Histol Histopathol (2011) 26: 1353-1361
DOI: 10.14670/HH-26.1353

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Big roles of microRNAs in tumorigenesis and tumor development

Jun Tie and Daiming Fan
State Key Laboratory of Cancer Biology and Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, Shaanxi, China

Summary. MicroRNAs (miRNAs) are a class of endogenous non-protein-coding small RNAs that are evolutionarily conserved and widely distributed among species. Their major function is to negatively regulate target gene expression. A single miRNA can regulate multiple target genes, indicating that miRNAs may regulate multiple signaling pathways and participate in a variety of physiological and pathological processes. Currently, approximately 50% of identified human miRNA-coding genes are located at tumor-related fragile chromosome regions. Abnormal miRNA expression and/or mutations have been found in almost all types of malignancies. These abnormally expressed miRNAs play roles similar to tumor suppressor genes or oncogenes by regulating the expression and/or function of tumor-related genes. Therefore, miRNAs, miRNA target genes, and the genes regulating miRNAs form a regulatory network with miRNAs in the hub. This network plays a pivotal role in tumorigenesis and tumor development.

Key words: MicroRNAs, Gene expression regulations, Neoplasms

Molecular properties and functional characteristics of miRNAs

Research history of miRNAs

Lee et al. (1993) and Wightman et al. (1993) simultaneously discovered an approximately 22-nucleotide, non-protein-coding small RNA in *C. elegans* in 1993. It is now known as *lin-4*, and binds the 3'-untranslated region (3' UTR) of the *lin-14* gene, inhibits the translation of the lin-14 mRNA, and regulates the transformation from the first larval stage to the second larval stage in nematode development. At that time, lin-4 was considered unique to *C. elegans*, so this type of small RNA did not attract much attention for 7 years after the discovery of lin-4. In 2000, another non-coding small RNA, let-7, was discovered to regulate nematode development. This finding became the prelude of miRNA research (Reinhart et al., 2000). Similar to lin-4, let-7 is also an endogenous non-coding RNA with a length of 22 nucleotides; it regulates the transformation of nematode from late-stage larva to adult nematode, similar to lin-4. This phenomenon drew considerable attention. In October 2001, Lagos-Quintana et al. (2001) reported in the journal Science the cloning of dozens of small RNA genes that are similar to lin-4 from *C. elegans, D. melanogaster*, and human in three laboratories. These small RNAs are collectively called miRNAs, information about which is included in the miRBase (http://microrna.sanger.ac.uk/). Subsequently, miRNAs were found to be present in many animal and plant cells. At present, 14,197 miRNAs have been registered at miRBase (http://www.mirbase.org), including 1240 mature miRNAs encoded by 940 genes in the human genome.

Properties of miRNA genes

The majority of human miRNA-coding genes are located in the introns, non-coding exons, or 3' untranslated regions (3' UTRs) of protein-coding genes (Rodriguez et al., 2004). A small number of miRNA genes are located within transcripts in the genome (Bartel, 2004). miRNA genes usually occur in clusters. Multiple miRNA genes are transcribed into a common precursor miRNA (pri-miRNA) that is processed into multiple mature miRNAs. For example, 54 miRNA genes form a gene cluster on chromosome 19. miRNA

Offprint requests to: Jun Tie and Daiming Fan, State Key Laboratoy of Cancer Biology and Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, Shaanxi 710032, China. e-mail: Fandaim@fmmu.edu.cn

expression exhibits temporal and tissue-specific patterns. For example, lin-4 is mainly expressed at the first and second larval stages in *C. elegans*, whereas let-7 is primarily expressed at the third and fourth larval stages and the adult stage. In humans, miR-1 is only expressed in myocardial cells, whereas miR-122 is only expressed in hepatocytes. miRNAs are highly conserved during evolution. Among all the identified miRNAs, nearly 12% are present throughout the entire evolutionary process (Tabara et al., 1999). The broad distribution and evolutionary conservation of miRNAs suggest their indispensable regulatory functions in vital biological processes.

Biogenesis of miRNAs

The biogenesis of miRNAs *in vivo* occurs as follows. First, miRNA-coding genes are transcribed by RNA polymerase II into pri-miRNAs that are spliced in the nucleus by an RNase III, the enzyme Drosha, into precursor miRNAs (pre-miRNAs) to a length of 60-70 nucleotides. The 5' terminus of the pre-miRNA contains a phosphate group, whereas its 3' terminus forms a hairpin structure with a two-nucleotide overhang (Lee et al., 2002; Denli et al., 2004). The pre-miRNAs are transported into the cytoplasm by the transporter protein Ran-GTP, where they are cleaved by the enzyme Dicer into small double-stranded RNAs at a length of 21-25 nucleotides. The double-stranded RNAs are then transformed by helicase into single-stranded small RNA molecules, also known as mature miRNAs. Mature miRNAs contain a phosphate group at the 5' terminus and a hydroxyl group at the 3' terminus. This feature can distinguish mature miRNAs from most of the other oligonucleotides and degradation fragments of functional RNAs (Hutvagner et al., 2001). Mature miRNAs bind the members of the Argonaute protein family and form the RNA-induced silencing complex. This complex binds the 3' UTR of target mRNAs and negatively regulates target gene expression.

Functional mechanism of miRNA function

miRNAs are a large class of gene expression regulatory factors. They function through complementary binding to target mRNAs and negatively regulate gene expression at the post-transcriptional level. miRNAs bind the 3' UTR of target mRNAs through base-pairing, resulting in the cleavage of target mRNAs or translational suppression. The degree of complementarity between miRNAs and their target sequences determines the outcome of target mRNA cleavage or translational suppression. When a miRNA and its target mRNA are almost completely complementary, the double-stranded RNAs that they form trigger the RNA-mediated interference (RNAi) pathway, leading to target mRNA degradation. This miRNA-mediated gene silencing mechanism is relatively common in plants (Llave et al., 2002; Palatnik et al., 2003), whereas in most mammals miRNAs do not cause target mRNA degradation. Rather, they bind the 3' UTR of target mRNAs through incomplete base-pairing and suppress the translation of the mRNAs (Pillai et al., 2005). However, partial complementation between miRNAs and their target genes also leads to mRNA degradation, although it is still unclear whether translational suppression occurs before mRNA degradation (Hornstein et al., 2005). The regulation of target genes by miRNAs is not one-to-one. A single miRNA can act on multiple target genes at the same time, and a gene can be simultaneously regulated by multiple miRNA molecules, suggesting the presence of multiple combination models in the gene regulation by miRNAs (Hornstein et al., 2005). miRNAs participate in a series of important biological processes, including development, hematopoiesis, organogenesis, cell proliferation, apoptosis, and death, by regulating target gene expression; they are also closely related to the pathogenesis of cancer, heart diseases, diabetes, AIDS, and many other diseases.

miRNAs in tumorigenesis and tumor development

miRNAs and tumorigenesis

Tumors result from pathological changes caused by dysregulation of cell proliferation and apoptosis. Abnormal expression of oncogenes and tumor suppressor genes has been widely accepted as the molecular mechanism of tumorigenesis. However, this traditional concept is being challenged by the discovery of non-coding RNAs. Recent studies have shown that miRNAs play important regulatory roles in tumorigenesis. miRNAs are expressed in a variety of tumors, exhibiting abnormal functions; they are involved in tumorigenesis and tumor development by negatively regulating protein-coding genes related to these processes. On one hand, downregulation or null expression of a tumor suppressor miRNA can lead to the expression of the miRNA target genes that promote tumorigenesis, causing excessive cell proliferation and abnormal differentiation and resulting in tumorigenesis. On the other hand, overexpression of oncogenic miRNAs can lead to decreased expression of the target genes with tumor suppressor functions, thereby promoting tumorigenesis and tumor development. For example, overexpression of miR-221 or miR-222 inhibits the expression of the Kit protein, resulting in the dedifferentiation of thyrocytes and tumorigenesis (He et al., 2005). Deficiency of miR-15a and miR-16-1 expression can lead to overexpression of Bcl-2, an important anti-apoptotic factor, resulting in decreased apoptosis, thereby promoting tumorigenesis and tumor development. We have found that miR-150 is significantly overexpressed in gastric cancer tissues and promotes gastric cancer cell proliferation through EGR2 (Wu et al., 2010). Moreover, the expression of miR-34 is decreased in gastric cancer. Restoring miR-34

expression in the gastric cancer cells with a mutant p53 significantly inhibits the protein expression of Bcl-2, Notch, and HMGA2, causing cell cycle arrest in G1 phase and cell growth inhibition. It also increases the activity of caspase-3 to promote apoptosis and inhibits the formation and growth of gastric cancer stem cell spheroids (Ji et al., 2008).

An interesting phenomenon found in recent studies of tumor miRNA expression profiles is that the miRNAs abnormally expressed in tumors usually exhibit reduced expression levels compared to their expression levels in normal tissues (Lu et al., 2005). This phenomenon may reflect a higher proliferation rate and a less differentiated state of tumor cells. Another explanation is that the cells with low levels of miRNAs are selected for during tumorigenesis due to their proliferative and survival advantages. These two possibilities are not mutually exclusive. In fact, both of them are supported by experimental evidence. For instance, after differentiation induction, HL60 cells show significantly increased miRNA expression, consistent with the fact that the differentiation state of the cells is maintained by the enhancement of miRNA transcription. Moreover, studies in lung cancer models have indicated that inhibiting miRNA biogenesis by genetic approaches or RNAi can promote tumorigenesis and tumor development (Kumar et al., 2007). In addition, c-Myc can induce the universal silencing of miRNA transcription (Chang et al., 2008), providing a possible mechanism for miRNA downregulation in malignant cells. These findings suggest that the majority of miRNAs may function as tumor suppressors in tumorigenesis.

miRNAs and tumor angiogenesis

miRNAs participate in tumorigenesis and tumor development through multiple mechanisms, one of which is regulating tumor angiogenesis. For example, the expression of the c-Myc oncogene is increased in most B cell lymphomas and some solid tumors. c-Myc is an important transcription factor that can lead to overexpression of the miR-17-92 family members. The miR-17-92 family consists of seven miRNAs, miR-17-5p, miR-17-3p, miR-18, miR-19a, miR-20, miR-19b-1, and miR-92-1, whose coding genes are located within the gene *C13orf25*. They are generated through the splicing of a common transcript. Among these members, miR-19a can bind the 3' UTR of the endogenous angiogenesis inhibitor thrombospondin 1 gene (*tsp-1*) to inhibit *tsp-1* expression and promote angiogenesis. miR-18 can directly inhibit the expression of connective tissue growth factor to affect tumor angiogenesis. In addition, miR-17-5p, miR-18, and miR-20 can also directly reduce the expression of the tumor suppressor gene *Rb2/p130* to regulate cell proliferation and differentiation (Wang et al., 2008). Thus, the mir-17-92 family plays important roles in tumor angiogenesis. miR-15b and miR-16 can regulate the expression of vascular endothelial growth factor (VEGF) and play a crucial regulatory role in angiogenesis. For example, the cellular levels of miR-15b and miR-16 are downregulated under hypoxic conditions, leading to diminished inhibitory effects of miR-15b and miR-16 on VEGF, thereby promoting tumor angiogenesis (Hua et al., 2006).

miRNAs and tumor metastasis

miRNAs can not only promote the development of primary tumors, but also affect tumor progression, including tumor metastasis (Ma and Weinberg, 2008; Dumont and Tlsty, 2009; Nicoloso et al., 2009; Zhang et al., 2009; Baranwal and Alahari, 2010; Ding et al., 2010; Khew-Goodall and Goodall, 2010; Li et al., 2010; Ma et al., 2010; Sachdeva and Mo, 2010; Santarpia et al., 2010; Schmittgen, 2010; Tian et al., 2010; Zhang et al., 2010). Both mutation (Gardner and Vinther, 2008) and misexpression (Santarpia et al., 2010; Zhang et al., 2010) of miRNAs can affect their normal functions, leading to abnormal expression of their target genes. Thus, tumor metastasis may be affected when the target genes are related to tumor cell migration, invasion, anoikis resistance, and other metastatic phenotypes.

Among several known miRNAs promoting tumor metastasis, miR-10b and miR-373 are particularly prominent. miR-10b is a direct target gene of the transcription factor Twist1 that promotes epithelial-mesenchymal transition and tumor metastasis. The expression of miR-10b is markedly upregulated in human breast cancer cells of high metastatic potential; the invasiveness of these cells decreases by 10-fold if the activity of miR-10b is blocked by an antisense oligonucleotide. Overexpression of miR-10b in breast cancer cells with low metastatic potential leads to a significant increase in the invasiveness of the tumor cells. Six weeks after orthotopic inoculation of highly miR-10b-expressing breast cancer cells into the breast of immunodeficient young female mice, tumors were found at the inoculation sites in all of the inoculated mice, together with apparent interstitial and vascular infiltration; distant metastasis appeared in all of these mice 11 weeks after the inoculation. No invasion or metastasis was observed when using low miR-10b–expressing breast cancer cells. miR-10b promotes the invasion and metastasis of breast cancer cells through inhibiting its target gene *HOXD10* to increase the expression of RhoC (Ma et al., 2007). miR-335 can induce cell morphological changes to reduce the invasiveness and metastasis of breast cancer cells through directly regulating its targets, the progenitor cell-regulating transcription factor SOX4 and the extracellular matrix component cadherin C (Tavazoie et al., 2008). miR-29c can inhibit the metastasis of nasopharyngeal carcinoma by regulating a variety of extracellular matrix proteins, including inhibiting collagen and laminin γ1 (Sengupta et al., 2008). miR-373 was identified from *in vitro* screening for enhanced tumor cell migration phenotypes (Huang et al., 2008),

and its role in promoting metastasis has been confirmed *in vivo*. It should be noted that miR-373 also exhibits the characteristics of an oncogene during the tumorigenesis of testicular germ cell tumors (Voorhoeve et al., 2006). It has been suggested that its oncogenic characteristics of promoting metastasis are a result of regulating different target genes.

We have conducted a series of studies to explore the relationship between miRNAs and gastric cancer metastasis and identified a group of gastric cancer metastasis-related miRNAs through screening. The expression of miR-218 was significantly decreased in gastric cancer cells with high metastatic potential, and the extent of the decrease is closely related to gastric cancer metastasis and the prognosis in gastric cancer patients. These results were confirmed in cells and clinical tissue samples. Based on bioinformatic analysis of the correlation between miR-218 expression and its function, we found that miR-218 inhibits the invasion and metastasis of gastric cancer through regulating the membrane receptor Robo1. In addition, we found that miR-218 is encoded by two genes, miR-218-1 and miR-218-2, located within introns of the genes *Slit2* and *Slit3,* respectively. Slit is the ligand of Robo1. In previous studies, we have demonstrated that *Robo1* is a target gene of miR-218, whereas *Slit,* encoding the ligand of Robo1, is the host gene of miR-218. *Slit* is co-transcribed and co-expressed with miR-218, thereby forming a Slit–miR-218–Robo1 negative-feedback loop. In gastric cancer, downregulation of the host gene *Slit3* leads to miR-218 expression deficiency, loss of inhibition of Robo1 translation, and Robo1 overexpression. Overexpressed Robo1 interacts with its other ligand, Slit2, to promote the invasion and metastasis of gastric cancer cells (Tie et al., 2010).

miRNAs and cancer drug resistance

In addition to the findings that miRNAs are associated with tumor pathogenesis and development, miRNAs are closely related to the drug resistance of tumor cells (Lwin et al., 2010; Ma et al., 2010; Zheng et al., 2010; Akao et al., 2011). miRNAs affect the sensitivity of tumor cells to cytotoxic drugs (Xia et al., 2008), biologically targeted drugs (Miller et al., 2008; Weiss et al., 2008), endocrine drugs (Garofalo et al., 2008; Zhao et al., 2008), and cytokine drugs (Kovalchuk et al., 2008). For example, Kovalchuk et al. (2008) found that the expression of miR-451 is significantly reduced in the doxorubicin-resistant breast cancer cell line MCF27/DOX and that restoring miR-451 expression by transfection can increase the sensitivity of MCF27/DOX cells to doxorubicin. Si et al. (2007) discovered that a miR-21 inhibitor can significantly increase the sensitivity of breast cancer cells to topotecan. The studies by Venturini et al. (2007) showed that miR-17-19b transfection can remarkably increase the imatinib-induced apoptosis of the acute myeloid leukemia cells

Table 1. Reported studies on miRNAs and tumor drug resistance.

Cancer type	miRNA	Target gene	Reference
Breast cancer	miR-451	Mdr1/P-glycoprotein	Kovalchuk et al., 2008
	miR-221/222	p27 (Kip1)	Miller et al., 2008
	miR-200 family	E-cadherin	Tryndyak et al., 2009
	miR-328	BCRP/ABCG2	Pan et al., 2009
Gastric cancer	miR-15a	Bcl-2	Xia et al., 2008
	miR-16		
Non–small cell lung cancer	miR-221/222	Kit, $p27^{(Kip1)}$	Zhao et al., 2008
Ovarian cancer	miR-214	PTEN	Yang et al., 2008
	miR-130a	M-CSF	Sorrentino et al., 2008
	miR-27a	Mdr1/p-glycoprotein	Zhu et al., 2008
	miR-451		
Prostate cancer	Mi-34a	Sirt1	Fujita et al., 2008
	miR-148a	MSK1	Fujita et al., 2010
Colon cancer	miR-519c	ABCG2	To et al., 2008
	MiR-34a	Sirt1/E2F3	Akao et al., 2011
Cervix carcinoma	miR-27a	Mdr1/p-glycoprotein	Zhu et al., 2008
	miR-451		
Esophageal Carcinoma	miR-27a	Mdr1/P-glycoprotein	Zhang et al., 2009
		Bcl-2	
B-cell lymphomas	miR-181a	Bim	Lwin et al., 2010

K562. We summarized the published studies on miRNAs and tumor drug resistance in Table 1; miRNAs appear to play important roles in tumor drug resistance by regulating the expression of drug resistance proteins, and targeted intervention of miRNAs may effectively reverse tumor drug resistance.

The incidence rate of gastric cancer varies in different races and geographical regions. Compared with the US and European countries, the incidence is significantly higher in China and some Asian countries (such as Japan and South Korea); therefore, gastric cancer drug resistance is rarely studied in the US and European countries. By comparing the miRNA expression profile of drug-resistant gastric cancer cells with their parental cells, we were among the first in the world to identify a group of differentially expressed miRNAs in drug-resistant gastric cancer cells. Among these differentially expressed miRNAs, the expression levels of miR-15b and miR-16 were significantly decreased in drug-resistant gastric cancer cells. Transfection of miR-15b and miR-16 precursor sequences restored their expression in drug-resistant gastric cancer cells and significantly enhanced the sensitivity of the transfected gastric cancer cells to the antitumor drugs VCR, ADR, VP-16, and CDDP. Further studies showed that the level of Bcl-2 is negatively correlated with the levels of miR-15b and miR-16 in gastric cancer, and that silencing Bcl-2 expression by RNAi in drug-resistant gastric cancer cells alters the drug resistance phenotype, similar to the changes induced by the transfection of miR-15b and miR-16. Further experiments using a fluorescent reporter gene also confirmed that miR-15b and miR-16 negatively regulate Bcl-2. Thus, these results suggest that miR-15b and miR-16 regulate multidrug resistance in gastric cancer cells through the Bcl-2 pathway (Xia et al., 2008).

miRNAs in tumor diagnosis and treatment

miRNAs and tumor diagnosis

miRNAs can be used to identify benign and malignant lesions and to determine the prognosis of cancer patients. High-throughput miRNA detection technologies have been emerging in recent years, including miRNA chips, miRNA expression profiling using magnetic bead–based flow cytometry, and miRNA qPCR assays. These methods have been used to identify a variety of tumor-specific miRNAs through parallel comparison between cancer tissues and the adjacent tissues. miRNAs are differentially expressed between tumor cells and normal cells, as well as among the tumor cells originating from different tissues. The discovery of miRNA expression features can help not only distinguish between benign and malignant lesions, but also determine the degree of tumor malignancy and prognosis, thereby providing the basis for personalized therapy (Alvarez-Garcia and Miska, 2005; Calin et al., 2005). For example, Calin et al. (2005) selected 13 out of 190 miRNAs as a set of miRNA expression markers for chronic lymphocytic leukemia; this miRNA set can not only distinguish between malignant and normal B lymphocytes, but also identify the chronic lymphocytic leukemia cases with high expression of 70-kD zeta-associated protein (ZAP70) or a mutated IgV (H) gene, which are associated with high malignancy, strong invasiveness, and poor prognosis. In non-small cell lung cancer, miR-155 upregulation and let-7 downregulation indicate poor prognosis. In colon cancer patients, high expression of miR-21 indicates poor prognosis. In our previous studies, we found that a characteristic spectrum composed of seven miRNAs (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p, miR-126) is sufficient to predict the survival of gastric cancer patients (Li et al., 2010). Because miRNAs are relatively stable, it becomes more and more promising to use miRNAs as new tumor markers.

miRNAs can distinguish different malignant phenotypes of the same tumor

Different malignant phenotypes of tumors originating from the same tissue can result in markedly different therapeutic outcomes and prognosis. miRNAs can distinguish not only normal cells from tumor cells, but also the different malignant phenotypes of tumor cells originating from the same tissue. For example, Budhu et al. (2008) examined the miRNA expression profiles in cancer tissues and the corresponding adjacent tissues from 241 hepatic cancer cases using miRNA microarray. These cancer specimens included both invasive and non-invasive samples. Their results showed that the levels of 20 miRNAs can accurately predict hepatic cancer metastasis, which are closely related to postoperative relapse and the survival of hepatic cancer patients. Compared to their expression in drug-sensitive gastric cancer cells, miR-15a and miR-16b were downregulated in drug-resistant gastric cancer cells (Xia et al., 2008), and miR-218 was downregulated in the gastric cancer cells with high metastatic potential (Tie et al., 2010).

miRNAs reflect the origin of tumor tissues

By examining the samples from 540 cases, including 363 cases of six types of malignancies with high incidence rates and 177 normal controls, Volinia et al. (2006) discovered that specific miRNA expression profiles can not only distinguish normal cells from tumor cells, but also reflect the origin of tumor tissues. Lu et al. (2005) analyzed the miRNAs in 334 samples from 217 mammalian species and found that tumors originating from the organs that develop from embryonic endoderm, such as stomach, intestine, and liver, displayed similar miRNA expression patterns, whereas the miRNA expression patterns of leukemia were significantly different from those of solid tumors, suggesting that

characteristic miRNA expression profiles possess diagnostic value in the identification of tumor tissue origin and exhibit great potential in the diagnosis of metastatic tumors with unknown primary tumor origin. miRNA expression profiles can also accurately reflect the progression and differentiation state of tumor tissues, especially for the poorly differentiated malignant tumors. Compared with the previously reported mRNA expression profiles, miRNA expression profiles provide a more accurate reflection of tumor status (Alvarez-Garcia and Miska, 2005).

Circulating miRNAs, new tumor markers

The discovery of circulating miRNAs further promoted investigations of miRNAs as biological markers in tumor diagnosis and prognosis. Lawrie et al (2008). first observed high expression of miR-21 in sera from patients with diffuse large B cell lymphoma and the close correlation of miR-21 expression with disease recurrence and patient survival. Mitchell et al. (2008) isolated RNAs of 18-24 nucleotides from the plasma of healthy volunteers and constructed a small RNA library. They found 91 known and 4 unknown miRNAs by sequencing analysis. Subsequently, 25 patients with metastatic prostate cancer and 25 healthy volunteers were separated into different groups, whose sera were examined. They found that the plasma miR-141 levels in prostate cancer patients were significantly higher than those in the normal control group. Thus, prostate cancer patients can be effectively distinguished from healthy individuals based on their miR-141 expression levels, indicating that miR-141 can be used as a circulating miRNA marker for the detection of prostate cancer. Chen et al. (2008) analyzed the miRNA expression in the sera from patients with lung cancer, colon cancer, or diabetes using Solexa sequencing and quantitative PCR. The results showed that these diseases all have their characteristic serum miRNA expression patterns. For example, 63 miRNAs were expressed in the sera of patients with lung cancer, but not in the sera of healthy individuals. Ten miRNAs were common to the sera of lung cancer and colon cancer patients. Resnick et al. (2009) examined the miRNA expression profile in plasma from eight ovarian cancer patients and found 23 differentially expressed miRNAs, among which 10 molecules had been previously reported in solid ovarian tumors. Verification in plasma from 19 ovarian cancer patients by qRT-PCR showed that miR-21, miR-29a, miR-92, miR-93, and miR-126 were remarkably upregulated in these patients, whereas miR-155, miR-127, and miR-99b were significantly downregulated. Some of the patients with upregulated miR-21, miR-92, and miR-93 did not show changes in CA-125. Due to the small sample size, no statistical correlation was found between plasma miRNA expression profiles and their pathological and clinical stages. Nevertheless, that study suggests that specific plasma miRNAs may serve as molecule markers that are detectable earlier than the traditional tumor markers used in tumor diagnosis and in efficacy assessment. These studies demonstrated that miRNAs are broadly present in the sera of healthy individuals and of patients with different diseases, including cancer. The types and quantities of miRNAs present in serum and plasma vary with physiological and pathological conditions. Specific miRNA expression profiles in the sera of tumor patients provide a new method for early diagnosis, classification, prognosis, efficacy assessment, and relapse monitoring of tumors, bringing new hope to noninvasive tumor diagnosis.

miRNAs and tumor treatment

With the continuing discovery of the roles of miRNAs in tumorigenesis and tumor development, people have started to explore the use of miRNAs in tumor treatment. A single miRNA can simultaneously regulate multiple protein-coding genes and a number of signaling pathways associated with tumor growth and proliferation, metastasis, drug resistance, and other malignant phenotypes. Therefore, miRNAs exhibit advantages over individual protein-coding genes for treating tumors that involve alterations in multiple genes. For example, miR-31 can simultaneously regulate five proteins involved in tumor metastasis, RhoA, RDX, MMP16, Fzd3, and ITGA5, in breast cancer cells. It inhibits multiple steps in metastasis, including tumor cell migration, invasion, anoikis resistance, and colony formation in distant organs. Exogenous overexpression of miR-31 can significantly suppress the metastasis of breast cancer cells both *in vitro* and *in vivo* (Valastyan et al., 2009, 2010; Valastyan and Weinberg, 2010). In head and neck tumors, miR-204 can simultaneously regulate more than 60 target proteins involved in multiple tumor-associated signaling pathways (Lee et al., 2010), suggesting that miR-204 is a promising target for the treatment of head and neck tumors.

Tumor-associated miRNAs can be divided into two categories: tumor-promoting miRNAs and tumor-suppressing miRNAs. Tumor-promoting miRNAs are highly expressed in tumors. A number of methods can be used to downregulate or to suppress the expression of tumor-promoting miRNAs, including antagomirs, anti-miRNA oligonucleotides and miRNA sponges. Tumor-suppressing miRNAs are not expressed or are expressed at low levels in tumors. Thus, tumor treatment can be exerted by introducing corresponding exogenous miRNAs. The fact that a single miRNA can regulate multiple target genes provides new approaches for gene therapy based on RNAi. Because tumorigenesis and tumor progression are often regulated by multiple genes, an artificial miRNA can be designed to target multiple genes that are highly expressed in tumors, thereby simultaneously suppressing the expression of multiple oncoproteins. This effect cannot be achieved by traditional RNAi technology. Moreover, miRNA genes are often distributed in clusters. For example, the pri-miRNA of the miR-17-92 cluster contains seven

independent mature miRNAs. Therefore, we can simulate the structure of the miR-17-92 precursor, design a structure for the expression of multiple miRNAs from a single promoter, and induce the interference of multiple oncogenes. However, further experimental verification is required to test this possibility.

Issues and prospects

Despite significant progress in the study of miRNAs in the field of cancer research, miRNA research is still in its infancy, with many important issues to be solved. First, many tumor-promoting or tumor-suppressing miRNAs still need further experimental confirmation. For example, except for the observation that abnormal expression of miR-155 in mice induces the pathogenesis of B cell leukemia, no sufficient evidence exists that an oncogenic miRNA alone can initiate malignant transformation. Similarly, although many miRNAs are silenced in tumor cells and likely possess tumor-suppressing functions, no loss-of-function studies have been conducted in mouse models for any miRNAs. Second, despite the identification of miRNA target genes at an accelerated pace, the regulatory network of miRNAs is still poorly understood, especially for the tumor-related miRNAs. We cannot expect to fully understand a specific miRNA through one or several target mRNAs, especially when it comes to the biological functions of a miRNA cluster. miRNA regulation is more likely the result of complex regulatory networks involving multiple molecules and pathways. The improvements in experimental methods and approaches to predict miRNA target genes will facilitate the comprehensive understanding of the underlying mechanisms of miRNA functions and the related signaling pathways. Third, currently, the vast majority of studies have merely focused on the regulatory effects of miRNAs on mRNAs. The expression of miRNAs and their regulatory factors are still poorly understood. The answers to these questions will contribute to the understanding of the definitive roles of miRNAs in biological processes and will promote the broad application of miRNAs in tumor diagnosis and treatment.

References

Akao Y., Noguchi S., Iio A., Kojima K., Takagi T. and Naoe T. (2011). Dysregulation of microRNA-34a expression causes drug-resistance to 5-FU in human colon cancer DLD-1 cells. Cancer Lett 300, 197-204.

Alvarez-Garcia I. and Miska E.A. (2005). MicroRNA functions in animal development and human disease. Development 132, 4653-4662.

Baranwal S. and Alahari S.K. (2010). miRNA control of tumor cell invasion and metastasis. Int. J. Cancer 126, 1283-1290.

Bartel D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281-297.

Budhu A., Jia H.L., Forgues M., Liu C.G., Goldstein D., Lam A., Zanetti K.A., Ye Q.H., Qin L.X., Croce C.M., Tang Z.Y. and Wang X.W. (2008). Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 47, 897-907.

Calin G.A., Ferracin M., Cimmino A., Di Leva G., Shimizu M., Wojcik S.E., Iorio M.V., Visone R., Sever N.I., Fabbri M., Iuliano R., Palumbo T., Pichiorri F., Roldo C., Garzon R., Sevignani C., Rassenti L., Alder H., Volinia S., Liu C.G., Kipps T.J., Negrini M. and Croce C.M. (2005). A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N. Engl. J. Med. 353, 1793-1801.

Chang T.C., Yu D., Lee Y.S., Wentzel E.A., Arking D.E., West K.M., Dang C.V., Thomas-Tikhonenko A. and Mendell J.T. (2008). Widespread microRNA repression by Myc contributes to tumorigenesis. Nat. Genet. 40, 43-50.

Chen X., Ba Y., Ma L., Cai X., Yin Y., Wang K., Guo J., Zhang Y., Chen J., Guo X., Li Q., Li X., Wang W., Wang J., Jiang X., Xiang Y., Xu C., Zheng P., Zhang J., Li R., Zhang H., Shang X., Gong T., Ning G., Zen K. and Zhang C.Y. (2008). Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 18, 997-1006.

Denli A.M., Tops B.B., Plasterk R.H., Ketting R.F. and Hannon G.J. (2004). Processing of primary microRNAs by the Microprocessor complex. Nature 432, 231-235.

Ding J., Huang S., Wu S., Zhao Y., Liang L., Yan M., Ge C., Yao J., Chen T., Wan D., Wang H., Gu J., Yao M., Li J., Tu H. and He X. (2010). Gain of miR-151 on chromosome 8q24.3 facilitates tumour cell migration and spreading through downregulating RhoGDIA. Nat. Cell Biol. 12, 390-399.

Dumont N. and Tlsty T.D. (2009). Reflections on miR-ing effects in metastasis. Cancer Cell 16, 3-4.

Gardner P.P. and Vinther J. (2008). Mutation of miRNA target sequences during human evolution. Trends Genet. 24, 262-265.

Garofalo M., Quintavalle C., Di Leva G., Zanca C., Romano G., Taccioli C., Liu C.G., Croce C.M. and Condorelli G. (2008). MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. Oncogene 27, 3845-3855.

He H., Jazdzewski K., Li W., Liyanarachchi S., Nagy R., Volinia S., Calin G.A., Liu C.G., Franssila K., Suster S., Kloos R.T., Croce C.M. and de la Chapelle A. (2005). The role of microRNA genes in papillary thyroid carcinoma. Proc. Natl. Acad. Sci. USA 102, 19075-19080.

Hornstein E., Mansfield J.H., Yekta S., Hu J.K., Harfe B.D., McManus M.T., Baskerville S., Bartel D.P. and Tabin C.J. (2005). The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development. Nature 438, 671-674.

Hua Z., Lv Q., Ye W., Wong C.K., Cai G., Gu D., Ji Y., Zhao C., Wang J., Yang B.B. and Zhang Y. (2006). MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS ONE 1, e116.

Huang Q., Gumireddy K., Schrier M., le Sage C., Nagel R., Nair S., Egan D.A., Li A., Huang G., Klein-Szanto A.J., Gimotty P.A., Katsaros D., Coukos G., Zhang L., Pure E. and Agami R. (2008). The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat. Cell Biol 10, 202-210.

Hutvagner G., McLachlan J., Pasquinelli A.E., Balint E., Tuschl T. and Zamore P.D. (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293, 834-838.

Ji Q., Hao X., Meng Y., Zhang M., Desano J., Fan D. and Xu L. (2008). Restoration of tumor suppressor miR-34 inhibits human p53-mutant

gastric cancer tumorspheres. BMC Cancer 8, 266.

Khew-Goodall Y. and Goodall G.J. (2010). Myc-modulated miR-9 makes more metastases. Nat. Cell Biol. 12, 209-211.

Kovalchuk O., Filkowski J., Meservy J., Ilnytskyy Y., Tryndyak V.P., Chekhun V.F. and Pogribny I.P. (2008). Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. Mol. Cancer Ther. 7, 2152-2159.

Kumar M.S., Lu J., Mercer K.L., Golub T.R. and Jacks T. (2007). Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat. Genet 39, 673-677.

Lagos-Quintana M., Rauhut R., Lendeckel W. and Tuschl T. (2001). Identification of novel genes coding for small expressed RNAs. Science 294, 853-858.

Lawrie C.H., Gal S., Dunlop H.M., Pushkaran B., Liggins A.P., Pulford K., Banham A.H., Pezzella F., Boultwood J., Wainscoat J.S., Hatton C.S. and Harris A.L. (2008). Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br. J. Haematol. 141, 672-675.

Lee R.C., Feinbaum R.L. and Ambros V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843-854.

Lee Y., Jeon K., Lee J.T., Kim S. and Kim V.N. (2002). MicroRNA maturation: stepwise processing and subcellular localization. EMBO J. 21, 4663-4670.

Lee Y., Yang X., Huang Y., Fan H., Zhang Q., Wu Y., Li J., Hasina R., Cheng C., Lingen M.W., Gerstein M.B., Weichselbaum R.R., Xing H.R. and Lussier Y.A. (2010). Network modeling identifies molecular functions targeted by miR-204 to suppress head and neck tumor metastasis. PLoS Comput Biol 6, e1000730.

Li X., Zhang Y., Ding J., Wu K. and Fan D. (2010). Survival prediction of gastric cancer by a seven-microRNA signature. Gut 59, 579-585.

Li Y., Vandenboom T.G. 2nd, Wang Z., Kong D., Ali S., Philip P.A. and Sarkar F.H. (2010). miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res 70, 1486-1495.

Llave C., Xie Z., Kasschau K.D. and Carrington J.C. (2002). Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science 297, 2053-2056.

Lu J., Getz G., Miska E.A., Alvarez-Saavedra E., Lamb J., Peck D., Sweet-Cordero A., Ebert B.L., Mak R.H., Ferrando A.A., Downing J.R., Jacks T., Horvitz H.R. and Golub T.R. (2005). MicroRNA expression profiles classify human cancers. Nature 435, 834-838.

Lwin T., Lin J., Choi Y.S., Zhang X., Moscinski L.C., Wright K.L., Sotomayor E.M., Dalton W.S. and Tao J. (2010). Follicular dendritic cell-dependent drug resistance of non-Hodgkin lymphoma involves cell adhesion-mediated Bim down-regulation through induction of microRNA-181a. Blood 116, 5228-5236.

Ma J., Dong C. and Ji C. (2010). MicroRNA and drug resistance. Cancer Gene Ther 17, 523-531.

Ma L. and Weinberg R.A. (2008). Micromanagers of malignancy: role of microRNAs in regulating metastasis. Trends Genet 24, 448-456.

Ma L., Teruya-Feldstein J. and Weinberg R.A. (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449, 682-688.

Ma L., Young J., Prabhala H., Pan E., Mestdagh P., Muth D., Teruya-Feldstein J., Reinhardt F., Onder T.T., Valastyan S., Westermann F., Speleman F., Vandesompele J. and Weinberg R.A. (2010). miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat. Cell Biol. 12, 247-256.

Miller T.E., Ghoshal K., Ramaswamy B., Roy S., Datta J., Shapiro C.L., Jacob S. and Majumder S. (2008). MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. J. Biol. Chem. 283, 29897-29903.

Mitchell P.S., Parkin R.K., Kroh E.M., Fritz B.R., Wyman S.K., Pogosova-Agadjanyan E.L., Peterson A., Noteboom J., O'Briant K.C., Allen A., Lin D.W., Urban N., Drescher C.W., Knudsen B.S., Stirewalt D.L., Gentleman R., Vessella R.L., Nelson P.S., Martin D.B. and Tewari M. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. USA 105, 10513-10518.

Nicoloso M.S., Spizzo R., Shimizu M., Rossi S. and Calin G.A. (2009). MicroRNAs--the micro steering wheel of tumour metastases. Nat. Rev. Cancer 9, 293-302.

Palatnik J.F., Allen E., Wu X., Schommer C., Schwab R., Carrington J.C. and Weigel D. (2003). Control of leaf morphogenesis by microRNAs. Nature 425, 257-263.

Pillai R.S., Bhattacharyya S.N., Artus C.G., Zoller T., Cougot N., Basyuk E., Bertrand E. and Filipowicz W. (2005). Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science 309, 1573-1576.

Reinhart B.J., Slack F.J., Basson M., Pasquinelli A.E., Bettinger J.C., Rougvie A.E., Horvitz H.R. and Ruvkun G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 403, 901-906.

Resnick K.E., Alder H., Hagan J.P., Richardson D.L., Croce C.M. and Cohn D.E. (2009). The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol. Oncol. 112, 55-59.

Rodriguez A., Griffiths-Jones S., Ashurst J.L. and Bradley A. (2004). Identification of mammalian microRNA host genes and transcription units. Genome Res. 14, 1902-1910.

Sachdeva M. and Mo Y.Y. (2010). MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. Cancer Res. 70, 378-387.

Santarpia L., Nicoloso M. and Calin G.A. (2010). MicroRNAs: a complex regulatory network drives the acquisition of malignant cell phenotype. Endocr. Relat. Cancer 17, F51-75.

Schmittgen T.D. (2010). miR-31: a master regulator of metastasis? Future Oncol. 6, 17-20.

Sengupta S., den Boon J.A., Chen I.H., Newton M.A., Stanhope S.A., Cheng Y.J., Chen C.J., Hildesheim A., Sugden B. and Ahlquist P. (2008). MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc. Natl. Acad. Sci. USA 105, 5874-5878.

Si M.L., Zhu S., Wu H., Lu Z., Wu F. and Mo Y.Y. (2007). miR-21-mediated tumor growth. Oncogene 26, 2799-2803.

Tabara H., Sarkissian M., Kelly W.G., Fleenor J., Grishok A., Timmons L., Fire A. and Mello C.C. (1999). The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. Cell 99, 123-132.

Tavazoie S.F., Alarcon C., Oskarsson T., Padua D., Wang Q., Bos P.D., Gerald W.L. and Massague J. (2008). Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451, 147-152.

Tian Y., Luo A., Cai Y., Su Q., Ding F., Chen H. and Liu Z. (2010). MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines. J. Biol. Chem. 285, 7986-7994.

Tie J., Pan Y., Zhao L., Wu K., Liu J., Sun S., Guo X., Wang B., Gang Y., Zhang Y., Li Q., Qiao T., Zhao Q., Nie Y. and Fan D. (2010).

MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor. PLoS Genet. 6, e1000879.

Valastyan S., Chang A., Benaich N., Reinhardt F. and Weinberg R.A. (2010). Concurrent suppression of integrin alpha5, radixin, and RhoA phenocopies the effects of miR-31 on metastasis. Cancer Res. 70, 5147-5154.

Valastyan S., Reinhardt F., Benaich N., Calogrias D., Szasz A.M., Wang Z.C., Brock J.E., Richardson A.L. and Weinberg R.A. (2009). A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 137, 1032-1046.

Valastyan S. and Weinberg R.A. (2010). miR-31: A crucial overseer of tumor metastasis and other emerging roles. Cell Cycle 9,

Venturini L., Battmer K., Castoldi M., Schultheis B., Hochhaus A., Muckenthaler M.U., Ganser A., Eder M. and Scherr M. (2007). Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood 109, 4399-4405.

Volinia S., Calin G.A., Liu C.G., Ambs S., Cimmino A., Petrocca F., Visone R., Iorio M., Roldo C., Ferracin M., Prueitt R.L., Yanaihara N., Lanza G., Scarpa A., Vecchione A., Negrini M., Harris C.C. and Croce C.M. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. USA 103, 2257-2261.

Voorhoeve P.M., le Sage C., Schrier M., Gillis A.J., Stoop H., Nagel R., Liu Y.P., van Duijse J., Drost J., Griekspoor A., Zlotorynski E., Yabuta N., De Vita G., Nojima H., Looijenga L.H. and Agami R. (2006). A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 124, 1169-1181.

Wang Q., Li Y.C., Wang J., Kong J., Qi Y., Quigg R.J. and Li X. (2008). miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. Proc. Natl. Acad. Sci. USA 105, 2889-2894.

Weiss G.J., Bemis L.T., Nakajima E., Sugita M., Birks D.K., Robinson W.A., Varella-Garcia M., Bunn P.A. Jr., Haney J., Helfrich B.A., Kato H., Hirsch F.R. and Franklin W.A. (2008). EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Ann. Oncol. 19, 1053-1059.

Wightman B., Ha I. and Ruvkun G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75, 855-862.

Wu Q., Jin H., Yang Z., Luo G., Lu Y., Li K., Ren G., Su T., Pan Y., Feng B., Xue Z., Wang X. and Fan D. (2010). MiR-150 promotes gastric cancer proliferation by negatively regulating the pro-apoptotic gene EGR2. Biochem. Biophys. Res. Commun. 392, 340-345.

Xia L., Zhang D., Du R., Pan Y., Zhao L., Sun S., Hong L., Liu J. and Fan D. (2008). miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. Int. J. Cancer 123, 372-379.

Zhang H., Li Y. and Lai M. (2010). The microRNA network and tumor metastasis. Oncogene 29, 937-948.

Zhang X., Liu S., Hu T., He Y. and Sun S. (2009). Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. Hepatology 50, 490-499.

Zhao J.J., Lin J., Yang H., Kong W., He L., Ma X., Coppola D. and Cheng J.Q. (2008). MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer. J. Biol. Chem. 283, 31079-31086.

Zheng T., Wang J., Chen X. and Liu L. (2010). Role of microRNA in anticancer drug resistance. Int. J. Cancer 126, 2-10.

Accepted May 20, 2011