRNome in a cancer cell towards that of the normal tissue. NCI-60 cancer cell lines may serve as a platform for this screening, as their miRNA expression profiles have been published (68,175). By modulating multiple miRNAs

simultaneously, such a miRNome modifying approach may be much more effective for therapy than strategies that aim to regulate a single miRNA.

miRNAs affect the drug sensitivity of a cell (165,176) (Table 5). Expression or inhibition of a miRNA can therefore be combined with treatment of a drug or other cytotoxic therapy. One example is miR-21 inhibition together with a secreted form of tumor necrosis factor-related apoptosis inducing ligand (S-TRAIL), which resulted in a complete eradication of glioblastoma cells (177).

Collectively, preliminary results suggest that miRNAs could be useful for cancer therapy. However, there is still a significant gap between basic research on miRNAs and clinical application. Extensive preclinical and translational research is necessary to increase the efficacy and decrease the side effects of miRNAs in vivo. In parallel, we need to acquire more knowledge on the interaction of miRNAs with the gene expression programs and how these interactions are altered in tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

Other small RNAs?

Besides siRNAs and miRNAs, other types of small RNA have been reported in yeast, *Drosophila*, *C. elegans*, and plants. These small RNAs include tiny noncoding RNAs (tncRNAs), repeat associated small interfering RNAs (rasiRNAs), scan RNAs (scnRNAs), and others (reviewed in (178)). These RNAs are thought to be related to miRNA and siRNA, but are distinct from them. Recently, Piwi-interacting RNA (piRNA), another small RNA that is a little bigger than miRNA, were discovered from mammalian testes. Other classes of small RNA possibly exist but are yet to be discovered, given that a significant portion of transcripts are non-coding RNA. Whereas protein coding genes comprise only 1-2 % of the human genome, significant fraction (~15 %) of the human genome is transcribed and these transcripts still remain as a dark matter (179,180). The discovery of noncoding RNAs is expected to be greatly stimulated by development of high-throughput deep sequencing techniques.

Other diseases

Besides cancers, miRNAs (or miRNA machinery) have been implicated in other diseases, for example, Tourette's syndrome (103), Fragile X syndrome (181), Digeorge syndrome (23), myotonic dystrophy, spinocerebellar ataxia type 3 (SCA3) (182), and schizophrenia and schizoaffective disorder (183). Of particular interest is myotonic dystrophy type 1 (DM1) which is characterized by expansion of CTG repeat in the 3'UTR of dmpk gene. Tandem CAG sequences are found in some miRNA seed sequences and thus are able to interact with the CTG repeats, raising the possibility that these miRNAs may be involved in DM1 pathogenesis (184).

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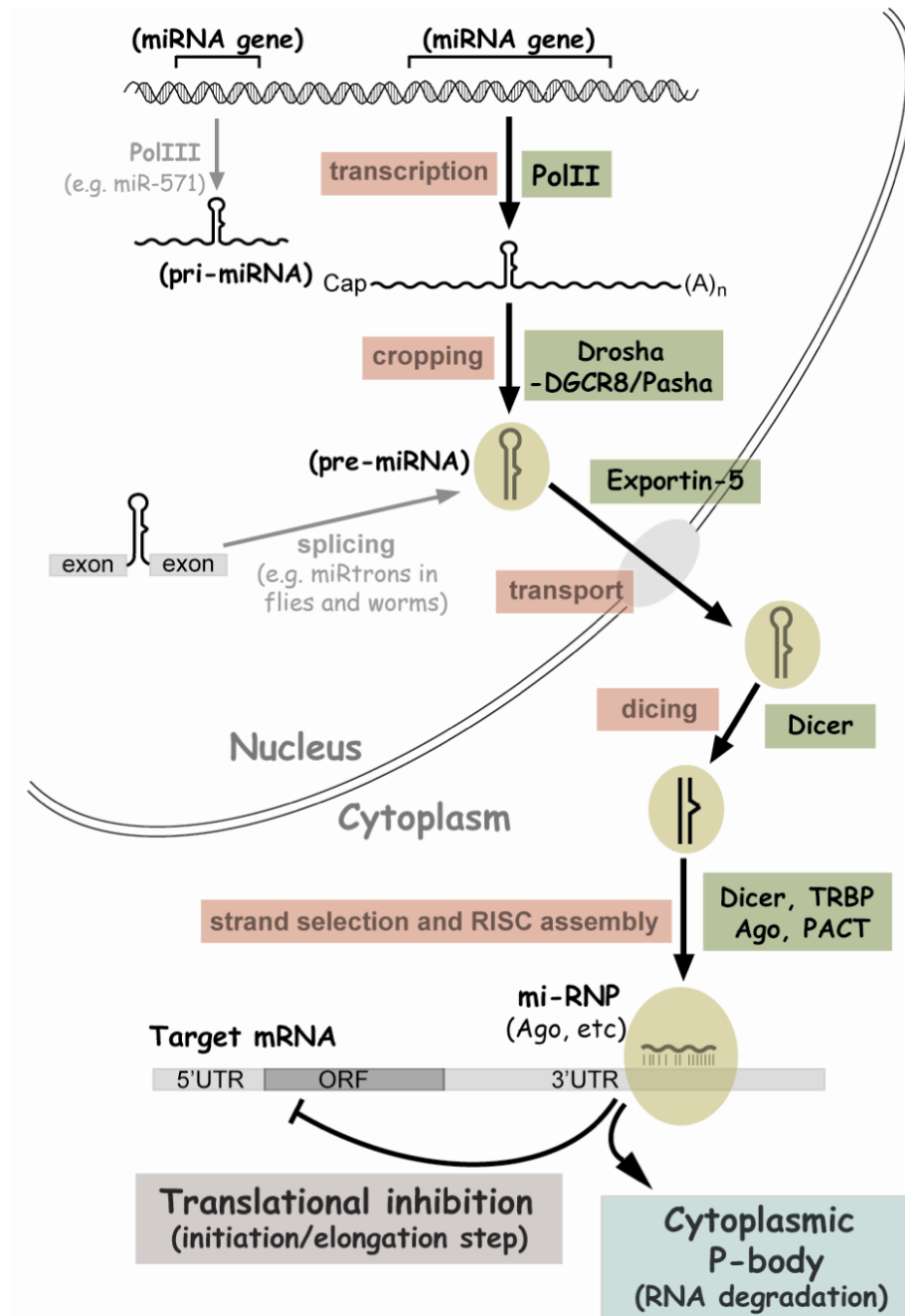


Figure 1. Pathways of miRNA biogenesis and action

Black lines indicate the canonical pathway, with minor pathways (grey lines) also depicted

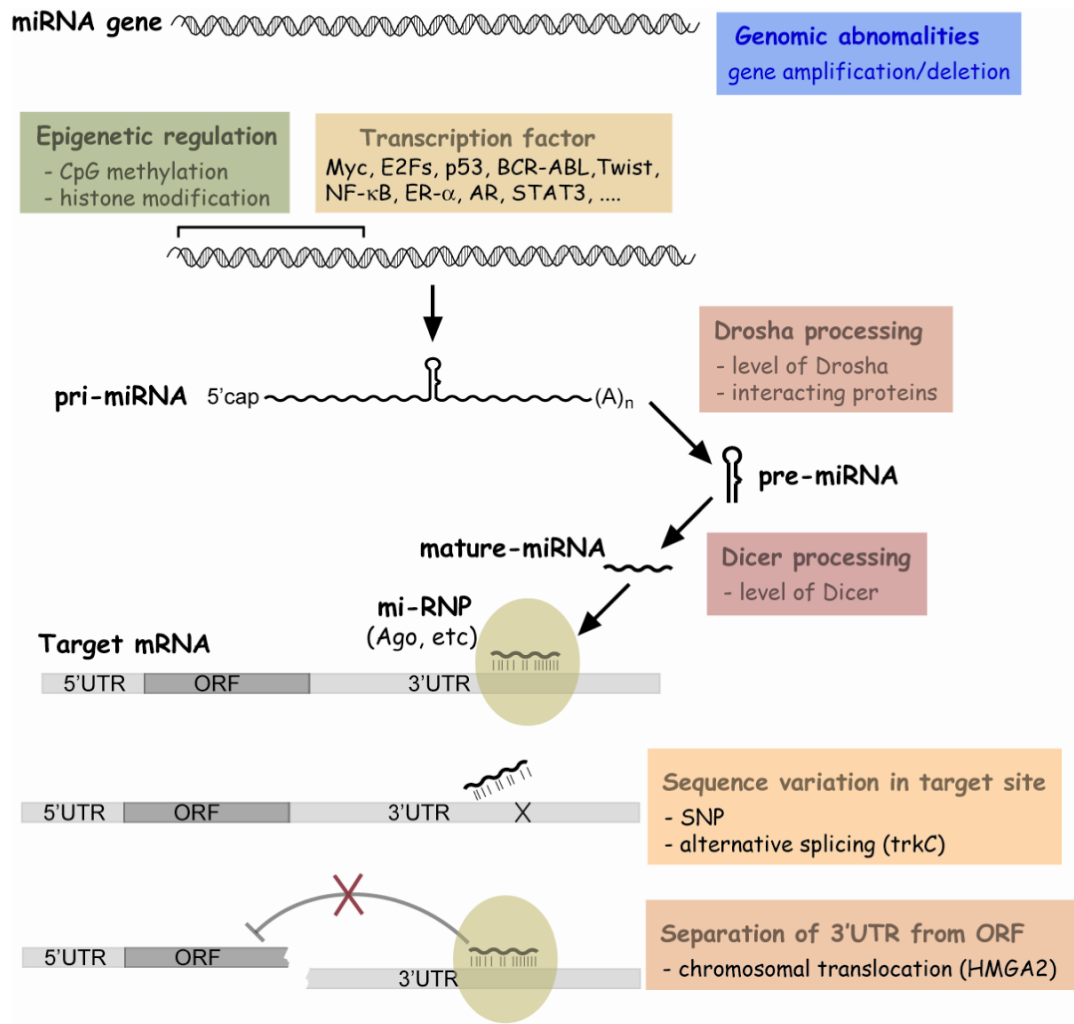


Figure 2. Various mechanisms for deregulation of miRNA expression or function

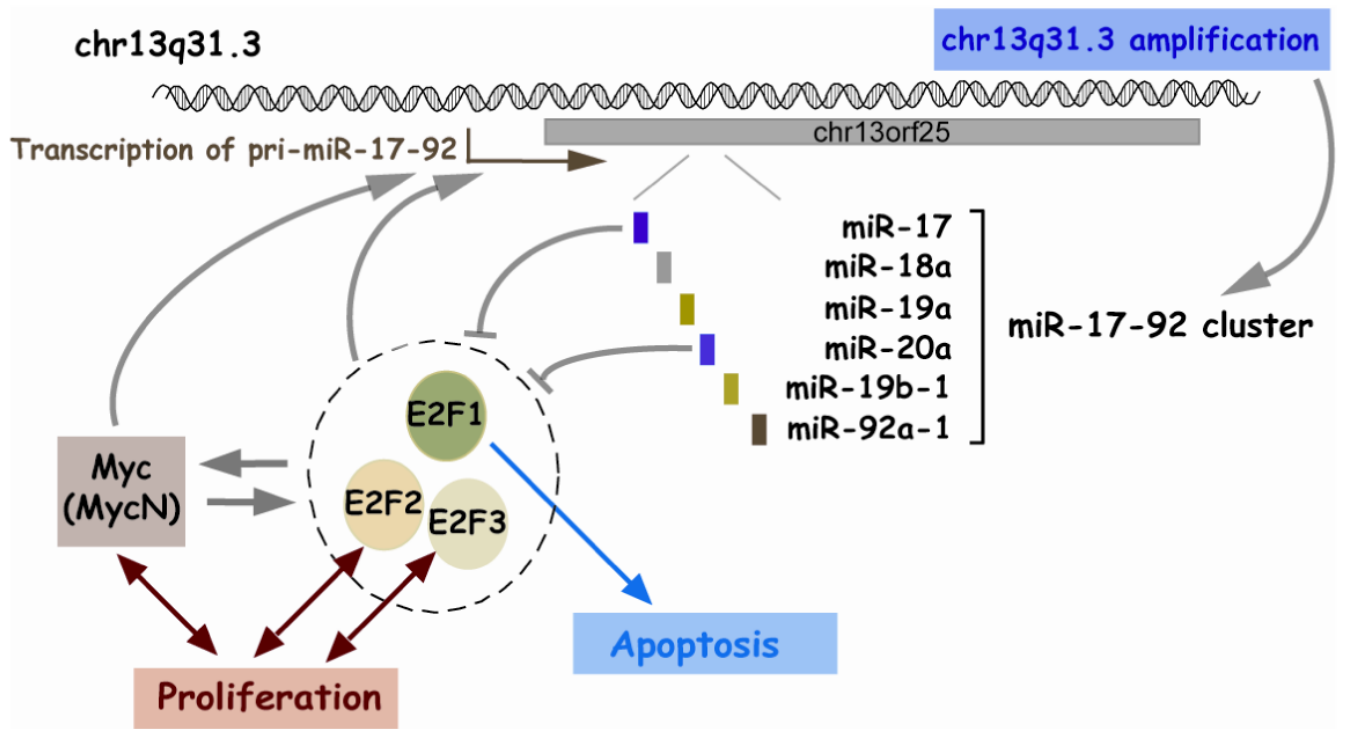


Figure 3. miR-17-92 pathway

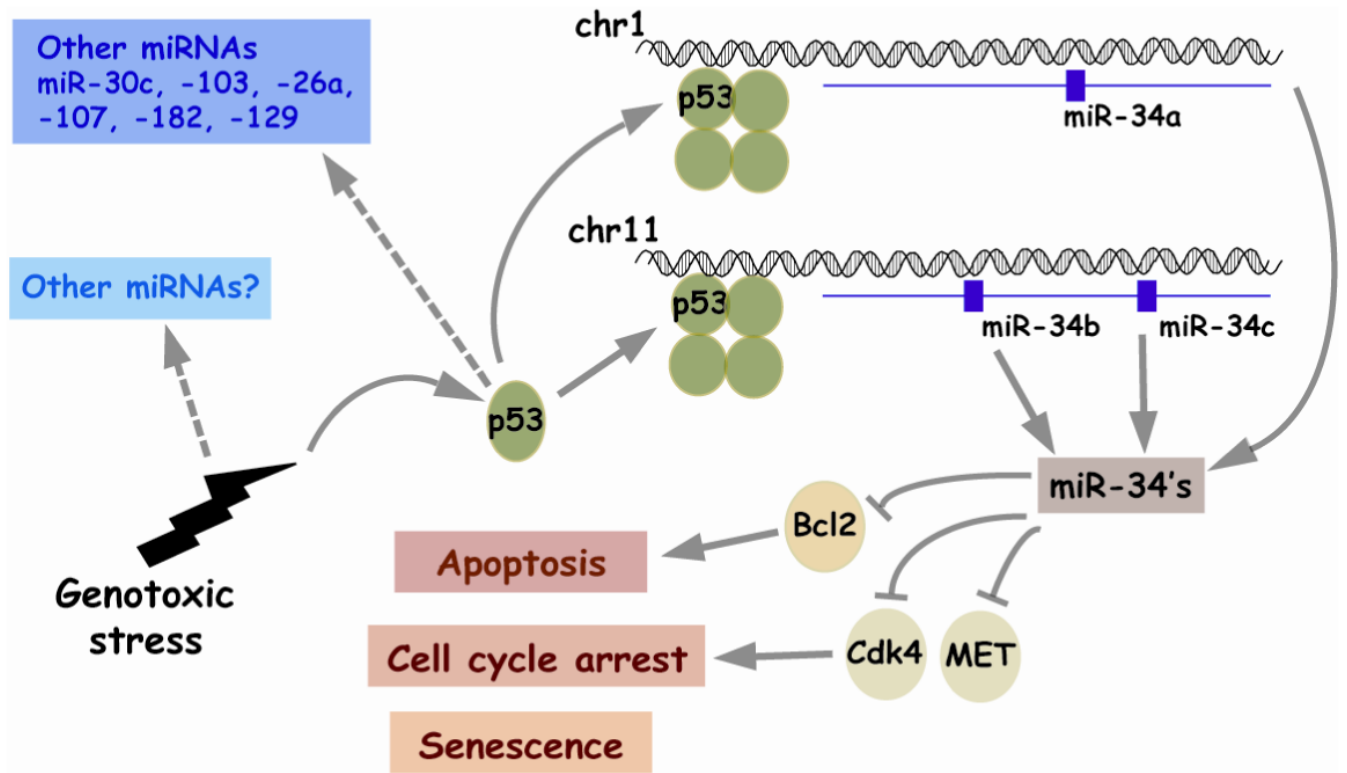


Figure 4. p53 and miR-34 pathway

Table 1

miRNome analyses in cancers

miRNA expression profiles from clinical specimens or cancer cell lines are listed according to tumor types. Profiling methods are bead based flow cytometric profiling (bead), qRT-PCR, microarray of miRNA chip (array), membrane spotted array (membrane), cloning, or miSAGE (serial analysis of gene expression of miRNA). miRNAs of notable change are indicated

Tumor type	Profiling method	miRNAs deregulated in tumor	note	ref
breast cancer	bead	up-regulated	classification of breast tumors into five sub-types	(93)
breast tumor	qRT-PCR array	miR-21		(165)
breast cancer	array		classification according to ErbB2/ER status	(158)
breast cancer	array	miR-21	clinicopathologic features	(185)
prostate cancer	array		<i>let-7c</i> , miR-125b, miR-145	(186)
prostate cancer	membrane		classification according to androgen dependency	(66)
prostate cancer	array			(158)
pancreatic cancer	array	miR-21, -221, -222, -181a, -181b, -181d, -155	vs. chronic pancreatitis and normal tissues	(187)
pancreatic cancer	array	miR-196a	pancreatic ductal adenocarcinoma vs. chronic pancreatitis and normal tissues	(160)
pancreatic cancer	qRT-PCR of pre-miRNA	<i>let-7i</i> , miR-100, -155, -221, -301, -21, -181a, -125b, -212, -376a	pancreatic adenocarcinoma, paired benign tissue, normal pancreas, chronic pancreatitis	(188)
pancreatic tumor	array	miR-103, -107, -23a, -26b, -342, -192, -204, -211, -21	endocrine tumors vs. acinar cell carcinoma	(189)
lung cancer	array	miR-21, -205	clinicopathological features	(190)
ovarian cancer	array	miR-200a, -141	clinicopathological features	(191)
cervical cancer	array		vs. Drosha overexpression	(95)
uterine leiomyoma	array	<i>let-7</i> , miR-21, -23b	clinicopathological features (tumor size, race, etc)	(192)
hepatocellular cancer	array	miR-21		(134)
hepatocellular cancer	array	miR-221	<i>let-7a</i> , miR-122a vs. liver cirrhosis	(193)
hepatocellular cancer	array	miR-224, -18	miR-199a, -199a*, -200a	(194)
thyroid cancer	array		miR-30d, -125b, -26a, and -30a-5p	(67)
thyroid cancer	array	miR-197, -346 in FTC relative to FA	anaplastic thyroid carcinomas vs. normal tissues	
thyroid cancer	array		follicular thyroid carcinoma (FTC) vs. follicular adenoma (FA)	(195)

Tumor type	Profiling method	miRNAs deregulated in tumor	note	ref
		up-regulated	down-regulated	
thyroid cancer	array	miR-221, -222, -181b		papillary thyroid carcinoma vs. normal tissues (159)
thyroid cancer	array	miR-221, -222, -146b		papillary thyroid carcinoma vs. normal tissues (100)
colorectal cancer	array	miR-25, -92 in MSS relative to MSI-H		microsatellite stability (MSS) vs. high microsatellite instability (MSI-H) (196)
colorectal cancer	qRT-PCR	miR-31, -96, -135b, -183	miR-133b, -145	miR-31 according to cancer stage (197)
colorectal cancer	cloning miSAGE			(17)
pituitary adenomas	array	miR-26a, -149	miR-21, -141, -144	clinicopathological features (198)
neuroblastoma	qRT-PCR			neuroblastoma subtypes (69)
glioblastoma	array	miR-221, -10b	miR-128, -181b, -181a	(199)
leukemia	bead	miR-128a, -128b in ALL compared with AML	<i>let-7b</i> , miR-223 in ALL compared with AML	acute lymphoblastic leukemia (ALL) vs. acute myeloid leukemia (AML) (200)
leukemia	cloning	miR-21, -150, -155	miR-92, -222	various chronic lymphocytic leukemias (CLL) (201)
leukemia	array		miR-29b, -181b in aggressive CLL with 11q deletion	three subtypes of CLL (202)
leukemia	array		miR-16-1, -15a	CLL according to ZAP-70 and IgVH (203)
leukemia	array			B-cell CLL (65)
various tumors	array			various sarcomas (204)
various tumors	cloning			solid tumors (lung, breast, stomach, prostate, colon, and pancreatic tumors) (122)
various tumors	bead			comprehensive collection of various cancers and normal tissues (64)

Table 2
miRNAs up- or down-regulated in various tumors relative to normal tissues

miRNA	up/down	cancer	ref
let-7	up	colon cancer	(205)
	down	breast cancer	(206)
	down	prostate cancer	(186)
	down	hepatocellular cancer	(193)
	down	gastric tumor	(157)
	up	uterine leiomyoma	(192)
	up	pancreatic cancer	(188)
	up	hepatocellular carcinoma	(152)
	down	lung cancer	(98)
	down	lung cancer	(207)
miR-9	down	breast cancer	(79)
	up	breast cancer	(146)
	down	neuroblastoma	(107)
miR-10b	up	breast cancer	(146)
	up	glioblastoma	(199)
miR-17-5p	up	neuroblastoma	(124)
	down	breast cancer	(162)
miR-18	up	hepatocellular carcinoma	(194)
miR-21	up	breast cancer	(206)
	up	colorectal cancer	(145)
	up	ovarian cancer	(191)
	up	hepatocellular cancer	(134)
	up	cervical cancer	(208)
	up	pancreatic cancer	(187)
	up	chronic lymphocytic leukemia	(201)
	up	uterine leiomyoma	(192)
	up	pancreatic cancer	(188)
	down	pituitary adenomas	(198)
	up	breast tumor	(165)
	up	pancreatic tumor	(189)
	up	hepatocellular carcinoma	(152)
	up	cholangiocarcinoma	(176)
	up	lung cancer	(190)
	up	breast cancer	(185)
	up	glioblastoma	(209)
miR-23a	up	pancreatic tumor	(189)
miR-23b	up	uterine leiomyoma	(192)
miR-23	up	hepatocellular carcinoma	(152)
miR-26a	down	thyroid cancer	(67)
miR-26b	up	pancreatic tumor	(189)
miR-27a	up	breast cancer	(139)
miR-27b	down	breast cancer	(210)
miR-29s	down	cholangiocarcinoma	(84)
miR-30a	down	thyroid cancer	(67)
miR-30d	down	thyroid cancer	(67)
miR-31	up	colorectal cancer	(197)
miR-34s	down	lung cancer	(60)
	down	neuroblastoma	(76)
	down	pancreatic cancers	(61)
	up	renal cell carcinoma	(133)
	up	colon cancers	(58)
miR-92	up	hepatocellular carcinoma	(134)
	up	neuroblastoma	(124)
miR-96	down	chronic lymphocytic leukemia	(201)
	up	colorectal cancer	(197)
miR-100	up	pancreatic cancer	(188)
miR-103	up	pancreatic tumor	(189)
miR-107	up	pancreatic tumor	(188)
miR-122a	down	hepatocellular cancer	(193)
miR-122	down	hepatocellular carcinoma	(152)
miR-124a	down	colon cancer, lung cancer	(80)
miR-125a	down	neuroblastoma	(107)
miR-125b	up	prostate cancer	(211)
	down	prostate cancer	(186)
	down	ovarian cancer	(191)
	down	thyroid cancer	(67)

miRNA	up/down	cancer	ref
	down	neuroblastoma	(107)
	up	pancreatic cancer	(188)
	down	breast cancer	(185)
miR-126*	down	lung cancer	(190)
miR-128	down	glioblastoma	(199)
miR-130	up	hepatocellular carcinoma	(152)
miR-133b	down	colorectal cancer	(197)
miR-135b	up	colorectal cancer	(197)
miR-140	down	ovarian cancer	(191)
miR-141	up	ovarian cancer	(191)
	down	pituitary adenomas	(198)
miR-143	down	B-cell malignancies	(212)
	down	cervical cancer	(208)
	down	colorectal neoplasia	(88)
miR-144	down	pituitary adenomas	(198)
miR-145	down	B-cell malignancies	(212)
	down	prostate cancer	(186)
	down	ovarian cancer	(191)
	down	colorectal cancer	(197)
	down	breast cancer	(185)
	down	colorectal neoplasia	(88)
miR-146b	up	papillary thyroid carcinoma	(100)
miR-149	up	pituitary adenomas	(198)
miR-150	up	chronic lymphocytic leukemia	(201)
	up	breast cancer	(146)
	up	pancreatic cancer	(187)
	up	chronic lymphocytic leukemia	(201)
	up	Pancreatic cancer	(188)
miR-155	down	pancreatic tumor	(189)
	up	Hodgkin's lymphomas, diffuse large B cell lymphoma, primary mediastinal B cell lymphoma	(128)
	up	diffuse large B cell lymphoma	(129)
	up	Burkitt lymphoma	(130)
miR-181a	up	pancreatic cancer	(187)
	up	pancreatic cancer	(188)
	down	glioblastoma	(199)
miR-181b	up	colon cancer	(205)
	up	pancreatic cancer	(187)
	up	papillary thyroid carcinoma	(159)
	down	glioblastoma	(199)
miR-181d	up	pancreatic cancer	(187)
miR-183	up	colorectal cancer	(197)
miR-184	down	neuroblastoma	(69)
miR-192	up	pancreatic tumor	(189)
miR-196a	up	pancreatic ductal adenocarcinoma	(160)
miR-197	up	thyroid cancer	(195)
miR-199a	down	ovarian cancer	(191)
	down	hepatocellular carcinoma	(194)
miR-199a*	down	hepatocellular carcinoma	(194)
miR-200a	up	ovarian cancer	(191)
	down	hepatocellular carcinoma	(194)
miR-200c	up	colon cancer	(205)
miR-204	up	pancreatic tumor	(189)
miR-205	up	lung cancer	(190)
miR-211	up	pancreatic tumor	(189)
miR-212	up	pancreatic cancer	(188)
miR-217	down	pancreatic ductal adenocarcinoma	(160)
miR-221	up	glioblastoma	(48)
	up	hepatocellular cancer	(193)
	up	pancreatic cancer	(187)
	up	pancreatic cancer	(188)
	up	papillary thyroid carcinoma	(159)
	up	papillary thyroid carcinoma	(100)
	up	glioblastoma	(199)
miR-222	up	glioblastoma	(48)
	up	pancreatic cancer	(187)
	up	papillary thyroid carcinoma	(159)
	down	chronic lymphocytic leukemia	(201)

miRNA	up/down	cancer	ref
	up	papillary thyroid carcinoma	(100)
miR-224	up	hepatocellular carcinoma	(194)
miR-301	up	pancreatic cancer	(188)
miR-320	up	neuroblastoma	(124)
miR-342	up	pancreatic tumor	(189)
miR-346	up	thyroid cancer	(195)
miR-372	up	testicular germ cell tumor	(213)
miR-373	up	testicular germ cell tumor	(213)
miR-375	down	pancreatic cancer	(188)
miR-376a	up	pancreatic cancer	(188)
	down	chronic lymphocytic leukemia	(203)
miR-16-1,	down	chronic lymphocytic leukemia	(140)
15a cluster	down	pituitary adenomas	(214)
	down	B-cell chronic lymphocytic leukemias	(63)
	up	erythroleukemia	(115)
miR-17	up	lung cancer cell line	(121)
cluster	up	lung cancer	(77)
	up	B-cell lymphomas	(119)
miR-106a	up	T-cell leukemia	(116)
cluster			

Table 3
miRNAs regulated by transcription factors

Oncogenic or tumor suppressive miRNAs that are directly modulated by transcription factors. The list contains miRNAs whose promoter region was analyzed by a luciferase reporter assay, and more importantly, was shown to bind to the transcription factor by chromatin immunoprecipitation assay

transcription factor	target miRNA	note (cancer type, etc)	ref
AR	miR-125b	prostate cancer	(211)
MYCN	miR-17-5p, -92, -320	neuroblastoma	(124)
Twist	miR-10b	breast cancer	(146)
p53	miR-34a, miR-34b-34c cluster	cell lines	(60)
p53	miR-34a	cell lines	(137)
p53	miR-34a, miR-34b-34c cluster	cell lines and in vivo	(215)
STAT3	miR-21	myeloma cell lines	(91)
HIF (hypoxia)	miR-26, -210		(150)
E2F3	miR-17-92 cluster		(125)
E2F1, 2, 3	miR-17-92 cluster		(126)
Myc	miR-17-92 cluster	angiogenesis in colon cancer model	(147)

Table 4
Oncogenic or tumor suppressive miRNAs and their direct target genes

The list are miRNAs and the target mRNAs where a directly regulatory connection was demonstrated by a luciferase assay using the 3'UTR of the mRNA

miRNA	target gene	note (cancer type, etc)	ref
let-7	RAS	lung cancer	(98)
let-7	CCND2, CDK6, CDC25A	cancer cell lines (A549, HepG2, HeLa)	(59)
let-7	HMGA2	ovarian cancer	(132)
let-7c	c-Myc	liver tumors	(83)
let-7	HMGA2	lung cancer cell lines	(38)
let-7g	c-Myc, k-RAS		(70)
let-7	HMGA2		(106)
let-7	NF2	cholangiocarcinoma cell lines	(216)
miR-9, -125a, -125b	tropomyosin-related kinase C (trkC)	neuroblastoma	(107)
miR-10b	HOXD10	breast cancer	(146)
miR-16-1, -15a	Bcl2	chronic lymphocytic leukemia	(140)
miR-17-5p	AIB1	breast cancer	(162)
miR-17-5p, -20	Transforming growth factor- β receptor type II (TBR11)		(74)
miR-18	CTGF (connective tissue growth factor)	colon cancer model of angiogenesis	(147)
miR-19	thrombospondin-1	colon cancer model of angiogenesis	(147)
miR-20a	E2F1, 2, 3		(126)
miR-21	Pdc4	colorectal cancer	(145)
miR-21	PTEN	hepatocellular cancer	(134)
miR-21	tropomyosin 1 (TPM1)		(47)
miR-21	PTEN	cholangiocarcinoma	(176)
miR-27b	CYP1B1	breast cancer	(210)
miR-29a, b, c	DNMT3A, DNMT3B	non small cell lung cancer	(82)
miR-29s	Mcl-1	cholangiocarcinoma cell line	(84)
miR-29b, -181b	TCL1	chronic lymphocytic leukemia	(202)
miR-34a	E2F3	neuroblastoma	(76)
miR-34a	cyclin E2 (CCNE2), hepatocyte growth factor receptor (MET)		(215)
miR-34a, -34b, -34c	Bcl2	non small cell lung cancer	(60)
miR-106a cluster	Myip (myosin regulatory light chain-interacting protein), Hipk3 (homeodomain-interacting protein kinase 3), Rbp1-like (retinoblastoma-binding protein 1-like)	T-cell leukemia	(116)
miR-122a	cyclin G1	hepatocellular cancer (HCC vs. liver cirrhosis)	(193)
miR-124a	cdk6	colon cancer, lung cancer	(80)
miR-125a, -125b	ERBB2 and ERBB3	breast cancer	(144)
miR-125b	Bak-1	prostate cancer	(211)
miR-127	Bcl6	bladder (and prostate) cancer	(217)
miR-206	ER alpha	breast cancer	(105)
miR-221, -222	p27 (Kip)	glioblastoma	(135)
miR-221, -222	p27 (Kip)	glioblastoma	(48)
miR-221, -222	p27 (Kip)	prostate cancer	(136)
miR-372, -373	LATS2	testicular germ cell tumor	(213)
miR-378	Sufu, Fus-1	U87 glioblastoma cell line	(148)
BART 16, 17-5p, 1-5p (EBV encoded miRNAs)	LMP1 (EBV encoded oncogene)	nasopharyngeal carcinoma	(111)
KSHV miRNAs miR-K12-1, -K12-3-3p, -K12-6-3p, -K12-11	thrombospondin 1 (THBS1)		(112)

Table 5
miRNAs that affect the properties of cancer cells

Phenotypes of cells are described after ectopic expression (denoted as “+” in the second column) or inhibition (“-” in the second column) of a miRNA

miRNA	effect on cell growth	Note (cancer type, etc)	ref
let-7	+inhibition of cell growth	lung cancer cell lines	(207)
let-7	+inhibition of cell growth (G1 arrest)	A549 lung cancer line or HepG2 cell line	(59)
	- enhanced cell growth	A549 lung cancer line	
let-7c	+inhibition of cell growth (G1 accumulation)	Hepa-1	(83)
let-7	+inhibition of cell growth	lung cancer cell lines	(38)
let-7g	+inhibition of cell growth		(70)
	- enhanced cell growth		
let-7a-3	+increased anchorage independent growth (soft agar assay)	A549 lung cancer line	(218)
let-7	- enhanced cytotoxicity (more apoptosis) by gemcitabine, 5-FU, camptothecin	cholangiocarcinoma cell lines	(216)
miR-9, -125a, -125b	+inhibition of cell growth (decreased BrdU incorporation)	neuroblastoma	(107)
	- enhanced cell growth (rescue of RA-induced growth arrest)		
miR-10b	+increased migration, invasion, intravasation, metastasis		(146)
	- decreased invasion		
miR-16-1, -15a	+increased apoptosis	megakaryocytic cell line MEG-01	(140)
miR-17-5p	+inhibition of cell growth (adhesion independent growth)	breast cancer	(162)
	- enhanced cell growth		
miR-17-5p and 20a	-inhibition of cell growth (increased apoptosis)	lung cancer cell line (Calu-6 and ACC-LC-172)	(121)
miR-17-5p, -20	+enhanced cell growth (adhesion independent growth, in vitro culture and engrafted tumor)		(74)
miR-20a	- increased apoptosis (in combination with doxorubicin)		(126)
miR-17-92 cluster	+enhanced cell growth	chronic myeloid leukemia cell line	(219)
miR-17-92 cluster	+increased angiogenesis	colon cancer model	(147)
miR-17-92 cluster	+enhanced cell growth	lung cancer cell line	(77)
miR-21	+increased invasion, intravasation, metastasis	colorectal cancer	(145)
	- decreased invasion, intravasation, metastasis		
miR-21	- increased apoptosis (reduced tumor growth upon inhibition by modified LNA in vivo)	glioblastoma	(177)
miR-21	+enhanced cell growth, increased migration	hepatocellular cancer cell lines and normal hepatocytes.	(134)
	- inhibition of cell growth, decreased cell migration and invasion		
miR-21	+decreased apoptosis upon IL-6 removal	myeloma cell line	(91)
miR-21	-inhibition of cell growth (in vitro culture and engrafted tumor), increased apoptosis	breast tumor	(165)
miR-21	+decreased cytotoxicity and less apoptosis upon gemcitabine treatment (in vitro culture)	cholangiocarcinoma	(176)
	- increased cytotoxicity and more apoptosis upon gemcitabine treatment (in vitro culture)		
miR-21	- increased apoptosis	glioblastoma cell line	(209)
miR-26, -210, -107	+increased apoptosis	HIF (hypoxia)-induced miRNAs	(150)
	- decreased apoptosis		
miR-27a	- increased apoptosis, inhibition of cell growth	breast cancer cell lines	(139)
miR-29a, b, c	+inhibition of cell growth (in vitro culture and engrafted tumor), increased apoptosis	lung cancer cells	(82)
miR-29b	+increased apoptosis	cholangiocarcinoma cell line (tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced cell death)	(84)
	- decreased apoptosis		
miR-34a	+inhibition of cell growth (in vitro culture and engrafted tumor), senescence-like phenotype		(58)

miRNA	effect on cell growth	Note (cancer type, etc)	ref
miR-34a	+inhibition of cell growth (G1 accumulation)		(137)
miR-34a	+inhibition of cell growth (increased apoptosis)		(61)
miR-34a	+inhibition of cell growth (increased apoptosis) -decreased apoptosis induced by genotoxic stress		(141)
miR-34a	+G1 accumulation		(60)
miR-34a	+inhibition of cell growth (increased apoptosis)	neuroblastoma cell lines	(76)
miR-34a, -34b, -34c	+inhibition of cell growth (G1 accumulation and senescence)		(215)
miR-34b and -34c	+inhibition of cell growth (adhesion independent growth)		(143)
miR-98	+more resistant to doxorubicin	primary human oral squamous cell carcinoma cell lines	(149)
miR-106a cluster	+transformation of NIH3T3 (colony formation assay)		(116)
miR-125a, -125b	+inhibition of cell growth, decreased migration and invasion	breast cancer	(144)
miR-125b	+enhanced cell growth -inhibition of cell growth	prostate cancer cell lines	(211)
miR-125b	-inhibition of cell growth	differentiated cell lines	(173)
miR-125b, -26a	+inhibition of cell growth	FB-1 cells (thyroid cancer cell line)	(67)
miR-143, -145	+inhibition of cell growth	B-cell malignancies (Raji cell)	(212)
miR-184	+inhibition of cell growth (increased apoptosis)	neuroblastoma cell lines	(69)
miR-197, -346	-inhibition of cell growth	thyroid cancer	(195)
miR-221, -222	-inhibition of cell growth	glioblastoma	(135)
miR-221, -222	-inhibition of cell growth (G1 block)	glioblastoma	(48)
miR-221, -222	+enhanced cell growth (adhesion independent growth) -inhibition of cell growth (adhesion independent growth)	LNCaP prostate cancer cell line PC3 prostate cancer cell line	(136)
miR-221	+enhanced cell growth -inhibition of cell growth	papillary thyroid carcinoma	(159)
miR-372, -373	+increased cell growth (adhesion independent growth), decreased senescence	testicular germ cell tumor	(213)
miR-378	+increased cell growth (in vitro culture and engrafted tumor), reduced apoptosis, enhanced angiogenesis -inhibition of cell growth	U87 glioblastoma cell line	(148)
EBV encoded BART miRNAs	+decreased apoptosis	nasopharyngeal carcinoma	(111)