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MicroRNAs as biomarkers and therapeutic drugs in human cancer

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Abstract
MicroRNAs (miRNAs) are evolutionarily conserved, endogenous, noncoding small RNAs that act as post-transcriptional gene regulators. Experimental evidence has shown that miRNAs can play roles as oncogenes or tumor suppressor genes, suggesting their contribution to cancer development and progression. Expression profiles of human miRNAs demonstrated that many miRNAs are deregulated in cancers and are differentially expressed in normal tissues and cancers. Therefore, miRNA profiling is used to create signatures for a variety of cancers, indicating that the profile will help further establish molecular diagnosis, prognosis and therapy using miRNAs. This paper introduces the aberrant expression of miRNAs in human cancer, and discusses the potential of these miRNAs as biomarkers and targets/molecules for molecular therapy.

Keywords: *Biomarker, cancer, microRNA, therapeutic drugs*

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Introduction

MicroRNAs (miRNAs, miRs) are endogenous noncoding ~22 base pair (bp) RNAs that suppress gene expression in a sequence-specific manner and are important in a wide range of physiologic and pathologic processes (Bartel 2004, Stefani & Slack 2008), although a few miRNAs (e.g., miR-369-3) are known to directly activate translation of target genes on cell cycle (Vasudevan et al. 2007). In the first miRNA, described in 1993, the *Caenorhabditis elegans* heterochronic gene *lin-4* encoded small RNAs with antisense complementarity to *lin-14* (Lee et al. 1993). Based on miRBase release 11.0, >600 human miRNAs have been registered, a number that is expected to increase up to 1,000 (Bentwich et al. 2005), with a large number being evolutionarily conserved (Pasquinelli et al. 2000). These miRNAs are predicted to regulate expression of ~90% of all human genes (Miranda et al. 2006). Moreover, Calin et al. reported that a single cluster of microRNAs (namely miR-15a/16-1

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cluster) has been found to regulate, directly or indirectly, ~14% of all human genome (Calin et al. 2008).

In mammalian cells, the primary transcript for miRNAs (pri-miRNA) is generated in the form of long, polycistronic messages by a type II polymerase (Cai et al. 2004, Lee et al. 2004). The pri-miRNAs have short and imperfectly base-paired stem loop structures that are processed in the nucleus by Drosha, an RNase III enzyme, into 60 – 110 bp called precursor miRNAs (pre-miRNA). Exportin 5 transports the pre-miRNA into the cytoplasm, where Dicer processes the molecule into double-stranded, 18 – 24 bp mature miRNAs (Ketting et al. 2001, Yi et al. 2003). The double-strand mature miRNA is composed of two complementary single-strand molecules, known as guide strand, which is integrated in the miRNA-protein complex (miRNP), and antiguide or passenger strand (marked as *), which is degraded as a miRNP complex substrate (Gregory et al. 2005). Perfect-to-near-perfect base pairing leads to degradation of the targeted mRNA. Alternatively, the miRNA can bind to the complementary sequence in the 3′ untranslated region (3′UTR) of target mRNAs (Bartel 2004, Liu et al. 2005, Saxena et al. 2003, Sen & Blau 2005). This base pairing subsequently causes degradation of the mRNA and/or inhibition of protein translation. The potential mechanisms underlying this process were recently reviewed (Pillai et al. 2007). Protein levels of the target gene are consequently reduced, whereas mRNA levels may or may not be decreased. In humans, miRNAs mainly inhibit protein translation of their target genes and only infrequently cause degradation or cleavage of the mRNA (Bartel 2004).

Expression of miRNA is highly specific for tissues and developmental stages, and its functions have been appreciated in various fundamental biological processes such as cell proliferation (Hayashi et al. 2005), stem cell division (Zhang et al. 2006) and apoptosis (Jovanovic & Hangartner 2006). Recently, it has been revealed that altered expression of specific miRNA genes contributes to the initiation and progression of cancer (Calin et al. 2002, Calin et al. 2004a, He et al. 2005, Lu et al. 2005).

In this review, we introduce aspects of aberrant expression of miRNAs in human cancer tissues, and describe the potential for miRNAs as diagnostic markers and molecules for therapy in human cancers.

miRNAs as biomarkers to diagnose human cancer

New approaches that can complement and improve on current strategies for cancer detection are urgently needed. Many independent studies on different tissues have demonstrated that cancer cells have different miRNA profiles compared with normal cells (Table I), suggesting that miRNA expression could be used for the diagnosis of cancer. In a report by Chan et al. (2005), expression of miR-21 was evaluated by northern blotting and membrane array in human brain tumors. The expression level was shown to have increased 5- to 100-fold in human glioblastoma multiforme tissue compared with control non-neoplastic brains. Furthermore, they found a similarly robust increase in miR-21 expression in six commonly used model cell lines derived from human glioblastomata. Takamizawa et al. showed by northern blotting the frequent occurrence (43.8%) of a significant reduction ($>80.0\%$) in let-7 miRNA expression in lung cancers when compared with that in corresponding normal lung tissues (Takamizawa et al. 2004). As shown in Table I, upregulation of miR-21 and downregulation of let-7 were reported not only in glioblastoma or lung cancer,

Table I. Cancer-associated microRNAs and their potential diagnostic markers.

Cancer type	miRNAs		Ref.
	upregulated	downregulated	
Brain, GBM	miR-21, 221	miR-128, 181	Chan et al 2005, Takeshita et al. 2005
Breast Ca	miR-9-1, 10b, 17-5p21, 21, 29b-2, 34, 146, 155, 181b-1, 213	let-7, miR-15a, 16, 125a, 125b, 127, 145, 204	Calin & Croce 2006, Saito et al. 2006, Takamizawa et al. 2004, Volinia et al. 2006, Zhang et al. 2007a
Lung Ca	miR-17-5p, 17-92, 21, 24-2, 106a, 128b, 146, 150, 155, 191, 192, 197, 199a-1, 203, 205, 210, 212, 214	let-7, miR-9, 26a-1-p, 27b, 29b-2, 32, 33, 30a-5p, 95, 101-1, 124, 124a-3, 125a, 125a-p, 126, 140, 143, 145, 181c-p, 198, 192-p, 199b-p, 216-p, 218-2, 219-1, 220, 224	Takamizawa et al. 2004, Volinia et al. 2006, Yanaihara et al. 2006
Esophageal Ca	miR-21, 93	miR-203, 205	Feber et al. 2008
Gastric Ca	miR-21, 24-1, 24-2, 25, 92-2, 107, 191, 214, 221, 223	let-7	Volinia et al. 2006, Zhang et al. 2007b
Colorectal Ca	miR-17-5p, 20a, 21, 24-1, 24-2, 29b-2, 30c, 31, 32, 96, 106a, 107, 128b, 135b, 155, 183, 191, 221, 223	let-7, miR-34, 127, 133b, 143, 145	Bandres et al. 2006, Gramantieri et al. 2007, Michael et al. 2003, Volinia et al. 2006
Hepatocellular Ca	miR-15b, 18a, 21, 106b, 221, 222, 224	let-7, miR-101, 122a, 125a, 195, 199a, 200a	Gramantieri et al. 2007, Meng et al. 2007, Murakami et al. 2006
Pancreatic Ca	miR-17-5p, 20a, 21, 24-1, 24-2, 25, 29b-2, 30c, 32, 92-2, 100, 106a, 107, 125b, 128b, 146, 155, 181a, 181b-1, 191, 196a, 196b, 199a-1, 212, 214, 221, 223, 301, 376a	miR-139, 142-p, 345, 375	Volinia et al. 2006,32,65
Prostate Ca	miR-17-5p, 20a, 21, 25, 30c, 32, 92-2, 106a, 146, 181b-1, 191, 199a-1, 214,223	miR-15a, 16, 143, 145, 218-2	Volinia et al. 2006
Cervical Ca	miR-21, 199a	miR-143, 145	Esquela-kerscher & Slack 2006, Lui et al. 2007
CLL	miR-17-92, 155	miR-15a, 16, 143, 145, 192, 213, 220	Akao et al. 2007, Cailin et al. 2002, Calin et al. 2005

Ref, references; GBM, glioblastoma maltiforme; Ca, cancer; B-CLL, B cell chronic lymphocytic leukemia.

respectively, but also in a variety of human cancers, suggesting that overexpression of miR-21 and/or underexpression of let-7 might be common markers in cancer diagnosis.

Large profiling studies using tumor tissues have proven the utility of miRNA profiling for diagnosis. Microarray analysis is the most comprehensive method for

disclosing expression levels of miRNAs and has been invaluable in evaluating human cancers. Iorio et al. (2005) demonstrated the existence of a breast-cancer-specific miRNA signature with a genome-wide miRNA expression profiling in a large set of normal and tumor breast tissues, in which 29 miRNAs were differentially expressed in breast cancer versus normal tissues. Among them, confirmed by northern blot, miR-21 and miR-155 were upregulated, whereas miR-10b, miR-125b and miR-145 were downregulated, suggesting that these miRNAs may potentially be diagnostic markers. The same group further examined the miRNA expression signature of human solid tumors including breast cancer. The authors identified 27 miRNAs that were differentially expressed in breast cancer versus adjacent normal tissues, that is, 15 miRNAs were upregulated and 12 were downregulated in breast cancer versus normal tissues (Volinia et al. 2006).

In lung cancer, Yanaihara et al. confirmed a unique miRNA expression profile using microarray (Yanaihara et al. 2006). Forty-three miRNA were found to be up- or downregulated and could accurately distinguish non-tumoral versus cancer tissues (Table I), even in Stage I cancer. Moreover, they also found a molecular signature for subsets of lung cancer.

Murakami et al. reported that three miRNAs were upregulated and five were downregulated in hepatocellular carcinomas (HCCs) compared to non-tumoral adjacent regions using microarray technology (Murakami et al. 2006). Hepatocellular carcinoma was distinguishable with high accuracy by the miRNA expression pattern from non-tumoral tissue including liver cirrhosis. Moreover, they also reported that the expression level was correlated with differentiation of the tumor. However, they were unable to find differences between hepatitis-B-positive and hepatitis-C-positive samples. Subsequently, Li et al. reported that eight miRNAs were differentially expressed in HCC compared to adjacent non-tumoral tissues (Li et al. 2007). Among them, however, only one in each report was reproducible. This discrepancy may reflect the application of different miRNA platforms, various techniques and the varieties of clinical backgrounds of the resected samples.

Bloomston et al. identified global expression patterns in 65 pancreatic ductal adenocarcinomas with matched benign adjacent pancreas and 42 chronic pancreatitis tissues (Bloomston et al. 2008). In pancreatic cancers, 21 miRNAs were differentially overexpressed and 4 were underexpressed relative to chronic pancreatitis and adjacent benign pancreas tissue. Szafranska et al. also reported miR-196a and miR-196b were overexpressed in pancreatic ductal adenocarcinomas but not in non-tumoral tissues including pancreatitis (Szafranska et al. 2007).

Calin et al. reported genome-wide expression profiling of miRNAs in 38 human chronic lymphocyte leukemia (CLL) samples (Calin et al. 2004b). Expression levels of miR-15a and miR-16, encoded within the 13q14 region, a deletion found in $>65\%$ of CLL cases, were downregulated in 75% of CLL cases that harbored this chromosomal abnormality. Inversely, He et al. reported that the miR-17-92 polycistron, which is located in 13q31–32, a region commonly amplified in B cell lymphoma, was upregulated in 65% of the B cell lymphoma patients (Calin et al. 2004a). These findings highlighted the potential of miRNA profiling in cancer diagnosis.

Detection of miRNA has also been applied to early detection of cancer or monitoring of cancer recurrence using peripheral blood. Mitchell et al. identified

miR-141 from serum samples as a specific marker of human prostate cancer (Mitchell et al. 2008). They found that serum levels of miR-141 were, in general, substantially higher in cancer patients ($n = 25$) compared with the healthy control group ($n = 25$). Furthermore, serum levels of miR-141 were able to detect individuals with cancer with 60% sensitivity at 100% specificity. Another study, reported by Taylor et al., demonstrated that 8 miRNAs (miR-21, -141, -200a, -200b, -200c, -203, -205 and -214) detected from peripheral blood were also identified as markers of ovarian cancer (Taylor et al. 2008). In addition, they revealed that these circulating miRNAs in the blood were incorporated in tumor-derived exosomes, which were small (50 – 100 nm) membrane vesicles of endocytic origin. Chen et al. recently reported that the expression levels of two miRNAs (miR-25 and miR-223) in serum are significantly higher in 152 non-small cell lung cancer patients than in 75 healthy volunteers (Chen et al. 2008). Interestingly, the authors revealed that serum miRNAs are resistant to RNase A digestion, treatment of HCl (pH = 1) and NaOH (pH = 13) and 10-cycle repeats of freeze–thaw, indicating that serum miRNAs are stable even after drawing blood, which is very important for a reliable biomarker.

Taken together, it is suggested that detection of cancer-associated specific miRNAs in each malignant tumor from tissue sample and/or serum will contribute to early detection and accurate diagnosis for cancer. Therefore, the miRNAs shown in Table I should be robust targets in the development of a molecular diagnosis of cancer, although further studies are needed to evaluate which miRNAs would be valid diagnostic markers in each cancer.

miRNAs in cancer classification and prognosis

Lu et al. asked the question whether global miRNA expression profiles could classify human cancer. In reply to this question, they used a bead-based flow cytometric miRNA expression profiling method to present a systematic expression analysis of miRNAs from human cancer tissue specimens (Lu et al. 2005). The authors established and applied miRNA expression profiles to an independent series of 17 poorly differentiated tumors of unknown origin. Based on the differential expression of 217 miRNAs, a correct diagnosis could be established in 12 out of 17 of the tumors. In contrast, when the same samples were profiled by expression of 16,000 mRNAs, they did not accurately classify the tumors. It is suggested that a small number of miRNAs define a cancer better than expression data from a large number of mRNAs. Volinia et al. also described a large-scale microarray analysis in 540 samples from 6 solid types of the most frequent human cancers (lung, breast, stomach, prostate, colon and pancreatic cancers) and found a common signature composed of 57 miRNAs (Volinia et al. 2006). In this study, out of the 228 miRNAs analyzed, 36 were overexpressed and 21 were underexpressed in cancer versus normal cells. Hierarchical clustering analysis showed that such an miRNA signature enabled the tumor samples to be classified based on their tissue of origin.

There is emerging evidence that miRNAs can be used for cancer prognosis (Table II). In the majority of CLL patients, the prognosis is relatively good and the treatment after diagnosis is started only if poor prognostic markers are evident. By performing an miRNA profiling screen on 144 CLL patients, a unique signature of 13 miRNAs was

Table II. Cancer-associated microRNAs and their poor prognostic markers

	miRNAs		
Cancer type	upregulated	downregulated	Ref.
Brain, GBM	miR-10b, 21, 221	miR-128, 181	Ciafre et al. 2005, Yanaihara et al. 2005
Breast Ca	miR-21, 155	miR-125,145	Bandres et al. 2006a, Iorio et al. 2005, Volinia et al. 2006
Lung Ca	miR-17-3p, 93, 106a, 155	let-7, miR-145,	Takamizawa et al. 2004, Yanaihara et al. 2006
Colorectal Ca	miR-21, 31, 96, 135b, 183	miR-133b, 143, 145	Akao et al. 2006, Bandres et al. 2006b, Cummins et al. 2006, Schetter et al. 2008
Hepatocellular Ca	miR-18, 224	miR-122, 125a, 195, 199a, 200a	Kutay et al. 2006, Murakami et al. 2006
Pancreatic Ca	miR-21, 103, 107, 196a	miR-204	Bloomston et al. 2007, Roldo et al. 2006
CLL	miR-21, 23a, 23b, 24-2, 146, 150, 155, 181a, 221	miR-15a, 16, 29c, 192, 222	Calin et al. 2002, Calin et al. 2005, Eis et al. 2004, Fulci et al. 2007

Ref, references; GBM, glioblastoma maltiforme; Ca, cancer; CLL, B cell chronic lymphocytic leukemia.

shown to differentiate cases on the basis of a good or bad prognosis or on the presence or absence of disease progression (Calin et al. 2005). Among them, miR-16 and miR-15a were expressed at lower levels in patients with a good prognosis, in agreement with early reports that 13q14.3 genomic deletions at the locus harboring these genes are related with a favorable course of the disease. Iorio et al. reported that miRNA expression profiling in breast cancer has also demonstrated prognostic value (Iorio et al. 2005). Analysis of 76 primary breast cancer samples demonstrated a consistently altered expression, including decreased miR-125b and -145, and increased miR-21 and -155. More significantly, most differentially expressed miRNAs were associated with invasive breast cancer biopathological features, including estrogen and progesterone receptor expression, tumor stage, vascular invasion or proliferation index.

Yanaihara et al. determined miRNA expression with 352 miRNA probes in 144 lung cancer tumor–normal pairs. Malignant lung tissues were consistently differentiated from normal tissues, according to the expression profile of 43 miRNAs. Further, univariant regression analysis identified 8 miRNAs (miRs-17-3p, -21, -93, -106a, -145, -155, let-7a-2 and let-7b) as independent survival prognosticators in lung adenocarcinoma (Yanaihara et al. 2006). In particular, reduced expression of let-7 and high expression of miR-155 in human lung cancers were associated with significant poor survival. Thus, these miRNAs may be used to identify those patients for closer monitoring and adjunct therapy.

In pancreatic cancer, a subset of six miRNAs was shown to be predictive of long-term survival in lymphnode-positive patients who show a poor prognosis. On the other hand, overexpression of miR-196a was associated with a poorer prognosis (Bloomston et al. 2007). Further correlations with existing biomarkers and miRNA expression are likely to improve our understanding of the prognostic relevance of miRNA profiling in human cancers.

If miRNAs prove useful for pathological diagnosis, their key advantage might be their high stability. In contrast to most mRNAs, miRNAs are long-lived *in vivo* (Lim et al. 2005) and very stable *in vitro* (Tang et al. 2006), which might allow an analysis of paraffin-embedded samples for routine diagnostic applications. In fact, Rosenfeld et al. reported that miRNAs, which were extracted from 333 formalin-fixed paraffin-embedded (FFPE) archival human cancer tissue samples, were used to identify cancer tissue origin by measuring the miRNA expression levels using an miRNA microarray (Rosenfeld et al. 2008). Two-thirds of the samples were classified with high confidence, with >90% accuracy. These data strongly suggested that identification of miRNAs expression profiling might be useful for diagnosis as biomarkers, even if miRNAs are from FFPE samples. Identification of miRNAs expression profiling using FFPE samples is considered to be effective, especially in histological diagnosis of metastatic cancer of an unknown primary lesion because a delayed diagnosis fails to prompt appropriate curatives and leads to a poor prognosis.

Application of miRNA for cancer therapy

It has been reported that miRNAs play a crucial role in the initiation and progression of human cancer. Deregulation of miRNAs is associated with genetic or epigenetic alterations, including deletion, amplification, point mutation and aberrant DNA methylation (Calin & Croce 2006). Calin et al. first made the connection between microRNAs and cancer by showing that miR-15 and miR-16 are located at 13q14 within a 30kb region of loss in CLL, and that both genes were deleted or downregulated in ~68% of CLL cases (Calin et al. 2002). More than 50% of miRNAs are located in cancer-associated genomic regions or in fragile sites (Calin et al. 2004a). Toyota et al. reported that miR-34b and miR-34c, two components of the p53 network, are epigenetically silenced in colorectal cancer, whereas 5-aza-2′-deoxycytidine treatment rapidly restores miR-34b/c expression (Toyota et al. 2008). Moreover, Lujambio et al. revealed that reintroduction of miR-34b/c in human cancer cells with epigenetic inactivation reduced tumor growth and inhibited cell motility and metastasis formation in xenograft model (Lujambio et al. 2008). Therefore, inhibitors of DNA methylation could induce expression of some miRNAs that may act as tumor suppressors. These results suggested that aberrant expression of miRNAs might be important in human cancer pathogenesis and these miRNAs might be targets for cancer therapy.

The most direct way for molecules to correct altered miRNA–mRNA interactions is RNA oligonucleotides. Therapeutic potentials using these RNA oligonucleotides have been proposed, although our understanding of the miRNAs' role in cancer is still very limited. There are two possible approaches: blocking oncogenic miRNAs (oncomiRs) or overexpressing miRNAs with tumor suppressor activity. Theoretically, antimiRNA oligonucleotides (AMOs) can be used to suppress miRNA activity if the AMOs can bind strongly to the miRNA and are stable enough in physiological conditions (Krutzfeldt et al. 2007). AntimiRNA oligonucleotides have complementary sequences to miRNAs and contain several chemical modifications to achieve that goal. Two types of modifications were developed to attain strong

binding: 2′-O-methylation of RNA nucleotides (Krutzfeldt et al. 2005, Hutvagner et al. 2004) and locked nucleic acid (LNA) DNA nucleotides (Vester et al. 2004). These modifications are also used in the opposite approach where modified oligonucleotides are delivered into cells that underexpress miRNAs with tumor suppressor activity. This approach showed promising results in cell culture (Johnson et al. 2005) and needs to be tested in animal models. It is important for *in vivo* trials that these oligonucleotides be chemically modified to allow for stability in serum and cellular uptake. Modified antisense oligonucleotides are already being developed to utilize the intrinsic RNAi pathway for delivery of a gene therapy. Krutzfeldt et al. demonstrated that modified cholesterol-conjugated antisense RNAs designated 'antagomiRs' could effectively inhibit miRNA function *in vivo* in the adult mouse (Krutzfeldt et al. 2005). The authors applied three daily i.v. injections of antagomiRs and achieved effective inhibition of four miRNAs over a period of weeks in most tissues.

Not only RNA oligonucleotides' modification but also a drug delivery system (DDS) of nucleic acid including siRNA using atelocollagen has been reported by our group (Ochiya et al. 1999, Ochiya et al. 2001). Atelocollagen was the first biomaterial with the potential for use as a carrier for gene delivery. Atelocollagen is liquid at low temperature, making admixing of nucleic acid solutions easy. Because the surface of atelocollagen molecules is positively charged, the molecules can bond electrostatically with negatively charged nucleic acid molecules. Tazawa et al. demonstrated in a mice model that direct intratumoral injection of an miR-34a/atelocollagen complex successfully suppressed the growth of tumors derived from human colon cancer cells (HCT116 and RKO) (Tazawa et al. 2007). Furthermore, significant reduction of the tumor volume was observed until day 6 after miR-34a administration. Interestingly, the authors showed that expression of miR-34a was downregulated in more than one-third of human colon cancers compared with counterpart normal colon mucosae. Therefore, these data suggested that restoring decreased miRNAs into cancer cells could suppress progression of cancer *in vivo*.

One problem for systemic treatment *in vivo* is that some serum RNases can degrade RNA oligonucleotide. However, siRNA, probably as well as miRNA, complexed with atelocollagen is resistant to nucleases and is transduced efficiently into cells, thereby allowing long-term gene silencing (Minakuchi et al. 2004). In fact, we previously reported the usefulness of atelocollagen DDS in systemic delivery of synthetic siRNA for cancer treatment (Takeshita et al. 2005). An siRNA/atelocollagen complex was administered through the tail vein of mice with bone metastasis of prostate cancer cells to examine the delivery of the siRNA to the systemic metastasis foci. The siRNA/atelocollagen was able to be efficiently delivered to tumors 24 h after injection and was able to exist intact for $\geq$3 days. Furthermore, atelocollagen-mediated systemic administration of siRNAs such as enhancer of zeste homolog 2 and phosphoinositide 3′-hydroxykinase p110-α-subunit, which were selected as candidate targets for inhibition of bone metastasis, resulted in an efficient inhibition of metastatic tumor growth in bone tissues. These results suggest that miRNA/atelocollagen could also be available for systemic delivery to suppress tumor growth and to inhibit cancer metastasis.

The great challenge for these approaches is the specific delivery of functional oligonucleotides into a tumor tissue. As these molecules are equally active in both healthy and cancer cells their side effects must be minimized before they can be considered for clinical trials.

Conclusion

miRNA has emerged in the field of cancer research in recent years. Many aberrant expressions of miRNA have been reported in a variety of human malignancies. Although the potential of miRNA as a diagnostic and/or prognostic marker in cancer has been identified, further studies are needed to identify and validate useful miRNAs in each cancer. Evaluation of the potential for miRNAs as diagnostic and prognostic

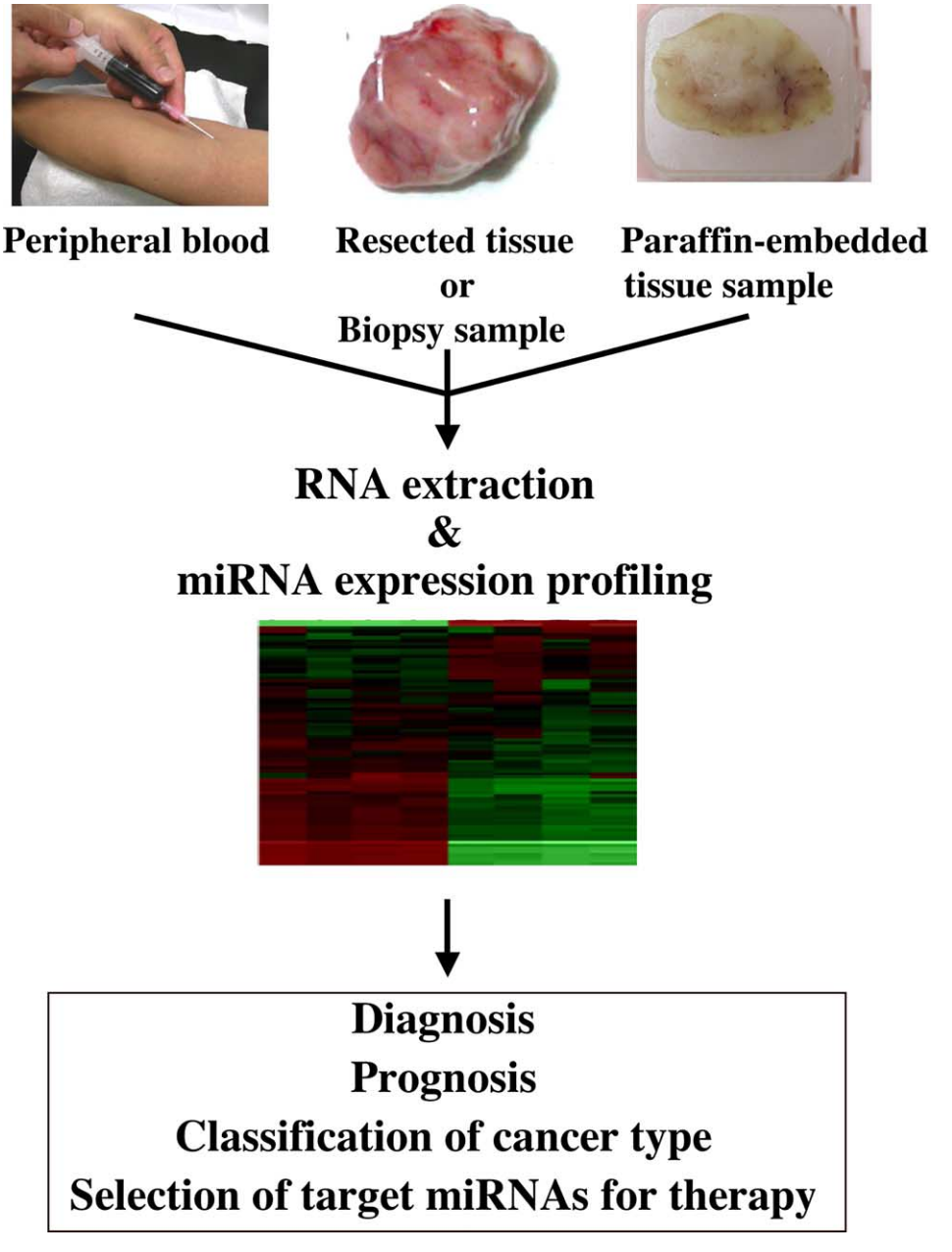

Figure 1. Clinical application of miRNA expression profiling.

markers or therapeutic molecules or targets has just begun. Therefore, not only retrospective studies but also prospective ones using clinical samples will serve to identify relevant markers. Moreover, the development of animal models will be useful for *in vivo* evaluation of miRNA molecules as anticancer drugs.

Discussion

In recent years, much evidence on miRNAs has accumulated in the field of cancer research. How will miRNAs in cancer diagnosis and therapy be applied in the future? Our prediction is illustrated in Figure 1. A rapid and accurate cancer diagnosis and prognosis will be possible by examining miRNA expression profiling using tissue specimens, even if they are biopsy samples, formalin-fixed samples and various fluids. Moreover, abnormal expression of miRNA might be detected from a peripheral blood sample as well as conventional tumor markers such as CEA (carcinoembryonic antigen), CA19-9 and PSA (prostate-specific antigen). From the point of cancer therapy, it might be possible to correct the abnormal expression of miRNAs associated with cancer by injecting tumor suppressor miRNAs or molecules inhibiting oncomiRs. The combination of miRNAs to be injected will depend on the miRNA expression profiling of each cancer patient, which indicates the possibility for ultimate personalized medicine. It will be also possible to use miRNAs in combination with chemotherapy and radiotherapy to attenuate drug resistance of cancer cells and/or to relieve side effect to normal tissue.

In this review, although we described the possibilities of miRNAs on diagnosis, prognosis and therapy in cancer, there might be another possibility of using miRNA expression data for cancer prevention. miRNAs will bring about many revolutionary changes in cancer diagnosis, therapy and prevention.

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References

Akao Y, Nakagawa Y, Naoe T. 2006. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biological and Pharmaceutical Bulletin 29:903–906.

Akao Y, Nakagawa Y, Kitade Y, et al. 2007. Downregulation of microRNAs-143 and -145 in B-cell malignancies. Cancer Science 98:1914–1920.

Bandres E, Cubedo E, Agirre X, et al. 2006a. Identification by real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Molecular Cancer 5:29.

Bandres E, Cubedo E, Agirre X, et al. 2006b. Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Molecular Cancer 5:29.

Bartel DP. 2004. MicroRNAs: Genomics biogenesis, mechanism, and function. Cell 116:281–297.

Bentwich I, Avniel A, Karov Y, et al. 2005. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37:766–770.

Bloomston M, Frankel WL, Petrocca F, et al. 2007. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. Journal of the American Medical Association 297:1901–1908.

Cai X, Hagedorn CH, Cullen BR. 2004. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 12:1957–1966.

Calin GA, Dumitru CD, Shimizu M, et al. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences USA 99:15524–15529.

Calin GA, Croce CM. 2006. MicroRNA signatures in human cancers. Nature Reviews Cancer 6:857–866.

Calin GA, Sevignani C, Dumitru CD, et al. 2004a. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences USA 101:2999–3004.

Calin CA, Liu CG, Sevignani C, et al. 2004b. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proceedings of the National Academy of Sciences USA 101: 11755–11760.

Calin GA, Ferracin M, Cimmino A, et al. 2005. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. New England Journal of Medicine 353:1793–1801.

Calin GA, Cimmino A, Fabbri M, et al. 2008. MiR-15a and miR-16-1 cluster functions in human leukemia. Proceedings of the National Academy of Sciences USA 105:5166–5171.

Chan JA, Krichevsky AM, Kosik KS. 2005. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Research 65:6029–6033.

Chen X, Ba Y, Ma L, et al. 2008. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Research (In Press).

Ciafre SA, Galardi S, Mangiola A, et al. 2005. Extensive modulation of a set of microRNAs in primary glioblastoma. Biochemical and Biophysical Research Communications 334:1351–1358.

Cummins JM, He Y, Leary RJ, et al. 2006. The colorectal microRNAome. Proceedings of the National Academy of Sciences USA 103:3687–3692.

Eis PS, Tam W, Sun L, et al. 2005. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proceedings of the National Academy of Sciences USA 102:3627–3232.

Esquela-kerscher A, Slack FJ. 2006. Oncomirs-microRNAs with a role in cancer. Nature Reviews Cancer 6:259–269.

Feber A, Xi L, Luketich JD, et al. 2008. MicroRNA expression profiles of esophageal cancer. Journal of Thoracic and Cardiovascular Surgery 135:255–260.

Fulci V, Chiaretti S, Goldoni M, et al. 2007. Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. Blood 109:4944–4951.

Gramantieri L, Ferracin M, Fornari F, et al. 2007. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Research 67:6092–6099.

Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R.. 2005. Human risc couples microRNA biogenesis and posttranscriptional gene silencing. Cell 123:631–640.

Hayashi Y, Osada H, Tatematsu Y, et al. 2005. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Research 65:9628–9632.

He L, Thomson JM, Hemman MT, et al. 2005. A microRNA polycistron as a potential human oncogene. Nature 435:828–833.

Hutvagner G, Simard MJ, Mello CC, Zamore PD. 2004. Sequence-specificinhibition of small RNA function. PLoS Biology 2:E98.

Iorio MV, Ferracin M, Liu CG, et al. 2005. MicroRNA gene expression deregulation in human breast cancer. Cancer Research 65:7065–7070.

Johnson SM, Grosshans H, Shingara J, et al. 2005. RAS is regulated by the let-7 microRNA family. Cell 120:635–647.

Jovanovic M, Hangartner MO. 2006. MiRNA and apoptosis: RNAs to die for. Oncogene 25:6176–6187.

Ketting RF, Fischer SE, Bernstein E, et al. 2001. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes and Development 15:2654–2659.

Krutzfeldt J, Kuwajima S, Braich R, et al. 2007. Specificity, duplex degradation and subcellular localization of antagomirs. Nucleic Acids Research 35:2885–2892.

Krutzfeldt J, Rajewsky N, Braich R, et al. 2005. Silencing of microRNAs in vivo with 'antagomirs'. Nature 438:685–689.

Kutay H, Bai S, Datta J, et al. 2006. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. Journal of Cellular Biochemistry 99:671–678.

Lee EJ, Gusev Y, Jiang J, et al. 2006. Expression profiling identifies microRNA signature in pancreatic cancer. International Journal of Cancer 120:1046–1054.

Lee RC, Feinbaum RL, Ambros V. 1993. The *C.elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854.
Lee Y, Kim M, Han J, et al. 2004. MicroRNA genes are transcribed by RNA polymerase II. EMBO Journal 23:4051–4060.
Li W, Xie L, He X, et al. 2008. Diagnostic and prognositic implications of microRNAs in human hepatocellular carcinoma. International Journal of Cancer 123:1616–1622.
Lim LP, Lau NC, Garrett-Engele P, et al. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433:769–773.
Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. 2005. MicroRNA-dependent localization of targeted mRNAs to mammalian p-bodies. Nature Cell Biology 7:719–723.
Lu J, Getz G, Miska EA, et al. 2005. MicroRNA expression profiles classify human cancers. Nature 435:834–838.
Lui WO, Pourmand N, Patterson BK, Fire A. 2007. Patterns of known and novel small RNAs in human cervical cancer. Cancer Research 67:6031–6043.
Lujambio A, Calin GA, Villanueva A, et al. 2008. A microRNA DNA methylation signature for human cancer metastasis. Proceedings of the National Academy of Sciences USA 105:13556–13561.
Meng F, Henson R, Wehbe-Janek H, et al. 2007. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 133:647–658.
Michael MZ, O'Connor SM, van Holst Pellekaan NG, et al. 2003. Reduced accumulation of specific microRNAs in colorectal neoplasia. Molecular Cancer Research 1:882–891.
Minakuchi Y, Takeshita F, Kosaka N, et al. 2004. Atelocollagen-mediated synthetic small interfering RNA delivery for effective gene silencing in vitro and in vivo. Nucleic Acids Research 32:e109.
Miranda KC, Huynh T, Tay Y, et al. 2006. A pattern-based method for the identification of microRNA binding sites and their corresponding heteroduplexes. Cell 126:1203–1217.
Mitchell PS, Parkin RK, Kroh EM, et al. 2008. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences USA 105:10513–10518.
Murakami Y, Yasuda T, Saigo K, et al. 2006. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 25:2537–2545.
Ochiya T, Nagahara S, Sano A, et al. 2001. Biomaterials for gene delivery: atelocollagen-mediated controlled release of molecular medicines. Current Gene Therapy 1:31–52.
Ochiya T, Takahama Y, Nagahara S, et al. 1999. New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet. Nature Medicine 5:707–710.
Pasquinelli AE, Reinhart BJ, Slack F, et al. 2000. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 408:86–89.
Pillai RS, Bhattacharyya SN, Filipowicz W. 2007. Repression of protein synthesis by miRNAs: how many mechanisms? Trends in Cell Biology 17:118–126.
Roldo C, Missiaglia E, Hagan JP, et al. 2006. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. Journal of Clinical Oncology 24:4677–4684.
Rosenfeld N, Aharonov R, Meiri E, et al. 2008. MicroRNA accurately identify cancer tissue origin. Nature Biotechnology 26:462–469.
Saito Y, Liang G, Egger G, et al. 2006. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL-6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 9:435–443.
Saxena S, Jonsson ZO, Dutta A. 2003. Small RNAs with imperfect match to endogenous mRNA repress translation. Implications for off-target activity of small inhibitory RNA in mammalian cells. Journal of Biological Chemistry 278:44312–44319.
Schetter AJ, Leung SY, Sohn JJ, et al. 2008. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. Journal of the American Medical Association 299: 425–436.
Sen GL, Blau HM. 2005. Argonaute 2/risc resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nature Cell Biology 7:633–636.
Stefani G, Slack FJ. 2008. Small noncoding RNAs in animal development. Nature Reviews Molecular Cell Biology 9:219–230.
Szafranska AE, Davison TS, John J, et al. 2007. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic duvtal adenocarcinoma. Oncogene 26: 4442–4452.
Takamizawa J, Konishi H, Yanagisawa K, et al. 2004. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Research 64:3753–3756.

Takeshita F, Minakuchi Y, Nagahara S, et al. 2005. Efficient delivery of small interfering RNA to bone-metastatic tumors by using atelocollagen in vivo. Proceedings of the National Academy of Sciences USA 102:12177–12182.

Tang F, Hajkova P, Barton SC, et al. 2006. MicroRNA expression profiling of single whole embryonic stem cells. Nucleic Acids Research 34:e9.

Taylor DD, Gercel-Taylor C. 2008. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncology 110:13–21.

Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. 2007. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proceedings of the National Academy of Sciences USA 104:15472–15477.

Toyota M, Suzuki H, Sasaki Y, et al. 2008. Epigenetic scilencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Research 68:4123–4132.

Vasudevan S, Tong Y, Steitz JA. 2007. Switching from repression to activation: MicroRNAs can up-regulate translation. Science 318:1931–1934.

Vester B, Wengel J. 2004. LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. Biochemistry 43:13233–13241.

Volinia S, Calin GA, Liu CG, et al. 2006. A microRNA expression signiture of human solid tumors defines cancer gene targets. Proceedings of the National Academy of Sciences USA 103:2257–2261.

Yanaihara N, Caplen N, Bowman E, et al. 2006. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 9:189–198.

Yi R, Qin Y, Makara IG, Curren BR. 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes and Development 17:3011–3016.

Zhang B, Pan X, Anderson TA. 2006. MicroRNA: a new player in stem cells. J Cell Physiol 209:266–269.

Zhang B, Pan X, Cobb GP, Anderson TA. 2007a. microRNAs as oncogenes and tumor suppressors. Developmental Biology 302:1–12.

Zhang HH, Wang XJ, Li GX, et al. 2007b. Detection of let-7a microRNA by real-time PCR in gastric carcinoma. World Journal of Gastroenterology 13:2883–2888.