Review

microRNAs as oncogenes and tumor suppressors

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Abstract

microRNAs (miRNAs) are a new class of non-protein-coding, endogenous, small RNAs. They are important regulatory molecules in animals and plants. miRNA regulates gene expression by translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-guided rapid deadenylation. Recent studies show that some miRNAs regulate cell proliferation and apoptosis processes that are important in cancer formation. By using multiple molecular techniques, which include Northern blot analysis, real-time PCR, miRNA microarray, up- or down-expression of specific miRNAs, it was found that several miRNAs were directly involved in human cancers, including lung, breast, brain, liver, colon cancer, and leukemia. In addition, some miRNAs may function as oncogenes or tumor suppressors. More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs may play a more important role in the pathogenesis of a limited range of human cancers than previously thought. Overexpressed miRNAs in cancers, such as *mir-17–92*, may function as oncogenes and promote cancer development by negatively regulating tumor suppressor genes and/or genes that control cell differentiation or apoptosis. Underexpressed miRNAs in cancers, such as *let-7*, function as tumor suppressor genes and may inhibit cancers by regulating oncogenes and/or genes that control cell differentiation or apoptosis. miRNA expression profiles may become useful biomarkers for cancer diagnostics. In addition, miRNA therapy could be a powerful tool for cancer prevention and therapeutics.
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Introduction

Cancer is one of the most serious diseases in the United States and around the world. There are five major steps for cancer development: initiation, promotion, malignant conversion, progression, and metastasis. Many factors influence the development of cancers: some inhibit tumor development (tumor suppressors), and some promote cancer development (cancer inducers). The formation of cancer is the combined interaction of both tumor suppressors and cancer inducers. Scientists have been trying to elucidate the molecular mechanisms that cause cancer development and cancer prevention. Although several genes, including oncogenes and tumor suppressor genes, have been identified in human and/or other model animal genomes, the mechanism of cancer formation is yet to be identified. A recently identified class of non-protein-coding small RNAs, microRNAs (miRNAs), may provide new

insight in cancer research. A recent study demonstrated that more than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites (Calin et al., 2004b), suggesting that miRNAs may play a more important role in the pathogenesis of a limited range of human cancers than previously thought.

microRNAs are an abundant class of endogenous small RNA molecules, 20–25 nucleotides in length (Ambros, 2001; Bartel, 2004; Carrington and Ambros, 2003). All pre-miRNAs have hairpin secondary structure (Ambros, 2001; Bartel, 2004; Carrington and Ambros, 2003) with high minimal folding free energy index (MFEI) (Zhang et al., 2006d). Some miRNAs are highly conserved from species to species in animals (Pasquinelli et al., 2000) and plants (Floyd and Bowman, 2004; Zhang et al., 2006b). More than a decade ago, a group led by Ambros found that a small RNA, lineage-deficient-4 (*lin-4*), had antisense nearly perfect complementarity to *lin-14*, and was involved in the regulation of developmental timing in *Caenorhabditis elegans* (Lee et al., 1993). At that time, this finding did not attract much attention from scientists; most investigators

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considered this small RNA as an oddity in worm genetics. However, hundreds more of these miRNAs were discovered in *C. elegans* and other animals by different laboratories (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001; Pasquinelli et al., 2000). miRNAs regulate gene expression by resulting in direct cleavage of the targeted mRNAs or inhibiting translation through perfect or nearly perfect complementarity to targeted mRNAs at the 3′ untranslated regions (UTRs) of the targets in animals (de Moor et al., 2005; Lai, 2002; Robins and Press, 2005; Stark et al., 2005; Sun et al., 2005). These targeted genes control multiple biological processes, including developmental timing (Reinhart et al., 2000), stem cell division (Cheng et al., 2005b; Hatfield et al., 2005; Houbaviy et al., 2003; Suh et al., 2004; Zhang et al., 2006f), apoptosis (Cheng et al., 2005a; Cimmino et al., 2005; Tanno et al., 2005), disease (Abelson et al., 2005; Alvarez-Garcia and Miska, 2005), and cancer (Meltzer, 2005; Volinia et al., 2006) in animals.

At present, thousands of miRNAs have been predicted in animals, plants and viruses by different approaches (Zhang et al., 2006c), including experimental methods (Lee and Ambros, 2001), computational approaches (Brown and Sanseau, 2005), and expressed sequence tag (EST) and genomic survey sequence (GSS) analysis (Zhang et al., 2005, 2006a). However, only a few of them have been experimentally validated (Griffiths-Jones et al., 2006). Computational analysis indicates that the total number of miRNAs may be more than 1% of the total protein coding genes (Lai et al., 2003; Lim et al., 2003a,b); more than 30% of protein-coding genes may be targeted by miRNAs (Berezikov et al., 2005; Lewis et al., 2005).

In this review, we briefly described miRNA biogenesis and the principle approaches for studying the function of miRNAs in cancers. Then, we focused on miRNAs as oncogenes and tumor suppressors by outlining the evidence for the involvement of miRNAs in several common human cancers, with several specific examples from both experimental and clinical analysis. Finally, we finished this review with conclusions regarding miRNAs and human cancers including the potential application of miRNAs as biomarkers, diagnosis, and potential therapeutic tools of human cancers.

miRNA biogenesis

miRNA biogenesis has been thoroughly reviewed by many investigators (Bartel, 2004; Chen, 2005; Kim, 2005; Tomari and Zamore, 2005). Briefly, miRNAs are transcribed by the RNA polymerase II enzyme to produce a primary-miRNA (pri-miRNA) (Lee et al., 2002, 2004; Zeng et al., 2005). Pri-miRNAs are usually long nucleotide sequences, and some of them have several hundred to a thousand nucleotides; pri-miRNA is usually capped at the 5′ end and poly-adenylated at the 3′ end, similar to protein-coding mRNAs (Cai et al., 2004). Then, pri-miRNAs form specific hairpin-shaped stem–loop secondary structures and enter a microprocessor complex (500–650 kDa) consisting of a Drosha (a RNase III endonuclease) and an essential cofactor DGCR8/Pasha (protein containing two double-stranded RNA binding domains) (Denli et al., 2004; Gregory et al., 2004; Han et al., 2004; Landthaler et al., 2004). There they are processed into a 60-to70-nt pre-miRNA with a 5′ phosphate and a 3′ 2 nt overhang. The pre-miRNAs are then transported to the cytoplasm by Exportin-5 (Exp5) (a member of the Ran transport receptor family) (Bohnsack et al., 2004; Lund et al., 2004; Yi et al., 2003). Once in the cytoplasm, pre-miRNAs are further processed to a short double strand miRNA:miRNA* duplex by Dicer, a second RNase III endonuclease (Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001). Finally, the miRNA:miRNA* duplex is unwound into a mature miRNA and miRNA* by a helicase. The mature miRNAs are asymmetrically incorporated into the RNA-induced silencing complex (RISC) where they regulate gene expression by mRNA degradation or translational repression while the miRNA* is quickly degraded (Gregory et al., 2005; Lin et al., 2005).

Principle approaches for studying the function of miRNAs in cancer

Currently, almost all of the miRNA-related studies on cancers based on the different expression profile of miRNAs in cancer cells vs. normal cells. Thus, methods used for detecting mRNA expression can also be used in studies on the potential role(s) of miRNAs in cancers.

Up- or down-regulated expression of miRNAs

Up- and/or down-regulated expression of the candidate miRNAs is a good approach to study the function of miRNAs in cancer pathogenesis. Knockdown or overexpression of a specific miRNA allows to study the specific roles of the miRNA in cancer initiation and development.

There are several methods to conduct this study, such as antisense inhibitors, transgenics, specific promoters, and point mutants. Using antisense inhibitors to block the targeted miRNA function is a good example. In this strategy, an artificial antisense RNA competes with cellular mRNAs to bind miRNAs. The antisense RNA pairs with the miRNA and inhibits the miRNA function. This has been adopted by two independent research groups to sequence-specifically inhibit miRNA- and siRNA-induced RNA silencing (Hutvagner et al., 2004; Meister et al., 2004), and inhibit four miRNAs *in vivo* by modified antisense RNAs (Krutzfeldt et al., 2005).

Point mutants of miRNAs or their targets can also be employed to study the function of miRNAs in cancers. One obvious advantage of point mutants is to study the direct interaction of miRNAs and their targeted genes. Several studies have shown that the "seed sequence" is important for miRNAs to recognize their targets, and increasing the mismatch in the seed sequences will significantly decrease the gene regulation function of miRNAs (Lewis et al., 2003, 2005). Thus, we can use this principle to design point mutants of miRNAs or their targets. One or two nucleotide changes in the "seed region" of a specific candidate miRNA will dramatically decrease the possibility of the miRNA binding to its targets, resulting in the overexpression of the targets of the studied miRNAs. If these miRNAs or their targets are involved in cancer formation, this point mutation will affect the formation of cancer.

Northern blot analysis

Northern blot analysis is a reliable technique to detect gene expression at the mRNA level; it is widely used in gene expression analysis. Early on, it was adopted to study the expression of miRNA genes (Lee et al., 1993; Wightman et al., 1993), and now is used as a method for detecting miRNA expression in cancer cells. For example, Hayashita et al. (2005) found that the miR-17–92 cluster is significantly over-expressed in lung cancer, especially with small-cell lung cancer, when compared with miRNA expression in normal cells (Hayashita et al., 2005).

Real-time PCR

Real-time PCR can also be employed to quantify miRNA expression profiles and study the potential function of miRNAs in cancer pathogenesis. Real-time PCR recently was employed to measure miRNA precursors and to study the expression of 23 miRNA precursors in six cell lines (Schmittgen et al., 2004). More recently, the real-time PCR assay was expanded to 222 miRNA precursor analysis in human cancer cell lines; different expression profiles of miRNA precursors in human cancers do exist (Jiang et al., 2005). These PCR-based analyses quantify miRNA precursors and not the active mature miRNAs. The relationship between pri-miRNA and mature miRNA expression has not been thoroughly addressed. This relationship is critical in order to use real-time PCR analysis to study the function of miRNAs in cancers.

microRNA microarray

Although northern blotting is a widely used method for miRNA analysis, it has some limitations, such as unequal hybridization efficiency of individual probes (Thomson et al., 2004), and difficulty in detecting multiple miRNAs simultaneously. For cancer studies, it is important to be able to compare the expression pattern of all known miRNAs between cancer cells and normal cells. Thus, it is better to have methods which detect all miRNA expression at a single time. Two-color fluorescence-based microarray technology (DNA microarray) has been widely used to detect gene expression simultaneously. Several laboratories have modified DNA microarray technology to form miRNA microarray technology (Babak et al., 2004; Barad et al., 2004; Liang et al., 2005; Liu et al., 2004; Nelson et al., 2004a,b; Thomson et al., 2004). Thomson et al. (2004) developed a custom dual-channel miRNA microarray platform, and employed it to monitor expression levels of 124 mammalian miRNAs. They observed that the expression patterns of miRNAs are different between adult mouse tissue and embryonic stem cells (Thomson et al., 2004). More recently, Lu et al. (2005) developed a novel strategy to detect abundant miRNA expression profiles in different cell types, including several human cancers. To overcome the concerns about probe specificity in miRNA microarray analysis, they first performed hybridization in solution, and then quantified the polymer heads that are hybridized to miRNA species using multicolor flow-sorting (Lu et al., 2005). This method can be used to detect miRNA expression profiles in cancers, and even in poorly differentiated tumors (Lu et al., 2005).

miRNA microarray analysis has become a comprehensive technology to help us better understand the relationship between cancer tissues and normal tissues (Liang et al., 2005; Liu et al., 2004). In addition, miRNA microarray technology has been widely used to study the roles of miRNA in cancers (Calin et al., 2005; Iorio et al., 2005; Lu et al., 2005), with several miRNA oncogenes and tumor suppressor genes identified.

Table 1 summarizes the strengths and weaknesses of these principle approaches for studying the functions of miRNAs in cancer pathogenesis. Although Northern blot analysis, real-time PCR, and miRNA microarray can detect the expression of certain miRNAs and determine which miRNAs may be associated with cancer formation, it is difficult to determine whether or not miRNAs play a unique role in cancers. Also, these techniques cannot directly determine the correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease. These problems

Table 1
Comparison of principle techniques for studying the functions of miRNAs in cancer pathogenesis

Technique	Strengths	Weaknesses
Antisense inhibitor	Inhibiting specific miRNA expression to study miRNA functions in cancers.	Need to design and transform specific antisense inhibitor into targeted cells.
Transgenics	Regulating specific miRNA expression to study miRNA functions in cancers.	Need to obtain transgenic cell line to study specific miRNA functions.
Specific promoter	Regulating specific miRNA expression to study miRNA functions in cancers.	It is difficult to select a promoter which really functions in cancer tissues or related tissues.
Point mutant	Directly affecting the miRNA binding to targeted mRNAs, and studying the interaction of miRNAs and their targeted cancer-related genes.	Complicated design process. Need to obtain transgenic cell lines before study miRNA functions.
Northern blot	The most reliable techniques to study the expression of miRNAs in cancers.	No direct correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease.
Real-time PCR	Rapidly detect miRNA expression, especially pri-miRNA expression.	No direct correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease.
miRNA microarray	Simultaneously detect the expression of multiple miRNAs in cancers, may become a technique in cancer epidemiology and early cancer detection.	No direct correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease.

can be solved by up- and/or down-regulation of candidate miRNAs by using antisense inhibitors, transgenics, specific promoters, and point mutants. However, the techniques for up- and/or down-regulation of specific miRNAs are still under development, and it has a long way to go before being used for clinical purposes. To better understand the functions of miRNAs in cancers, a technique combining the strengths of current techniques needs to be developed.

MicroRNAs as oncogenes and tumor suppressors

Evidence for the involvement of miRNAs in cancers

When cells exhibit abnormal growth and loss of apoptosis function, it usually results in cancer formation. Several recent studies indicate that miRNA regulates cell growth and apoptosis (Cheng et al., 2005a; Tanno et al., 2005). For example, *miR-15* and *miR-16* induce apoptosis by targeting antiapoptotic gene B cell lymphoma 2 (*BCL2*) mRNA (Cimmino et al., 2005), which is a key player in many types of human cancers, including leukemias, lymphomas, and carcinomas (Sanchez-Beato et al., 2003). Nairz et al. (2006) demonstrated that misexpression of *miR-278* in developing eyes causes massive overgrowth in *Drosophila*, partially due to inhibition of apoptosis by *miR-278* (Nairz et al., 2006). This suggests that miRNAs are involved in some cancer formation through regulation of cell growth and apoptosis.

The initial evidence for the involvement of miRNAs in cancers came from a molecular study characterizing the 13q14 deletion in human chronic lymphocytic leukemia (CLL) (Calin et al., 2002), the most common form of adult leukemia in the Western world (Dohner et al., 2000). It was observed that two miRNAs, *miR-15a* and *miR-16a*, are located on chromosome 13q14, a region deleted in more than half of B cell chronic lymphocytic leukemia (B-CLL) cases. Detailed deletion analysis indicated that these two miRNAs are the only two genes within the small (30 kb) common region which are lost in CLL patients, and expression analysis indicated that *miR-15a* and *miR-16a* were either absent or down-regulated in the majority (~68%) of CLL patients (Calin et al., 2002). Recognition of miRNAs that are differentially expressed between tumor tissues and normal tissues may help to identify those miRNAs that are involved in human cancers and further establish the apparent pathogenic role of miRNAs in cancers (Iorio et al., 2005). Calin et al. (2004a,b) determined genome-wide expression profiles of miRNAs in human B cell CLL using a microarray containing 368 probes corresponding to 245 human and mouse miRNA genes. This miRNA microarray analysis further confirmed that *miR-16* and *miR-15* are reduced in human CLL (Calin et al., 2004a). microRNA microarray analysis also indicated that miRNA expression patterns were related to the biological and clinical behavior of CLL (Calin et al., 2004a). A recent study indicated that BCL2 is one of the targets of miR-15a and miR-16-1. miR-15a and miR-16-1 expression was inversely correlated to BCL2 expression in CLL; both miRNAs negatively regulate BCL2 at the posttranscriptional level (Cimmino et al., 2005). This was also confirmed in a leukemic cell line model (Cimmino et al., 2005), suggesting that *miR-15a* and *miR-16-1* could be used therapeutically to cure tumors overexpressing *BCL2*.

Lung cancer is one of the most common cancers of adults. It is also the leading cause of cancer-related deaths in many economically developed countries. Emerging evidence suggests that miRNA *let-7* may control lung cancer development, or at least play a critical role in the pathogenesis of lung cancer. Takamizawa et al. (2004) observed that the expression levels of *let-7* were frequently reduced in both *in vitro* and *in vivo* lung cancer studies; reduced *let-7* expression was significantly associated with shortened postoperative survival, independent of disease stage (Takamizawa et al., 2004). They also observed that overexpression of miRNA *let-7* in A549 lung adenocarcinoma cell lines inhibited cancer cell growth (Takamizawa et al., 2004). *let-7* negatively regulates the expression of RAS and MYC by targeting their mRNAs for translation repression (Johnson et al., 2005). Both RAS and MYC have been implicated, along with p53, as key oncogenes in lung cancer; they have multiple complementary sites to *let-7* in their 3′ UTR (Johnson et al., 2005). Johnson et al. (2005) also showed that lung tumor tissues display significantly reduced levels of *let-7* and significantly increased levels of RAS protein relative to normal lung tissue, suggesting *let-7* regulation of RAS as a mechanism for lung oncogenesis.

In contrast to miRNA *let-7*, the expression of miRNA cluster miR-17–92 is remarkably increased in lung cancer, especially in the most aggressive form, small-cell lung cancer (Hayashita et al., 2005). *miR-17–92* cluster also enhances lung cancer cell growth (Hayashita et al., 2005). Interestingly, the predicted targets of the *miR-17–92* cluster include two tumor suppressor genes, *PTEN* and *RB2* (Lewis et al., 2003). Unfortunately, no experiments have yet confirmed that *PTEN* and *RB2* are truly targets of the *miR-17–92* cluster. Whether the *miR-17–92* is cluster directly involved in lung cancer development or controls lung cancer by targeting lung cancer suppressor genes is still unknown. Additional investigations found a connection between the *miR-17–92* cluster and the c-*myc* oncogene, which is frequently amplified and/or overexpressed in small-cell lung cancer (O'Donnell et al., 2005). Hayashita et al. (2005) found that overexpression of the *miR-17–92* cluster without gene amplification was likely associated with upregulation of at least one member of the *myc* gene family. This suggests that the overexpression of the *miR-17–92* cluster may be caused by *myc* gene overexpression.

Breast cancer is one of the most important cancers in adult females. After evaluating hundreds of miRNA expression profiles of 10 normal and 76 neoplastic breast tissues using miRNA microarray, Iorio et al. (2005) found that the miRNA expression patterns were significantly different between normal and neoplastic breast tissues; *miR-125b*, *miR-145*, *miR-21,* and *miR-155* were significantly reduced in breast cancer tissues. They also observed that the expression of miRNAs was correlated with specific breast cancer biopathologic features, such as tumor stage, proliferation index, estrogen and progesterone receptor expression, and vascular invasion (Iorio et al., 2005).

Colorectal neoplasia (colon cancer) is also associated with alteration in miRNA expression. Michael et al. (2003) identified 28 different miRNAs in colonic adenocarcinoma and normal mucosa, and found that the expression of two mature miRNAs, miR-143, and miR-145, was consistently reduced at the adenomatous and cancer stages of colorectal neoplasia (Michael et al., 2003). However, the transcriptional level of the unprocessed hairpin precursors of miR-143 and miR-145 was not altered in precancerous and neoplastic colorectal tissue, suggesting that altered transcription is not responsible for the reduced mature miRNA levels (Michael et al., 2003).

miRNAs are also involved in human brain cancer. Glioblastoma multiforme (GBM) is the most frequent occurrence and malignant form of primary brain tumors. These are highly invasive, very aggressive, and one of the most incurable cancers in humans (Ciafre et al., 2005). However, an understanding of the molecular mechanisms involved with this tumor is still very poor. Recently, Ciafre et al. (2005) employed microRNA microarry analysis to examine the global expression levels of 245 miRNAs in GBM. They observed that miR-221 was strongly upregulated in glioblastoma samples from patients. They also found that miR-181a, miR-181b, and miR-181c were down-regulated in glioblastoma compare to normal brain controls (Ciafre et al., 2005). Using microRNA microarray and Northern blot analysis, Chan et al. (2005) also observed that miR-21 was strongly overexpressed (5- to 100-fold) in highly malignant human glioblastoma tumor tissues, early-passage glioblastoma cultures, and in six established glioblastoma cell lines compared with its expression in nonneoplastic controls. Their results also indicated that knockdown of miR-21 in cultured glioblastoma cells activated caspases and resulted in more cell death by an apoptotic pathway (Chan et al., 2005). This suggest that miR-21 is an antiapoptotic factor in human glioblastoma cells; aberrantly expressed miR-21 may result in malignant human brain cancer by blocking expression of critical apoptosis-related genes (Chan et al., 2005).

In several types of lymphomas, including Burkitt's lymphoma, the expression of miR-155 is increased compared to normal cells (Eis et al., 2005; Metzler et al., 2004). It is well known that the BIC gene is related to several cancers. Activation of the BIC gene accelerates the pathogenesis of lymphomas and leukemias, and the expression of BIC is elevated in Hodgkin and children's Burkitt lymphoma but is low in normal lymphoid tissues (Haasch et al., 2002; Metzler et al., 2004; van den Berg et al., 2003). This suggests that BIC is a proto-oncogene in these diseases (Tam et al., 2002). However, the molecular basis of BIC-related cancers is unknown. A recent study indicted that miR-155 is located in the only phylogenetically conserved region of the BIC gene (Tam, 2001), suggesting that miR-155 may be responsible for the oncogenic activity of the BIC gene (Eis et al., 2005). Eis et al. (2005) found that miR-155 is overexpressed in a wide range of lymphomas derived from B cells of different developmental stages, especially in aggressive B cell neoplasms such as diffuse large B cell lymphoma (DLBCL). An elevation of 10- to 60-fold in the amount of miR-155 was observed in aggressive (DLBCL) and more indolent (CLL and MZ) lymphomas (Eis et al., 2005). They also found that

significantly higher levels of miR-155 were observed in cells with the ABC phenotype than in the cells with the GC phenotype, suggesting that miR-155 may be useful diagnostically for curing the ABC-type lymphomas (Eis et al., 2005). One possible mechanism for miR-155 involvement in this type of cancer is due to that fact that miR-155 may down-regulate the expression of the transcription factor PU.1, which is required for later differentiation of B cells (John et al., 2004). In addition to miR-155, other miRNAs, such as miR-15a, are also underexpressed in DLBCL (Eis et al., 2005). An miRNA polycistron, mir-17–92, was found to be overexpressed in many kinds of lymphoma samples compared with normal tissues (He et al., 2005b).

Papillary thyroid carcinoma (PTC) is the most common malignancy in thyroid tissue; about 80% of incident thyroid cancers are PTC. Although PTC is usually associated with alterations in the RET/PTC-RAS-BRAF signaling pathway (Kimura et al., 2003; Melillo et al., 2005), the detailed molecular mechanism is unclear. Recently, He et al. (2005a) demonstrated that numerous miRNAs are overexpressed in PTC tumors compared with normal thyroid tissues. Of these miRNAs, miR-221, miR-222, and miR-146 were strongly overexpressed (11- to 19-fold) in thyroid tumors compared with unaffected thyroid tissues (He et al., 2005a). They also found that the levels of KIT mRNA and KIT protein are dramatically decreased while the levels of miR-221, miR-222, and miR-146 increased in thyroid cancers, suggesting that negative regulation of KIT by these three miRNAs may also contribute to thyroid cancers (He et al., 2005a). KIT has been predicted as one of the targets of the overexpressed miRNAs in PTC (John et al., 2004; Krek et al., 2005; Rehmsmeier et al., 2004).

More recently, two miRNAs (miR-372 and miR-373) were found to function as oncogenes in human testicular germ cell tumors (Voorhoeve et al., 2006). In vitro neoplastic transformation assays has been successfully used to model cancerous processes in primary human cells (Hahn et al., 1999). Using this assay, several tumor suppressor genes and genetic elements required in cancer have been identified (Kolfschoten et al., 2005; Voorhoeve and Agami, 2003; Westbrook et al., 2005). Voorhoeve et al. (2006) used this model system to perform a functional genetic screen to identify miRNAs that act as oncogenes in tumorigenesis. Their results indicated that miR-372 and miR-373 were overexpressed in human testicular germ cell tumors (TGCTs). The possible mechanism is that miR-372 and miR-373 neutralized p53-mediated CDK inhibition of TGCTs through direct inhibition of the expression of the tumor suppressor Large Tumor Suppressor homolog 2 (LAST2), and permitted the proliferation and tumorigenesis of primary human cells which have both oncogenic RAS and active wild-type p53 (Voorhoeve et al., 2006).

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in liver. Murakami et al. (2006) investigated miRNA expression profiles of HCC and adjacent nontumorous tissue and found that miR-18 and miR-224 were significantly overexpressed in HCC compared with nontumor tissues. In contrast, miR-199a*, miR-195, miR-199a, miR-200a, and miR-125a were underexpressed in HCC tissues (Murakami et al., 2006).

The aberrant expression of miRNAs in cancer supports the hypothesis that miRNAs play a role in cancers. Lu et al. (2005) used a novel microRNA microarray technology to examine systematic expression profiles of 217 miRNA genes in primary tumors, tumor-derived cell lines, and normal tissues. Their results demonstrated that miRNAs have different profiles in cancers compared with normal tissues, and the profiles vary among different cancers (Lu et al., 2005). They also observed that the miRNA profiles are surprisingly informative for reflecting the developmental lineage and differentiation state of the tumors compared with the traditionally used mRNA profiles (Lu et al., 2005). Using a high-resolution array-based comparative genomic hybridization technique, Zhang et al. (2006e) found that miRNAs exhibited high frequency genomic alterations in human cancers, including ovarian cancer, breast cancer, and melanoma. More importantly, miRNA expression profiles can successfully clarify even poorly differentiated tumors (Lu et al., 2005). These findings suggest that miRNA profiles may be potentially useful for cancer diagnosis, and they play an essential role in cancer pathogenesis. This finding is further supported by the fact that human miRNA genes are frequently located at fragile sites, and in minimal regions of heterozygosity loss, minimal regions of amplification, or common breakpoint regions that are genetically altered in

human cancers (Calin et al., 2004b). To study the possible correlations between the location of cancer-associated genomic regions and the genomic position of miRNA genes, Calin et al. (2004a) investigated 186 human miRNA genes (all the available miRNAs at the time). They found that more than half (52.5%, 98 of 186) of the human miRNA genes were located in cancer-associated genomic regions or in fragile sites (Calin et al., 2004b). Additional supporting evidence is the correlation between the location of miRNAs and Homeobox (HOX) genes (Wynter, 2006). HOX proteins are a family of transcription factors that play a crucial role in animal development and in oncogenesis. Several miRNAs, such as miR-196 and miR-10a, are located in HOX clusters (Calin et al., 2004b).

microRNAs as oncogenes

Several experiments and clinic analysis suggest that miRNAs may function as a novel class of oncogenes or tumor suppressor genes (Fig. 1).

Those miRNAs whose expression is increased in tumors may be considered as oncogenes. These oncogene miRNAs, called "oncomirs", usually promote tumor development by negatively inhibiting tumor suppressor genes and/or genes that control cell differentiation or apoptosis. Many miRNA genes have been
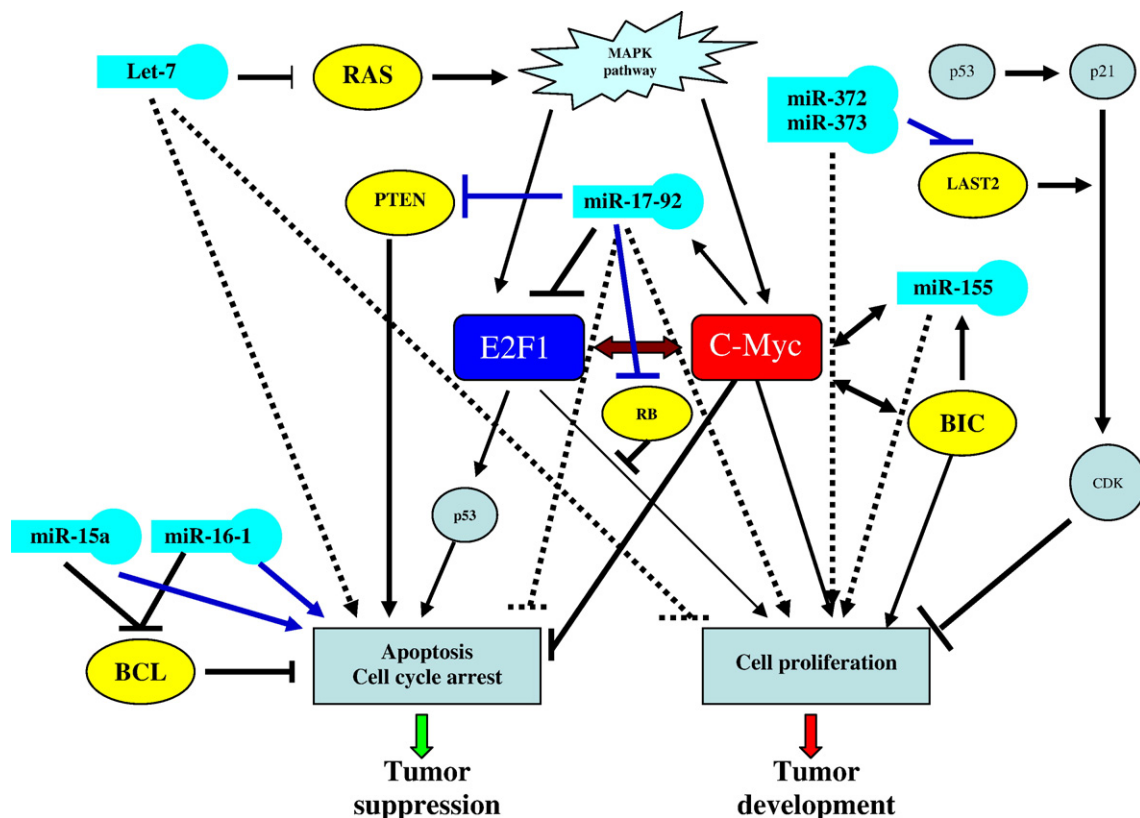

Fig. 1. A schematic model showing the molecular mechanisms of microRNA-involved cancer pathogenesis. miRNAs can be involved in cancers by directly regulating cell growth or indirectly controlling apoptosis through targeting transcription factors or signaling pathways. In this figure, *let-7*, *miR-15a*, and *miR-16-1* function as tumor suppressor genes while *miR-17–92*, *miR-155*, *miR-372*, and *miR-373* are considered as oncogenes. The dash lines represent indirect interactions. Some interactions have been confirmed (line in black color) by experiments; some have not (lines in blue color). See details in text. Abbreviations used in the figure, BCL2: B cell lymphoma 2, an antiapoptotic gene; BIC: an evolutionarily conserved non-coding RNA; CDK: cyclin dependent kinase; c-Myc; an oncogenes; E2F1: a cell cycle transcription factor; LAST2: Large Tumor Suppressor homolog 2, a serine–threonine kinase; MAPK: mitogen-activated protein kinase; p53: a critical tumor suppressor that is involved in most, if not all, tumorigenesis; PTEN: phosphatase and tensin homology, a tumor suppressor gene; RAS: a common proto-oncogene.

found that are significantly over-expressed in different cancers. All of them appear to function as oncogenes; however, only a few of them have been well characterized.

mir-17–92 is a good example for an oncogenic miRNA. *mir-17–92* cluster is a miRNA polycistron located at chromosome 13q31, a genomic locus that is amplified in lung cancer and several kinds of lymphoma, including diffuse large B-cell lymphoma (Hayashita et al., 2005; He et al., 2005b). Compared with normal tissues, the expression of *mir-17–92* is significantly increased in several cancer types, including lung cancer and lymphomas, especially in their most aggressive forms, such as small-cell lung cancer and human B-cell lymphomas (Hayashita et al., 2005; He et al., 2005b). The *miR-17–92* cluster also appears to enhance lung cancer cell growth (Hayashita et al., 2005). Overexpression of *miR-17–92* using transgenic mice (hematopoietic stem cells) significantly accelerated the formation of lymphoid malignancies (He et al., 2005b). Co-expression of *miR-17–19b*, a truncated portion of *miR-17–92*, strongly accelerated lymphomagenesis (Hammond, 2006). All of these findings suggest that *mir-17–92* functions as an oncogene in humans and other animal models.

Bioinformatic studies indicate that numerous genes are the targets of *miR-17–92*: more than 600 for *miR-19a* and *miR-20*, two members of *miR-17–92* cluster (Krek et al., 2005; Lewis et al., 2005). Two tumor suppressor genes *PTEN* (phosphatase and tensin homolog deleted on chromosome ten) and *RB2* were predicted to be targeted by *miR-17–92* cluster (Lewis et al., 2003). *PTEN* promotes apoptosis through the P13K-Akt-PKB pathway (Hammond, 2006). Unfortunately, no experiments have confirmed that *PTEN* and *RB2* are truly targets of the *miR-17–92* cluster. Whether the *miR-17–92* cluster is directly involved in lung cancer development or controls lung cancer by targeting suppressor genes is still unknown.

More recent studies indicate that the expression of *miR-17–92* is related to the expression of *c-Myc* gene; both *miR-17–92* and *c-Myc* regulate the expression of cell cycle transcription factor gene *E2F1* (Fig. 2) (O'Donnell et al., 2005). *c-Myc* is one of the best-characterized oncogenes. It is a helix–loop–helix leucine zipper transcription factor that regulates cell proliferation, growth, and apoptosis-mediated cell death by targeting about 10–15% of the genes in humans and other animals (Fernandez et al., 2003; Levens, 2002; Li et al., 2003; Orian et al., 2003). Misexpression or dysfunction of *c-Myc* usually causes human malignancy (Cole and McMahon, 1999). O'Donnell et al. (2005) demonstrated that *c-Myc* simultaneously activates the transcription of both *E2F1* and *miR-17–92* (O'Donnell et al., 2005). However, *miR-17-5p* and *miR-20a*, two miRNAs in the *miR-17–92* cluster, repress *E2F1* translation (O'Donnell et al., 2005). Transient overexpression of *miR-17–92* in HeLa cells resulted in a 50% decrease in *E2F1* protein levels without affecting *E2F1* mRNA abundance. In addition, mutants of *miR-20a* also caused a four-fold increase in *E2F1* protein levels without affecting *E2F1* mRNA abundance (O'Donnell et al., 2005). These findings suggest that *c-Myc*-regulated *miR-17–92* modulates *E2F1* expression (O'Donnell et al., 2005), that affects apoptosis-mediated cell death through the ARE-p53 pathway in which *miR-17–92* inhibits *Myc*-induced apoptosis (Hammond, 2006; O'Donnell et al., 2005).

miR-372 and *miR-373* are two additional examples of oncogenic miRNAs (considered oncogenes in human testicular germ cell tumors) (Voorhoeve et al., 2006). These two miRNAs promote cell proliferation and tumor development by neutralizing p53-mediated CDK inhibition, possibly through direct inhibition of expression of the tumor suppressor gene *LATS2* (Voorhoeve et al., 2006).

microRNAs as tumor suppressor genes

In oncogenesis, some miRNAs expression is decreased in cancerous cells. These types of miRNAs are considered tumor suppressor genes (Fig. 1). Tumor suppressor miRNAs usually prevent tumor development by negatively inhibiting oncogenes and/or genes that control cell differentiation or apoptosis. Currently, several miRNAs are considered as tumor suppressor genes, for example, miRNA *let-7*.

let-7 is one of the founding members of the miRNA family (Ambros, 2004; Bartel, 2004). *let-7* was originally observed in
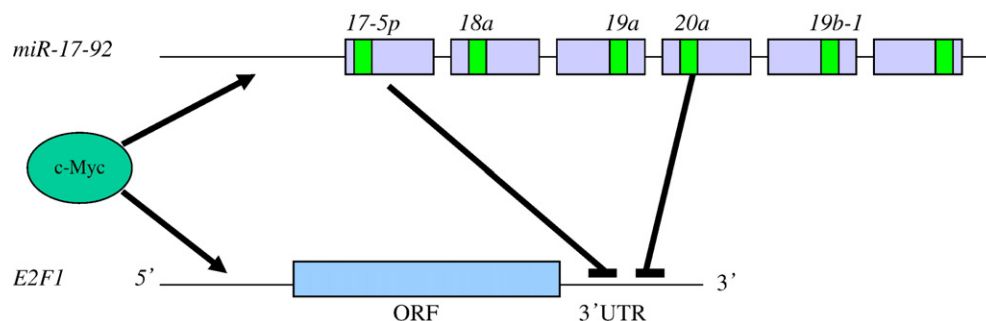

Fig. 2. Modulation of cell cycle transcription factor E2F1 by a *c-Myc*-regulated microRNA polycistron *miR-17–92* and its function in cancer pathogenesis (O'Donnell et al., 2005). The upper part of the panel shows the schematic representation of the microRNA polycistron *miR-17–92* cluster. The larger boxes represent the miRNA precursors (pre-miRNAs), and the smaller boxes within represent the mature miRNAs. *c-Myc* promotes the transcription of both *miR-17–92* and *E2F1* by binding at the CACGTG or CARGTG sites on the *miR-17–92* gene (O'Donnell et al., 2005) and promoter sites of *E2F1*. Both *miR-17-5p* and *miR-20a* negatively regulate *E2F1* gene expression through translational repression by directly binding *miR-17-5p* and *miR-20a* at the 3′UTR of *E2F1* mRNAs. Level expression results in the inhibition of apoptosis through the ARF-p53 pathway, and causes cancer pathogenesis in several organs, including lung cancer and lymphomas. In this regulatory mechanism, *miR-17–92* functions as an oncogene.

C. elegans. It is essential for the transition of the fourth larval stage into an adult (Pasquinelli et al., 2000; Reinhart et al., 2000). Subsequent studies determined that *let-7* is highly conserved in animals from worm to human (Pasquinelli et al., 2000), and its expression is dependent on developmental timing (Johnson et al., 2003; Johnston and Hobert, 2003; Miska et al., 2004; Thomson et al., 2004). In early stages of animal development, very low levels of *let-7* are detected. The highest levels of *let-7* expression occur in differentiated adult tissues (Miska et al., 2004; Thomson et al., 2004).

Inappropriate expression of *let-7* results in oncogenic loss of differentiation. In *let-7* mutants, seam cells cannot exit the cell cycle and terminally differentiate at the correct time (Reinhart et al., 2000). This is a hallmark trait of cancers (Johnson et al., 2005). In humans, *let-7* is located at a chromosome region that is usually deleted in human cancers (Calin et al., 2004b). Takamizawa et al. (2004) found that *let-7* was poorly expressed in lung cancers; reduced *let-7* expression was significantly associated with shortened postoperative survival independent of disease stage (Takamizawa et al., 2004). This suggests that miRNA *let-7* may be a tumor suppressor gene. To confirm this conclusion, Takamizawa et al. (2004) overexpressed miRNA gene *let-7* in A549 lung adenocarcinoma cell lines and found *let-7* inhibited lung cancer cell growth *in vitro* (Takamizawa et al., 2004).

Recent studies indicate that the *RAS* oncogene is a direct target of miRNA *let-7*; it negatively regulates *RAS* expression by pairing at the 3′ UTR of *RAS* mRNA for translational repression (Johnson et al., 2005). Lung tumor tissues display significantly reduced levels of *let-7* and significantly increased levels of RAS protein relative to normal lung tissue, suggesting that *let-7* regulation of *RAS* is a mechanism for *let-7* to function as a tumor suppressor gene in lung oncogenesis (Johnson et al., 2005).

Conclusions and future perspectives

Emerging evidence suggests that miRNAs play important roles in the pathogenesis of a limited range of human cancers (Table 2). Some miRNAs may be directly involved in cancer development by controlling cell differentiation and apoptosis, while others may be involved in cancers by targeting cancer oncogenes and/or tumor suppressors. Understanding of the function of miRNAs is providing the new insights on the molecular basis of cancers, and new biomarkers for cancer diagnoses and cancer therapy.

How many miRNAs are involved in human cancers?

Currently, investigations on the function of miRNAs in cancers are based on microRNA microarray analyses. Thus, findings can be limited by the known miRNAs in human. Although up to 1000 miRNAs have been estimated in the human genome, only about 200–300 miRNAs have been currently identified in humans (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006). A majority of miRNAs still await discovery, some of them may be cancer-specific miRNAs.

Table 2
Cancer-related miRNAs and their potential targeted genes

Cancer	miRNAs involved [a]	miRNA-targeted genes	Reference
Brain cancer	miR-21↑, miR-221↑, miR-181↓		Ciafre et al., 2005; Chan et al., 2005
Breast cancer	miR-125b↓, miR-145↓, miR-21↓, miR-155↓		Iorio et al., 2005
Chronic lymphocytic leukemia	miR-15↓, miR-16↓	BCL2	Calin et al., 2002, 2004a; Cimmino et al., 2005
Colorectal neoplasia	miR-143↓, miR-145↓		Michael et al., 2003
Hepatocellular carcinoma	miR-18↑, miR-224↑, miR-199↓, miR-195↓, miR-200↓, miR-125↓		Murakami et al., 2006
Lung cancer	let-7↓, miR-17–92↑	RAS, MYC	Takamizawa et al., 2004; Johnson et al., 2005; Hayashita et al., 2005; O'Donnell et al., 2005
Lymphomas	miR-155↑, miR-17–92↑	BIC	Eis et al., 2005; Metzler et al., 2004; He et al., 2005b
Papillary thyroid carcinoma	miR-221↑, miR-222↑, miR-146↑, miR-181↑	KIT	He et al., 2005b; Pallante et al., 2006
Testicular germ cell tumors	miR-372↑, miR-373↑	LAST2	Voorhoeve et al., 2006

[a] ↑ represents that the expression of the miRNAs increased in this type of cancer; ↓ represents that the expression of the miRNAs decreased in this type of cancer. See details in text.

Molecular mechanisms of miRNA-associated cancers

Although evidence shows that some miRNAs play an essential role in human cancers, the molecular mechanisms of miRNA-regulated pathogenesis are unclear. How miRNAs regulate the multiple stages (initiation, promotion, malignant conversion, progression, and metastasis) of cancers also awaits discovery. One possible mechanism is that miRNAs regulate cancer pathogenesis by targeting oncogenes and/or tumor suppressor genes, but only one miRNA target has been demonstrated experimentally. Whether or not other miRNAs regulate cancer pathogenesis by similarly targeting cancer oncogenes or tumor suppressor genes needs further study.

Cancer biomarkers and cancer diagnosis

Many miRNAs are uniquely and differentially expressed in certain cancer tissues as compared with normal adjacent tissues. For example, the expression of miRNA *let-7* is down-expressed in lung cancer but not in other cancers, such as breast or colon cancer (Eder and Scherr, 2005; Johnson et al., 2005; Takamizawa et al., 2004). microRNA microarray analysis indicates that miRNA expression profiles are a better indicator for distinguishing cancer tissues from normal tissues, and can

successfully classify even poorly differentiated tumors (Lu et al., 2005). These findings suggest that miRNAs can be used as biomarkers and a powerful diagnostic tool for detecting cancers.

Cancer therapeutic tools and microRNA therapy

Because miRNAs function as oncogenes or tumor suppressors, it might be possible to regulate miRNA expression and/or inject miRNAs to regulate cancer formation, similar to the use of antisense mRNAs and RNAi which are widely used as tools for studying gene functions and in some case of gene therapy. Artificial miRNAs could be synthesized to down-regulate oncogenes and prevent the formation of cancer. In plants, Zhang and colleagues hypothesized that improvements of crop yield and quality could be achieved by targeting certain genes using artificial miRNAs (Zhang et al., 2006c). This was confirmed to be applicable by a recent study (Schwab et al., 2006). In a mouse model, expressing *mir-17–92* in transgenic mice strongly inhibited c-*myc*-induced apoptosis, and resulted in accelerated tumor development (He et al., 2005b). The activation of a majority of oncogenes causes cancer formation. Thus, artificial miRNAs can be designed to block the expression of these oncogenes based on the complementary characteristics of miRNAs to their targeted mRNAs.

miRNAs have also significant potentials as therapeutic targets because that several miRNAs themselves function as oncogenes and/or tumor suppressor genes. Thus, antisense RNAs could be employed to block miRNAs which function as oncogenes. Recently, two research groups independently transfected 2′-O-methyl-modified antisense RNAs into several independent miRNAs and showed sequence-specific inhibition (Hutvagner et al., 2004; Meister et al., 2004). More interestingly, four miRNAs were successfully inhibited by injecting antagomirs (modified antisense RNAs) into adult mice (Krutzfeldt et al., 2005). Most notably, Dickins et al. (2005) found that miR-30-based shRNAs (called shRNA-mirs) suppressed gene expression when driven by Pol II promoters. They also observed that tumor growth could be controlled by tightly regulating Trp53 knock-down using tetracycline-based systems, and gene knock-down by expression of shRNA-mirs may be similar to overexpression of protein-coding cDNAs (Dickins et al., 2005). These results suggest that miRNAs could provide a powerful therapeutic tool for treating cancers.

There is a long way to go before artificial and natural miRNAs could be used as cancer therapeutic tools and miRNA therapy for clinical purposes. To achieve this goal, several obstacles need to be overcome. First, specific miRNAs in a specific type of cancer should be identified; only when the specific miRNAs are identified and their action mechanisms were elucidated in a specific cancer, we can manipulate these miRNAs for therapeutic purposes. How to deliver these miRNAs into targeted tissues and keep their continuous activity is another obstacle. Can the technique of RNAi be used to archive these goals? After these obstacles have been overcome, miRNA-related techniques will have a brilliant future and become new cancer therapeutic tools.

Acknowledgments

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