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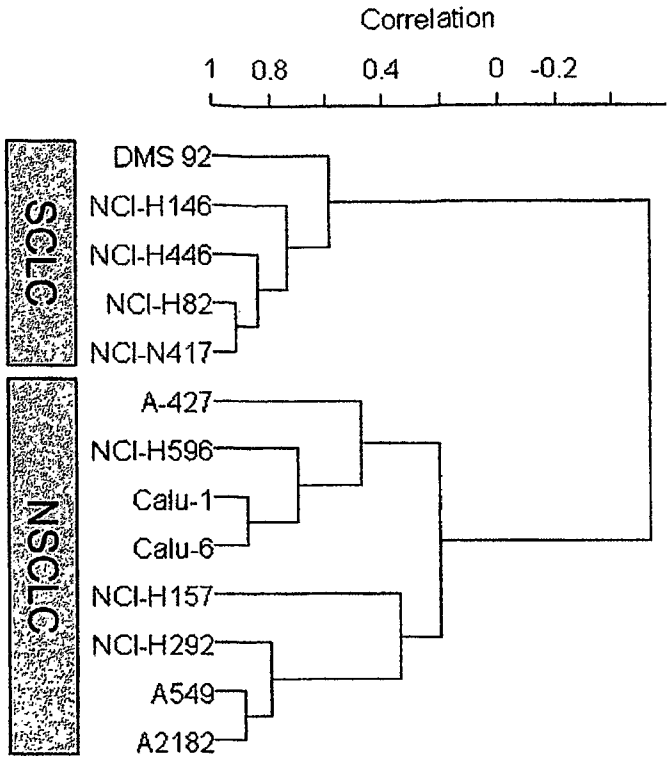
(54) **Title:** MICRORNA-BASED METHODS AND COMPOSITIONS FOR THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF LUNG CANCER



(57) **Abstract:** The present invention provides novel methods and compositions for the diagnosis, prognosis and treatment of lung cancer. The invention also provides methods of identifying anti-lung cancer agents.

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TITLE

MicroRNA-BASED METHODS AND COMPOSITIONS FOR THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF LUNG CANCER

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GOVERNMENT SUPPORT

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BACKGROUND OF THE INVENTION

Lung cancer causes more deaths worldwide than any other form of cancer (Goodman, G.E., *Thorax* 57:994-999 (2002)). In the United States, lung cancer is the primary cause of cancer death among both men and women. In 2002, the death rate from lung cancer was an estimated 134,900 deaths. Lung cancer is also the leading cause of cancer death in all European countries, and numbers of lung cancer-related deaths are rapidly increasing in developing countries as well.

The five-year survival rate among all lung cancer patients, regardless of the stage of disease at diagnosis, is only about 13%. This contrasts with a five-year survival rate of 46% among cases detected while the disease is still localized. However, only 16% of lung cancers are discovered before the disease has spread. Early detection is difficult as clinical symptoms are often not observed until the disease has reached an advanced stage. Currently, diagnosis is aided by the use of chest x-rays, analysis of the type of cells contained in sputum and fiberoptic examination of the bronchial passages. Treatment regimens are determined by the type and stage of the cancer, and include surgery, radiation therapy and/or chemotherapy. In spite of considerable research into therapies for this and other cancers, lung cancer remains difficult to diagnose and treat effectively. Accordingly, there is a great need for improved methods of detecting and treating such cancers.

Carbone, *J. Clin. Oncol. 23*:3219-3226 (2005); Granville and Dennis, *Cell Mol. Biol. 32*:169-176 (2005)). For example, defects in both the *p53* and *RB/p16* pathways are common in lung cancer. Several other genes, such as *K-ras*, *PTEN*, *FHIT* and *MYO18B*, are genetically altered in lung cancers, though less frequently (Minna *et al.*, *Cancer Cell 1*:49-52 (2002); Sekido *et al.*, *Annu. Rev. Med. 54*:73-87 (2003); Yokota and Kohno, *Cancer Sci. 95*:197-204 (2004)). Although focusing on known genes and proteins has yielded useful information, previously unknown markers of lung cancer may also lend insight into the biology of lung cancer.

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that control gene expression by hybridizing to and triggering either translational repression or, less frequently, degradation of a messenger RNA (mRNA) target. The discovery and study of miRNAs has revealed miRNA-mediated gene regulatory mechanisms that play important roles in organismal development and various cellular processes, such as cell differentiation, cell growth and cell death (Cheng, A.M., *et al.*, *Nucleic Acids Res. 33*:1290-1297 (2005)). Recent studies suggest that aberrant expression of particular miRNAs may be involved in human diseases, such as neurological disorders (Ishizuka, A., *et al.*, *Genes Dev. 16*:2497-2508 (2002)) and cancer. In particular, misexpression of miR-16-1 and/or miR-15a has been found in human chronic lymphocytic leukemias (Calin, G.A., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 99*:15524-15529 (2002)).

The development and use of microarrays containing all known human microRNAs has permitted a simultaneous analysis of the expression of every miRNA in a sample (Liu, C.G., *et al.*, *Proc Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004)). These microRNA microarrays have not only been used to confirm that miR-16-1 is deregulated in human CLL cells, but also to generate miRNA expression signatures that are associated with well-defined clinicopathological features of human CLL (Calin, G.A., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:1175-11760 (2004)).

Identification of microRNAs that are differentially-expressed in lung cancer cells would aid in diagnosing, prognosticating and treating lung cancer. Furthermore, the identification of putative targets of these miRNAs would help to unravel their pathogenic role. The present invention provides novel methods and compositions for the diagnosis, prognosis and treatment of lung cancer.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the identification of specific miRNAs associated with altered expression levels in lung cancer cells.

Accordingly, the invention encompasses methods of diagnosing whether a subject has, or is at risk for developing, lung cancer. According to the methods of the invention, the level of at least one miR gene product in a test sample from the subject is compared to the level of a corresponding miR gene product in a control sample. An alteration (e.g., an increase, a decrease) in the level of the miR gene product in the test sample, relative to the level of a corresponding miR gene product in a control sample, is indicative of the subject either having, or being at risk for developing, lung cancer. In certain embodiments, the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1. In a particular embodiment, the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126* and miR-224. In another embodiment, the at least one miR gene product is selected from the group consisting of miR-21, miR-205 and miR-216. In yet another embodiment, the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126*, miR-126, miR-24-2, miR-219-1, miR-95, miR-192-prec, miR-220, miR-216-prec, miR-204-prec, miR-188, miR-198, miR-145 and miR-224.

The level of the at least one miR gene product can be measured using a variety of techniques that are well known to those of skill in the art (e.g., quantitative or semi-quantitative RT-PCR, Northern blot analysis, solution hybridization detection). In a particular embodiment, the level of at least one miR gene product is measured by reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides, hybridizing the target oligodeoxynucleotides to one or more miRNA-specific probe oligonucleotides (e.g., a microarray that comprises

miRNA-specific probe oligonucleotides) to provide a hybridization profile for the test sample, and comparing the test sample hybridization profile to a hybridization profile generated from a control sample. An alteration in the signal of at least one miRNA in the test sample relative to the control sample is indicative of the subject either having, or being at risk for developing, lung cancer. In a particular embodiment, the microarray comprises miRNA-specific probe oligonucleotides for a substantial portion of all known human miRNAs. In a further embodiment, the microarray comprises miRNA-specific probe oligonucleotides for one or more miRNAs selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1.

The invention also provides methods of determining the prognosis of a subject with lung cancer, comprising measuring the level of at least one miR gene product, which is associated with an adverse prognosis in lung cancer, in a test sample from the subject. According to these methods, an alteration in the level of a miR gene product that is associated with an adverse prognosis, in the test sample, as compared to the level of a corresponding miR gene product in a control sample, is indicative of an adverse prognosis. In certain embodiments, the at least one miR gene product is selected from the group consisting of miR-155, miR-17-3p, miR-106a, miR-93, let-7a-2, miR-145, let-7b, miR-20 and miR-21. In a particular embodiment, the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-155 and let-7a-2.

The level of the at least one miR gene product can be measured as described herein (e.g., quantitative or semi-quantitative RT-PCR, Northern blot analysis, solution hybridization detection, microarray analysis). An alteration in the signal of at least one miRNA in the test sample, relative to the control sample is indicative of the subject either having, or being at risk for developing, a lung cancer with an adverse prognosis. In a particular embodiment, an alteration in the signal of miR-125a, miR-125b-1, miR-

224 and/or miR-21 is indicative of the subject either having, or being at risk for developing, a lung cancer with an adverse prognosis. In another embodiment, an alteration in the signal of miR-155 and/or let-7a-2 in a sample from a subject with lung adenocarcinoma is indicative of an adverse prognosis. In a certain embodiment, the microarray comprises miRNA-specific probe oligonucleotides for one or more miRNAs selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1.

The invention also encompasses methods of treating lung cancer in a subject, wherein at least one miR gene product is deregulated (e.g., down-regulated, up-regulated) in the cancer cells of the subject. When at least one isolated miR gene product is down-regulated in the lung cancer cells, the method comprises administering an effective amount of an isolated miR gene product, or an isolated variant or biologically-active fragment thereof, such that proliferation of cancer cells in the subject is inhibited. When at least one isolated miR gene product is up-regulated in the cancer cells, the method comprises administering to the subject an effective amount of at least one compound for inhibiting expression of the at least one miR gene product, such that proliferation of lung cancer cells is inhibited.

In a related embodiment, the methods of treating lung cancer in a subject additionally comprise the step of first determining the amount of at least one miR gene product in lung cancer cells from the subject, and comparing that level of the miR gene product to the level of a corresponding miR gene product in control cells. If expression of the miR gene product is deregulated (e.g., down-regulated, up-regulated) in lung cancer cells, the methods further comprise altering the amount of the at least one miR gene product expressed in the lung cancer cells. In one embodiment, the amount of the miR gene product expressed in the cancer cells is less than the amount of the miR gene product expressed in control cells, and an effective amount of the miR gene product, or an isolated variant or biologically-active fragment thereof, is

administered to the subject. In another embodiment, the amount of the miR gene product expressed in the cancer cells is greater than the amount of the miR gene product expressed in control cells, and an effective amount of at least one compound for inhibiting expression of the at least one miR gene is administered to the subject.

The invention further provides pharmaceutical compositions for treating lung cancer. In one embodiment, the pharmaceutical compositions comprise at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, and a pharmaceutically-acceptable carrier. In a particular embodiment, the at least one miR gene product corresponds to a miR gene product that has a decreased level of expression in lung cancer cells relative to suitable control cells. In certain embodiments the isolated miR gene product is selected from the group consisting of miR-126*, miR-143, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, let-7f-1 and a combination thereof.

In another embodiment, the pharmaceutical compositions of the invention comprise at least one miR expression-inhibition compound. In a particular embodiment, the at least one miR expression-inhibition compound is specific for a miR gene product whose expression is greater in lung cancer cells than control cells. In certain embodiments, the miR expression-inhibition compound is specific for one or more miR gene products selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

The invention also encompasses methods of identifying an anti-lung cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR gene product in the cell. In one embodiment, the method comprises providing a test agent to a cell and measuring the level of at least one miR gene product associated with decreased expression levels in lung cancer cells. An increase in the level of the miR gene product in the cell, relative to a suitable control cell, is indicative of the test agent being an anti-lung cancer agent. In a particular embodiment, the at least one miR gene product associated with decreased expression levels in lung cancer cells is selected from the group consisting of miR-126*, miR-143, miR-192, miR-224, miR-

126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, let-7f-1 and a combination thereof.

In other embodiments, the method comprises providing a test agent to a cell and measuring the level of at least one miR gene product associated with increased expression levels in lung cancer cells. A decrease in the level of the miR gene product associated with increased expression levels in lung cancer in the cell, relative to a suitable control cell, is indicative of the test agent being an anti-lung cancer agent. In a particular embodiment, the at least one miR gene product associated with increased expression levels in lung cancer cells is selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 shows graphs depicting the relative expression level of human miR-21 precursor (*hsa-mir-21*; top panels), human miR-126* precursor (*hsa-mir-126**; middle panels) and human miR-205 precursor (*hsa-mir-205*; bottom panels) in lung cancer (Ca) and noncancerous (N) tissues, as determined by real-time RT-PCR analysis. Cancer samples were either adenocarcinoma or squamous cell carcinoma (SCC). A paired *t* test was performed to ascertain statistical significance between the expression levels in lung cancer tissues and noncancerous lung tissues.

FIG. 2 depicts the expression of mature miRNAs for miR-21 (*hsa-mir-21*), miR-126* (*hsa-mir-126**) and miR-205 (*hsa-mir-205*) in lung cancer samples (i.e., adenocarcinomas (Adeno) and squamous cell carcinomas (SCC)), as detected by solution hybridization. Ca represents cancerous lung tissues and N represents noncancerous lung tissues. 5S rRNA served as a loading control.

FIG. 3A is a dendrogram depicting a hierarchical clustering based on

microRNA expression profiles of 13 lung cancer cell lines representing small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC).

FIG. 3B depicts a miRNA expression cluster view for 13 lung cancer cell lines (top), corresponding to those listed in FIG. 3A. The expression levels of various miRNAs, listed at the right of the figure, are indicated according to color. Blue indicates expression levels below the median, black indicates expression levels that are about equal to the median, and orange indicates expression levels that are greater than the median. Gray indicates missing data points.

FIG. 4 is a Kaplan-Meier survival curve for adenocarcinoma patients. Adenocarcinoma cases in which hybridization intensity was different from background (see Example 4) were classified according to *hsa-mir-155* expression, and the survival data were compared using the log-rank test. The mean expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The *hsa-mir-155* high expression group (i.e., group with an expression ratio of $\geq$ mean expression ratio (1.42); n=27) was compared with corresponding noncancerous lung tissues. The *hsa-mir-155* low expression group (i.e., group with an expression ratio of $<$ mean expression ratio (1.42); n=28) was compared with corresponding noncancerous lung tissues. The ratios represent the intensity of hybridization signal in the lung cancer sample relative to noncancerous controls.

FIG. 5 is a Kaplan-Meier survival curve for adenocarcinoma patients. Adenocarcinoma cases in which hybridization intensity was different from background (see Example 4) were classified according to *hsa-let-7a-2* expression, and the survival data were compared using the log-rank test. The mean expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The *hsa-let-7a-2* high expression group (i.e., group with an expression ratio of $\geq$ mean expression ratio (0.95); n=34) was compared with corresponding noncancerous lung tissues. The *hsa-let-7a-2* low expression group (i.e., group with an expression ratio of $<$ mean expression ratio (0.95); n=18) was compared with corresponding noncancerous lung tissues.

FIG. 6 is a Kaplan-Meier survival curve for adenocarcinoma patients. Thirty two adenocarcinoma cases from an original cohort were classified according to precursor *hsa-miR-155* expression, and the survival data were compared using the log-

rank test. The mean expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The precursor *hsa-miR-155* high expression group (i.e., group with an expression ratio of ≥ mean expression ratio (1.19); n=13) was compared with corresponding noncancerous lung tissues. The precursor *hsa-miR-155* low expression group (i.e., group with an expression ratio of < mean expression ratio (1.19); n=19) was compared with corresponding noncancerous lung tissues.

FIG. 7 is a Kaplan-Meier survival curve for adenocarcinoma patients. Thirty two adenocarcinoma cases from an original cohort were classified according to precursor *hsa-let-7a-2* expression, and the survival data were compared using the log-rank test. The mean expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The precursor *hsa-let-7a-2* high expression group (i.e., group with an expression ratio of ≥ mean expression ratio (0.92); n=18) was compared with corresponding noncancerous lung tissues. The precursor *hsa-let-7a-2* low expression group (i.e., group with an expression ratio of < mean expression ratio (0.92); n=14) was compared with corresponding noncancerous lung tissues.

FIG. 8 is a Kaplan-Meier survival curve for adenocarcinoma patients. Thirty two adenocarcinoma cases from an independent additional cohort were classified according to precursor *hsa-let-7a-2* expression, and the survival data were compared using the log-rank test. Precursor *hsa-mir-155* high expression group (n=14); precursor *hsa-mir-155* low expression group (n=18).

FIG. 9 is a Kaplan-Meier survival curve for adenocarcinoma patients. Thirty two adenocarcinoma cases from an independent additional cohort were classified according to precursor *hsa-let-7a-2* expression, and the survival data were compared using the log-rank test. Precursor *hsa-let-7a-2* high expression group (n=15); precursor *hsa-let-7a-2* low expression group (n=17).

FIG. 10 is a Kaplan-Meier survival curve for adenocarcinoma patients. Sixty four adenocarcinoma cases from a combination of 2 independent cohorts were classified according to precursor *hsa-mir-155* expression, as estimated by real-time RT-PCR analysis. The survival data were compared using the log-rank test. The mean

expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The precursor *hsa-miR-155* high expression group (i.e., group with an expression ratio of ≥ mean expression ratio (1.19); n=27) was compared with corresponding noncancerous lung tissues. The precursor *hsa-miR-155* low expression group (i.e., group with an expression ratio of < mean expression ratio (1.19); n=37) was compared with corresponding noncancerous lung tissues.

FIG. 11 is a Kaplan-Meier survival curve for adenocarcinoma patients. Sixty four adenocarcinoma cases from a combination of 2 independent cohorts were classified according to precursor *hsa-let-7a-2* expression, as estimated by real-time RT-PCR analysis. The survival data were compared using the log-rank test. The mean expression ratio is defined as mean expression ratio = mean of tumor expression/mean of noncancerous tissue expression. The precursor *hsa-let-7a-2* high expression group (i.e., group with an expression ratio of ≥ mean expression ratio (0.92); n=33) was compared with corresponding noncancerous lung tissues. The precursor *hsa-let-7a-2* low expression group (i.e., group with an expression ratio of < mean expression ratio (0.92); n=31) was compared with corresponding noncancerous lung tissues.

FIG. 12 depicts the expression of *MYO18B* mRNA after treatment with 5-aza-dC and/or TSA in two lung cancer cell lines (H157, A549), as determined by RT-PCR analysis. Lane 1, no treatment; lane 2, treatment with 1.0 μM 5-aza-dC for 72 hr; lane 3, treatment with 1.0 μM TSA for 24 hr; lane 4, treatment with 1.0 μM 5-aza-dC for 72 hours, followed by treatment with 1.0 μM TSA for 24 hr. *GAPDH* expression served as a loading control.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, on the identification of particular microRNAs having altered expression in lung cancer cells relative to normal control cells, and on association of these microRNAs with particular diagnostic, prognostic and therapeutic features.

As used herein interchangeably, a "miR gene product," "microRNA," "miR," or "miRNA" refers to the unprocessed or processed RNA transcript from a miR gene. As the miR gene products are not translated into protein, the term "miR gene products"

does not include proteins. The unprocessed miR gene transcript is also called a "miR precursor," and typically comprises an RNA transcript of about 70-100 nucleotides in length. The miR precursor can be processed by digestion with an RNAse (for example, Dicer, Argonaut, RNAse III (e.g., *E. coli* RNAse III)) into an active 19-25 nucleotide RNA molecule. This active 19-25 nucleotide RNA molecule is also called the "processed" miR gene transcript or "mature" miRNA.

The active 19-25 nucleotide RNA molecule can be obtained from the miR precursor through natural processing routes (*e.g.*, using intact cells or cell lysates) or by synthetic processing routes (*e.g.*, using isolated processing enzymes, such as isolated Dicer, Argonaut, or RNAse III). It is understood that the active 19-25 nucleotide RNA molecule can also be produced directly by biological or chemical synthesis, without having to be processed from the miR precursor. When a microRNA is referred to herein by name, the name corresponds to both the precursor and mature forms, unless otherwise indicated.

The present invention encompasses methods of diagnosing whether a subject has, or is at risk for developing, lung cancer, comprising measuring the level of at least one miR gene product in a test sample from the subject and comparing the level of the miR gene product in the test sample to the level of a corresponding miR gene product in a control sample. As used herein, a "subject" can be any mammal that has, or is suspected of having, lung cancer. In a preferred embodiment, the subject is a human who has, or is suspected of having, lung cancer.

The lung cancer can be any form of lung cancer, for example, lung cancers of differing histology (e.g., adenocarcinoma, squamous cell carcinoma). Furthermore, the lung cancer may be associated with a particular prognosis (e.g., low survival rate, fast progression).

Tables 1a and 1b depict the nucleotide sequences of particular precursor and mature human microRNAs.

Table 1a- Human microRNA Precursor Sequences

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
let-7a-1	CACUGUGGGAUGAGGUAGUAGGUUGUAUAGUUU AGGGUCACACCCACCACUGGGAGAUAACUAUACA AUCUACUGUCUUUCCUAACGUG	1

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
let-7a-2	AGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUA CAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUU UCCU	2
let-7a-3	GGGUGAGGUAGUAGGUUGUAUAGUUUGGGGCUCU GCCCUGCUAUGGGAUAACUAUACAAUCUACUGUC UUUCCU	3
let-7a-4	GUGACUGCAUGCUCCCAGGUUGAGGUAGUAGGUU GUAUAGUUUAGAAUUACACAAGGGAGAUAACUGU ACAGCCUCCUAGCUUUCCUUGGGUCUUGCACUAA ACAAC	4
let-7b	GGCGGGGUGAGGUAGUAGGUUGUGUGGUUUCAGG GCAGUGAUGUUGCCCCUCGGAAGAUAACUAUACA ACCUACUGCCUUCCCUG	5
let-7c	GCAUCCGGGUUGAGGUAGUAGGUUGUAUGGUUUA GAGUUACACCCUGGGAGUUAACUGUACAACCUUC UAGCUUUCCUUGGAGC	6
let-7d	CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUUAGG GCAGGGAUUUUGCCCACAAGGAGGUAACUAUACG ACCUGCUGCCUUUCUUAGG	7
let-7d-v1	CUAGGAAGAGGUAGUAGUUUGCAUAGUUUUAGGG CAAAGAUUUUGCCCACAAGUAGUUAGCUAUACGA CCUGCAGCCUUUUGUAG	8
let-7d-v2	CUGGCUGAGGUAGUAGUUUGUGCUGUUGGUCGGG UUGUGACAUUGCCCGCUGUGGAGAUAACUGCGCA AGCUACUGCCUUGCUAG	9
let-7e	CCCGGGCUGAGGUAGGAGGUUGUAUAGUUGAGGA GGACACCCAAGGAGAUCACUAUACGGCCUCCUAG CUUUCCCCAGG	10
let-7f-1	UCAGAGUGAGGUAGUAGAUUGUAUAGUUGUGGGG UAGUGAUUUUACCCUGUUCAGGAGAUAACUAUAC AAUCUAUUGCCUUCCCUGA	11
let-7f-2-1	CUGUGGGAUGAGGUAGUAGAUUGUAUAGUUGUGG GGUAGUGAUUUUACCCUGUUCAGGAGAUAACUAU ACAAUCUAUUGCCUUCCCUGA	12
let-7f-2-2	CUGUGGGAUGAGGUAGUAGAUUGUAUAGUUUUAG GGUCAUACCCCAUCUUGGAGAUAACUAUACAGUC UACUGUCUUUCCCACGG	13
let-7g	UUGCCUGAUUCCAGGCUGAGGUAGUAGUUUGUAC AGUUUGAGGGUCUAUGAUACCACCCGGUACAGGA GAUAACUGUACAGGCCACUGCCUUGCCAGGAACA GCGCGC	14
let-7i	CUGGCUGAGGUAGUAGUUUGUGCUGUUGGUCGGG UUGUGACAUUGCCCGCUGUGGAGAUAACUGCGCA AGCUACUGCCUUGCUAG	15

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-1b-1-1	ACCUACUCAGAGUACAUACUUCUUUAUGUACCCA UAUGAACAUACAAUGCUAUGGAAUGUAAAGAAGU AUGUAUUUUUGGUAGGC	16
miR-1b-1-2	CAGCUAACAACUUAGUAAUACCUACUCAGAGUAC AUACUUCUUUAUGUACCCAUAUGAACAUACAAUG CUAUGGAAUGUAAAGAAGUAUGUAUUUUUGGUAG GCAAUA	17
miR-1b-2	GCCUGCUUGGGAAACAUACUUCUUUAUAUGCCCA UAUGGACCUGCUAAGCUAUGGAAUGUAAAGAAGU AUGUAUCUCAGGCCGGG	18
miR-1b	UGGGAAACAUACUUCUUUAUAUGCCCAUAUGGAC CUGCUAAGCUAUGGAAUGUAAAGAAGUAUGUAUC UCA	19
miR-1d	ACCUACUCAGAGUACAUACUUCUUUAUGUACCCA UAUGAACAUACAAUGCUAUGGAAUGUAAAGAAGU AUGUAUUUUUGGUAGGC	20
miR-7-1a	UGGAUGUUGGCCUAGUUCUGUGUGGAAGACUAGU GAUUUUGUUGUUUUUUAGAUAACUAAAUCGACAAC AAAUCACAGUCUGCCAUAUGGCACAGGCCAUGCC UCUACA	21
miR-7-1b	UUGGAUGUUGGCCUAGUUCUGUGUGGAAGACUAG UGAUUUUGUUGUUUUUUAGAUAACUAAAUCGACAA CAAAUCACAGUCUGCCAUAUGGCACAGGCCAUGC CUCUACAG	22
miR-7-2	CUGGAUACAGAGUGGACCGGCUGGCCCCAUCUGG AAGACUAGUGAUUUUGUUGUUGUCUUACUGCGCU CAACAACAAAUCCCAGUCUACCUAAUGGUGCCAG CCAUCGCA	23
miR-7-3	AGAUUAGAGUGGCUGUGGUCUAGUGCUGUGUGGA AGACUAGUGAUUUUGUUGUUCUGAUGUACUACGA CAACAAGUCACAGCCGGCCUCAUAGCGCAGACUCC CUUCGAC	24
miR-9-1	CGGGGUUGGUUGUUAUCUUUGGUUAUCUAGCUGU AUGAGUGGUGUGGAGUCUUCAUAAAGCUAGAUAA CCGAAAGUAAAAAUAACCCCA	25
miR-9-2	GGAAGCGAGUUGUUAUCUUUGGUUAUCUAGCUGU AUGAGUGUAUUGGUCUUCAUAAAGCUAGAUAACC GAAAGUAAAAACUCCUUCA	26
miR-9-3	GGAGGCCCGUUUCUCUCUUUGGUUAUCUAGCUGU AUGAGUGCCACAGAGCCGUCAUAAAGCUAGAUAA CCGAAAGUAGAAAUGAUUCUCA	27
miR-10a	GAUCUGUCUGUCUUCUGUAUAUACCCUGUAGAUC CGAAUUUGUGUAAGGAAUUUUGUGGUCACAAAUU CGUAUCUAGGGGAAUAUGUAGUUGACAUAAACAC UCCGCUCU	28

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-10b	CCAGAGGUUGUAACGUUGUCUAUAUAUACCCUGU AGAACCGAAUUUGUGUGGUAUCCGUAUAGUCACA GAUUCGAUUCUAGGGGAAUAUAUGGUCGAUGCAA AAACUUCA	29
miR-15a-2	GCGCGAAUGUGUGUUUAAAAAAAAUAAAACCUUG GAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUU UUGAAAAGGUGCAGGCCAUAUUGUGCUGCCUCAA AAAUAC	30
miR-15a	CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGU GGAUUUUGAAAAGGUGCAGGCCAUAUUGUGCUGC CUCAAAAAUACAAGG	31
miR-15b-1	CUGUAGCAGCACAUCAUGGUUUACAUGCUACAGU CAAGAUGCGAAUCAUUAUUUGCUGCUCUAG	32
miR-15b-2	UUGAGGCCUUAAAGUACUGUAGCAGCACAUCAUG GUUUACAUGCUACAGUCAAGAUGCGAAUCAUUAU UUGCUGCUCUAGAAAUUUAAGGAAAUUCAU	33
miR-16-1	GUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGC GUUAAGAUUCUAAAAUUAUCUCCAGUAUUAACUG UGCUGCUGAAGUAAGGUUGAC	34
miR-16-2	GUUCCACUCUAGCAGCACGUAAAUAUUGGCGUAG UGAAAUAUAUAUUAAACACCAAUAUUACUGUGCU GCUUUAGUGUGAC	35
miR-16-13	GCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUA AGAUUCUAAAAUUAUCUCCAGUAUUAACUGUGCU GCUGAAGUAAGGU	36
miR-17	GUCAGAAUAAUGUCAAAGUGCUUACAGUGCAGGU AGUGAUAUGUGCAUCUACUGCAGUGAAGGCACUU GUAGCAUUAUGGUGAC	37
miR-18	UGUUCUAAGGUGCAUCUAGUGCAGAUAGUGAAGU AGAUUAGCAUCUACUGCCCUAAGUGCUCCUUCUG GCA	38
miR-18-13	UUUUUGUUCUAAGGUGCAUCUAGUGCAGAUAGUG AAGUAGAUUAGCAUCUACUGCCCUAAGUGCUCCU UCUGGCAUAAGAA	39
miR-19a	GCAGUCCUCUGUUAGUUUUGCAUAGUUGCACUAC AAGAAGAAUGUAGUUGUGCAAAUCUAUGCAAAAC UGAUGGUGGCCUGC	40
miR-19a-13	CAGUCCUCUGUUAGUUUUGCAUAGUUGCACUACA AGAAGAAUGUAGUUGUGCAAAUCUAUGCAAAACU GAUGGUGGCCUG	41
miR-19b-1	CACUGUUCUAUGGUUAGUUUUGCAGGUUUGCAUC CAGCUGUGUGAUAUUCUGCUGUGCAAAUCCAUGC AAAACUGACUGUGGUAGUG	42

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-19b-2	ACAUUGCUACUUACAAUUAGUUUUGCAGGUUUGC AUUUCAGCGUAUAUAUGUAUAUGUGGCUGUGCAA AUCCAUGCAAAACUGAUUGUGAUAAUGU	43
miR-19b-13	UUCUAUGGUUAGUUUUGCAGGUUUGCAUCCAGCU GUGUGAUAUUCUGCUGUGCAAAUCCAUGCAAAAC UGACUGUGGUAG	44
miR-19b-X	UUACAAUUAGUUUUGCAGGUUUGCAUUUCAGCGU AUAUAUGUAUAUGUGGCUGUGCAAAUCCAUGCAA AACUGAUUGUGAU	45
miR-20 (miR-20a)	GUAGCACUAAAGUGCUUAUAGUGCAGGUAGUGUU UAGUUAUCUACUGCAUUAUGAGCACUUAAAGUAC UGC	46
miR-21	UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUU GAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCU GACA	47
miR-21-17	ACCUUGUCGGGUAGCUUAUCAGACUGAUGUUGAC UGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCU GUCUGACAUUUUG	48
miR-22	GGCUGAGCCGCAGUAGUUCUUCAGUGGCAAGCUU UAUGUCCUGACCCAGCUAAAGCUGCCAGUUGAAG AACUGUUGCCCUCUGCC	49
miR-23a	GGCCGGCUGGGGUUCCUGGGGAUGGGAUUUGCUU CCUGUCACAAAUCACAUUGCCAGGGAUUUCCAAC CGACC	50
miR-23b	CUCAGGUGCUCUGGCUGCUUGGGUUCCUGGCAUG CUGAUUUGUGACUUAAGAUUAAAAUCACAUUGCC AGGGAUUACCACGCAACCACGACCUUGGC	51
miR-23-19	CCACGGCCGGCUGGGGUUCCUGGGGAUGGGAUUU GCUUCCUGUCACAAAUCACAUUGCCAGGGAUUUC CAACCGACCCUGA	52
miR-24-1	CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAU UUUACACACUGGCUCAGUUCAGCAGGAACAGGAG	53
miR-24-2	CUCUGCCUCCCGUGCCUACUGAGCUGAAACACAGU UGGUUUGUGUACACUGGCUCAGUUCAGCAGGAAC AGGG	54
miR-24-19	CCCUGGGCUCUGCCUCCCGUGCCUACUGAGCUGAA ACACAGUUGGUUUGUGUACACUGGCUCAGUUCAG CAGGAACAGGGG	55
miR-24-9	CCCUCCGGUGCCUACUGAGCUGAUAUCAGUUCUC AUUUUACACACUGGCUCAGUUCAGCAGGAACAGC AUC	56
miR-25	GGCCAGUGUUGAGAGGCGGAGACUUGGGCAAUUG CUGGACGCUGCCCUGGGCAUUGCACUUGUCUCGG UCUGACAGUGCCGGCC	57

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-26a	AGGCCGUGGCCUCGUUCAAGUAAUCCAGGAUAGG CUGUGCAGGUCCCAAUGGCCUAUCUUGGUUACUU GCACGGGGACGCGGGCCU	58
miR-26a-1	GUGGCCUCGUUCAAGUAAUCCAGGAUAGGCUGUG CAGGUCCCAAUGGGCCUAUUCUUGGUUACUUGCA CGGGGACGC	59
miR-26a-2	GGCUGUGGCUGGAUUCAAGUAAUCCAGGAUAGGC UGUUUCCAUCUGUGAGGCCUAUUCUUGAUUACUU GUUUCUGGAGGCAGCU	60
miR-26b	CCGGGACCCAGUUCAAGUAAUUCAGGAUAGGUUG UGUGCUGUCCAGCCUGUUCUCCAUUACUUGGCUC GGGGACCGG	61
miR-27a	CUGAGGAGCAGGGCUUAGCUGCUUGUGAGCAGGG UCCACACCAAGUCGUGUUCACAGUGGCUAAGUUC CGCCCCCCAG	62
miR-27b-1	AGGUGCAGAGCUUAGCUGAUUGGUGAACAGUGAU UGGUUUCCGCUUUGUUCACAGUGGCUAAGUUCUG CACCU	63
miR-27b-2	ACCUCUCUAACAAGGUGCAGAGCUUAGCUGAUUG GUGAACAGUGAUUGGUUUCCGCUUUGUUCACAGU GGCUAAGUUCUGCACCUGAAGAGAAGGUG	64
miR-27-19	CCUGAGGAGCAGGGCUUAGCUGCUUGUGAGCAGG GUCCACACCAAGUCGUGUUCACAGUGGCUAAGUU CCGCCCCCCAGG	65
miR-28	GGUCCUUGCCCUCAAGGAGCUCACAGUCUAUUGA GUUACCUUUCUGACUUUCCCACUAGAUUGUGAGC UCCUGGAGGGCAGGCACU	66
miR-29a-2	CCUUCUGUGACCCCUUAGAGGAUGACUGAUUUCU UUUGGUGUUCAGAGUCAAUAUAAUUUUCUAGCAC CAUCUGAAAUCGGUUAUAAUGAUUGGGGAAGAGC ACCAUG	67
miR-29a	AUGACUGAUUUCUUUUGGUGUUCAGAGUCAAUAU AAUUUUCUAGCACCAUCUGAAAUCGGUUAU	68
miR-29b-1	CUUCAGGAAGCUGGUUUCAUAUGGUGGUUUAGAU UUAAAUAGUGAUUGUCUAGCACCAUUUGAAAUCA GUGUUCUUGGGGG	69
miR-29b-2	CUUCUGGAAGCUGGUUUCACAUGGUGGCUUAGAU UUUUCCAUCUUUGUAUCUAGCACCAUUUGAAAUC AGUGUUUUAGGAG	70
miR-29c	ACCACUGGCCCAUCUCUUACACAGGCUGACCGAUU UCUCCUGGUGUUCAGAGUCUGUUUUUGUCUAGCA CCAUUUGAAAUCGGUUAUGAUGUAGGGGGAAAAG CAGCAGC	71

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-30a	GCGACUGUAAACAUCCUCGACUGGAAGCUGUGAA GCCACAGAUGGGCUUUCAGUCGGAUGUUUGCAGC UGC	72
miR-30b-1	AUGUAAACAUCCUACACUCAGCUGUAAUACAUGG AUUGGCUGGGAGGUGGAUGUUUACGU	73
miR-30b-2	ACCAAGUUUCAGUUCAUGUAAACAUCCUACACUC AGCUGUAAUACAUGGAUUGGCUGGGAGGUGGAUG UUUACUUCAGCUGACUUGGA	74
miR-30c	AGAUACUGUAAACAUCCUACACUCUCAGCUGUGG AAAGUAAGAAAGCUGGGAGAAGGCUGUUUACUCU UUCU	75
miR-30d	GUUGUUGUAAACAUCCCCGACUGGAAGCUGUAAG ACACAGCUAAGCUUUCAGUCAGAUGUUUGCUGCU AC	76
miR-30e	CUGUAAACAUCCUUGACUGGAAGCUGUAAGGUGU UCAGAGGAGCUUUCAGUCGGAUGUUUACAG	77
miR-31	GGAGAGGAGGCAAGAUGCUGGCAUAGCUGUUGAA CUGGGAACCUGCUAUGCCAACAUAUUGCCAUCUU UCC	78
miR-32	GGAGAUAUUGCACAUUACUAAGUUGCAUGUUGUC ACGGCCUCAAUGCAAUUUAGUGUGUGUGAUAUUU UC	79
miR-33b	GGGGGCCGAGAGAGGCGGGCGGCCCCGCGGUGCA UUGCUGUUGCAUUGCACGUGUGUGAGGCGGGUGC AGUGCCUCGGCAGUGCAGCCCGGAGCCGGCCCCUG GCACCAC	80
miR-33b-2	ACCAAGUUUCAGUUCAUGUAAACAUCCUACACUC AGCUGUAAUACAUGGAUUGGCUGGGAGGUGGAUG UUUACUUCAGCUGACUUGGA	81
miR-33	CUGUGGUGCAUUGUAGUUGCAUUGCAUGUUCUGG UGGUACCCAUGCAAUGUUUCCACAGUGCAUCACA G	82
miR-34-a	GGCCAGCUGUGAGUGUUUCUUUGGCAGUGUCUUA GCUGGUUGUUGUGAGCAAUAGUAAGGAAGCAAUC AGCAAGUAUACUGCCCUAGAAGUGCUGCACGUUG UGGGGCCC	83
miR-34-b	GUGCUCGGUUUGUAGGCAGUGUCAUUAGCUGAUU GUACUGUGGUGGUUACAAUCACUAACUCCACUGC CAUCAAAACAAGGCAC	84
miR-34-c	AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUG CUAAUAGUACCAAUCACUAACCACACGGCCAGGU AAAAAGAUU	85
miR-91-13	UCAGAAUAAUGUCAAAGUGCUUACAGUGCAGGUA GUGAUAUGUGCAUCUACUGCAGUGAAGGCACUUG UAGCAUUAUGGUGA	86

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-92-1	CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUG UGUUUCUGUAUGGUAUUGCACUUGUCCCGGCCUG UUGAGUUUGG	87
miR-92 -2	UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGU GUUCUAUAUAAAGUAUUGCACUUGUCCCGGCCUG UGGAAGA	88
miR-93-1 (miR-93-2)	CUGGGGGCUCCAAAGUGCUGUUCGUGCAGGUAGU GUGAUUACCCAACCUACUGCUGAGCUAGCACUUC CCGAGCCCCCGG	89
miR-95-4	AACACAGUGGGCACUCAAUAAAUGUCUGUUGAAU UGAAAUGCGUUACAUUCAACGGGUAUUUAUUGAG CACCCACUCUGUG	90
miR-96-7	UGGCCGAUUUUGGCACUAGCACAUUUUUGCUUGU GUCUCUCCGCUCUGAGCAAUCAUGUGCAGUGCCA AUAUGGGAAA	91
miR-97-6 (miR-30)*	GUGAGCGACUGUAAACAUCCUCGACUGGAAGCUG UGAAGCCACAGAUGGGCUUUCAGUCGGAUGUUUG CAGCUGCCUACU	92
miR-98	GUGAGGUAGUAAGUUGUAUUGUUGUGGGGUAGGG AUAUUAGGCCCCAAUUAGAAGAUAACUAUACAAC UUACUACUUUCC	93
miR-99b	GGCACCCACCCGUAGAACCGACCUUGCGGGGCCUU CGCCGCACACAAGCUCGUGUCUGUGGGUCCGUGU C	94
miR-99a	CCCAUUGGCAUAAACCCGUAGAUCCGAUCUUGUG GUGAAGUGGACCGCACAAGCUCGCUUCUAUGGGU CUGUGUCAGUGUG	95
miR-100-1/2	AAGAGAGAAGAUAUUGAGGCCUGUUGCCACAAAC CCGUAGAUCCGAACUUGUGGUAUUAGUCCGCACA AGCUUGUAUCUAUAGGUAUGUGUCUGUUAGGCAA UCUCAC	96
miR-100-11	CCUGUUGCCACAAACCCGUAGAUCCGAACUUGUG GUAUUAGUCCGCACAAGCUUGUAUCUAUAGGUAU GUGUCUGUUAGG	97
miR-101-1 /2	AGGCUGCCCUGGCUCAGUUAUCACAGUGCUGAUG CUGUCUAUUCUAAAGGUACAGUACUGUGAUAACU GAAGGAUGGCAGCCAUCUUACCUUCCAUCAGAGG AGCCUCAC	98
miR-101	UCAGUUAUCACAGUGCUGAUGCUGUCCAUUCUAA AGGUACAGUACUGUGAUAACUGA	99
miR-101-1	UGCCCUGGCUCAGUUAUCACAGUGCUGAUGCUGU CUAUUCUAAAGGUACAGUACUGUGAUAACUGAAG GAUGGCA	100

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-101-2	ACUGUCCUUUUUCGGUUAUCAUGGUACCGAUGCU GUAUAUCUGAAAGGUACAGUACUGUGAUAACUGA AGAAUGGUGGU	101
miR-101-9	UGUCCUUUUUCGGUUAUCAUGGUACCGAUGCUGU AUAUCUGAAAGGUACAGUACUGUGAUAACUGAAG AAUGGUG	102
miR-102-1	CUUCUGGAAGCUGGUUUCACAUGGUGGCUUAGAU UUUUCCAUCUUUGUAUCUAGCACCAUUUGAAAUC AGUGUUUUAGGAG	103
miR-102-7.1 (miR-102-7.2)	CUUCAGGAAGCUGGUUUCAUAUGGUGGUUUAGAU UUAAAUAGUGAUUGUCUAGCACCAUUUGAAAUCA GUGUUCUUGGGGG	104
miR-103-2	UUGUGCUUUCAGCUUCUUUACAGUGCUGCCUUGU AGCAUUCAGGUCAAGCAACAUUGUACAGGGCUAU GAAAGAACCA	105
miR-103-1	UACUGCCCUCGGCUUCUUUACAGUGCUGCCUUGU UGCAUAUGGAUCAAGCAGCAUUGUACAGGGCUAU GAAGGCAUUG	106
miR-104-17	AAAUGUCAGACAGCCCAUCGACUGGUGUUGCCAU GAGAUUCAACAGUCAACAUCAGUCUGAUAAGCUA CCCGACAAGG	107
miR-105-1	UGUGCAUCGUGGUCAAAUGCUCAGACUCCUGUGG UGGCUGCUCAUGCACCACGGAUGUUUGAGCAUGU GCUACGGUGUCUA	108
miR-105-2	UGUGCAUCGUGGUCAAAUGCUCAGACUCCUGUGG UGGCUGCUUAUGCACCACGGAUGUUUGAGCAUGU GCUAUGGUGUCUA	109
miR-106-a	CCUUGGCCAUGUAAAAGUGCUUACAGUGCAGGUA GCUUUUUGAGAUCUACUGCAAUGUAAGCACUUCU UACAUUACCAUGG	110
miR-106-b	CCUGCCGGGGCUAAAGUGCUGACAGUGCAGAUAG UGGUCCUCUCCGUGCUACCGCACUGUGGGUACUU GCUGCUCCAGCAGG	111
miR-107	CUCUCUGCUUUCAGCUUCUUUACAGUGUUGCCUU GUGGCAUGGAGUUCAAGCAGCAUUGUACAGGGCU AUCAAAGCACAGA	112
miR-108-1-small	ACACUGCAAGAACAAUAAGGAUUUUUAGGGGCAU UAUGACUGAGUCAGAAAACACAGCUGCCCCUGAA AGUCCCUCAUUUUUCUUGCUGU	113
miR-108-2-small	ACUGCAAGAGCAAUAAGGAUUUUUAGGGGCAUUA UGAUAGUGGAAUGGAAACACAUCUGCCCCCAAAA GUCCCUCAUUUU	114
miR-122a-1	CCUUAGCAGAGCUGUGGAGUGUGACAAUGGUGUU UGUGUCUAAACUAUCAAACGCCAUUAUCACACUA AAUAGCUACUGCUAGGC	115

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-122a-2	AGCUGUGGAGUGUGACAAUGGUGUUUGUGUCCAA ACUAUCAAACGCCAUUAUCACACUAAAUAGCU	116
miR-123	ACAUUAUUACUUUUGGUACGCGCUGUGACACUUC AAACUCGUACCGUGAGUAAUAAUGCGC	117
miR-124a-1	AGGCCUCUCUCUCCGUGUUCACAGCGGACCUUGA UUUAAAUGUCCAUACAAUUAAGGCACGCGGUGAA UGCCAAGAAUGGGGCUG	118
miR-124a-2	AUCAAGAUUAGAGGCUCUGCUCUCCGUGUUCACA GCGGACCUUGAUUUAAUGUCAUACAAUUAAGGCA CGCGGUGAAUGCCAAGAGCGGAGCCUACGGCUGC ACUUGAAG	119
miR-124a-3	UGAGGGCCCCUCUGCGUGUUCACAGCGGACCUUG AUUUAAUGUCUAUACAAUUAAGGCACGCGGUGAA UGCCAAGAGAGGCGCCUCC	120
miR-124a	CUCUGCGUGUUCACAGCGGACCUUGAUUUAAUGU CUAUACAAUUAAGGCACGCGGUGAAUGCCAAGAG	121
miR-124b	CUCUCCGUGUUCACAGCGGACCUUGAUUUAAUGU CAUACAAUUAAGGCACGCGGUGAAUGCCAAGAG	122
miR-125a-1	UGCCAGUCUCUAGGUCCCUGAGACCCUUUAACCU GUGAGGACAUCCAGGGUCACAGGUGAGGUUCUUG GGAGCCUGGCGUCUGGCC	123
miR-125a-2	GGUCCCUGAGACCCUUUAACCUGUGAGGACAUCC AGGGUCACAGGUGAGGUUCUUGGGAGCCUGG	124
miR-125b-1	UGCGCUCCUCUCAGUCCCUGAGACCCUAACUUGUG AUGUUUACCGUUUAAAUCCACGGGUUAGGCUCUU GGGAGCUGCGAGUCGUGCU	125
miR-125b-2	ACCAGACUUUUCCUAGUCCCUGAGACCCUAACUU GUGAGGUAUUUUAGUAACAUCACAAGUCAGGCUC UUGGGACCUAGGCGGAGGGGA	126
miR-126-1	CGCUGGCGACGGGACAUUAUUACUUUUGGUACGC GCUGUGACACUUCAAACUCGUACCGUGAGUAAUA AUGCGCCGUCCACGGCA	127
miR-126-2	ACAUUAUUACUUUUGGUACGCGCUGUGACACUUC AAACUCGUACCGUGAGUAAUAAUGCGC	128
miR-127-1	UGUGAUCACUGUCUCCAGCCUGCUGAAGCUCAGA GGGCUCUGAUUCAGAAAGAUCAUCGGAUCCGUCU GAGCUUGGCUGGUCGGAAGUCUCAUCAUC	129
miR-127-2	CCAGCCUGCUGAAGCUCAGAGGGCUCUGAUUCAG AAAGAUCAUCGGAUCCGUCUGAGCUUGGCUGGUC GG	130
miR-128a	UGAGCUGUUGGAUUCGGGGCCGUAGCACUGUCUG AGAGGUUUACAUUUCUCACAGUGAACCGGUCUCU UUUUCAGCUGCUUC	131

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-128b	GCCCGGCAGCCACUGUGCAGUGGGAAGGGGGGCC GAUACACUGUACGAGAGUGAGUAGCAGGUCUCAC AGUGAACCGGUCUCUUUCCCUACUGUGUCACACU CCUAAUGG	132
miR-128	GUUGGAUUCGGGGCCGUAGCACUGUCUGAGAGGU UUACAUUUCUCACAGUGAACCGGUCUCUUUUUCA GC	133
miR-129-1	UGGAUCUUUUUGCGGUCUGGGCUUGCUGUUCCUC UCAACAGUAGUCAGGAAGCCCUUACCCCAAAAAG UAUCUA	134
miR-129-2	UGCCCUUCGCGAAUCUUUUUGCGGUCUGGGCUUG CUGUACAUAACUCAAUAGCCGGAAGCCCUUACCCC AAAAAGCAUUUGCGGAGGGCG	135
miR-130a	UGCUGCUGGCCAGAGCUCUUUUCACAUUGUGCUA CUGUCUGCACCUGUCACUAGCAGUGCAAUGUUAA AAGGGCAUUGGCCGUGUAGUG	136
miR-131-1	GCCAGGAGGCGGGGUUGGUUGUUAUCUUUGGUUA UCUAGCUGUAUGAGUGGUGUGGAGUCUUCAUAAA GCUAGAUAACCGAAAGUAAAAAUAACCCCAUACA CUGCGCAG	137
miR-131-3	CACGGCGCGGCAGCGGCACUGGCUAAGGGAGGCC CGUUUCUCUCUUUGGUUAUCUAGCUGUAUGAGUG CCACAGAGCCGUCAUAAAGCUAGAUAACCGAAAG UAGAAAUG	138
miR-131	GUUGUUAUCUUUGGUUAUCUAGCUGUAUGAGUGU AUUGGUCUUCAUAAAGCUAGAUAACCGAAAGUAA AAAC	139
miR-132-1	CCGCCCCCGCGUCUCCAGGGCAACCGUGGCUUUCG AUUGUUACUGUGGGAACUGGAGGUAACAGUCUAC AGCCAUGGUCGCCCCGCAGCACGCCCACGCGC	140
miR-132-2	GGGCAACCGUGGCUUUCGAUUGUUACUGUGGGAA CUGGAGGUAACAGUCUACAGCCAUGGUCGCCC	141
miR-133a-1	ACAAUGCUUUGCUAGAGCUGGUAAAAUGGAACCA AAUCGCCUCUUCAAUGGAUUUGGUCCCCUUCAAC CAGCUGUAGCUAUGCAUUGA	142
miR-133a-2	GGGAGCCAAAUGCUUUGCUAGAGCUGGUAAAAUG GAACCAAAUCGACUGUCCAAUGGAUUUGGUCCCC UUCAACCAGCUGUAGCUGUGCAUUGAUGGCGCCG	143
miR-133	GCUAGAGCUGGUAAAAUGGAACCAAAUCGCCUCU UCAAUGGAUUUGGUCCCCUUCAACCAGCUGUAGC	144
miR-133b	CCUCAGAAGAAAGAUGCCCCCUGCUCUGGCUGGU CAAACGGAACCAAGUCCGUCUUCCUGAGAGGUUU GGUCCCCUUCAACCAGCUACAGCAGGGCUGGCAA UGCCCAGUCCUUGGAGA	145

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-133b-small	GCCCCCUGCUCUGGCUGGUCAAACGGAACCAAGUCCGUCUUCCUGAGAGGUUUGGUCCCCUUCAACCAGCUACAGCAGGG	146
miR-134-1	CAGGGUGUGUGACUGGUUGACCAGAGGGGCAUGCACUGUGUUCACCCUGUGGGCCACCUAGUCACCAACCCUC	147
miR-134-2	AGGGUGUGUGACUGGUUGACCAGAGGGGCAUGCACUGUGUUCACCCUGUGGGCCACCUAGUCACCAACCCU	148
miR-135a-1	AGGCCUCGCUGUUCUCUAUGGCUUUUUAUUCCUAUGUGAUUCUACUGCUCACUCAUAUAGGGAUUGGAGCCGUGGCGCACGGCGGGGACA	149
miR-135a-2 (miR-135-2)	AGAUAAAUUCACUCUAGUGCUUUAUGGCUUUUUAUUCCUAUGUGAUAGUAAUAAAGUCUCAUGUAGGGAUGGAAGCCAUGAAAUACAUUGUGAAAAAUCA	150
miR-135	CUAUGGCUUUUUAUUCCUAUGUGAUUCUACUGCUCACUCAUAUAGGGAUUGGAGCCGUGG	151
miR-135b	CACUCUGCUGUGGCCUAUGGCUUUUCAUUCCUAUGUGAUUGCUGUCCCAAACUCAUGUAGGGCUAAAAGCCAUGGGCUACAGUGAGGGGCGAGCUCC	152
miR-136-1	UGAGCCCUCGGAGGACUCCAUUUGUUUUGAUGAUGGAUUCUUAUGCUCCAUCAUCGUCUCAAAUGAGUCUUCAGAGGGUUCU	153
miR-136-2	GAGGACUCCAUUUGUUUUGAUGAUGGAUUCUUAUGCUCCAUCAUCGUCUCAAAUGAGUCUUC	154
miR-137	CUUCGGUGACGGGUAUUCUUGGGUGGAUAAUACGGAUUACGUUGUUAUUGCUUAAGAAUACGCGUAGUCGAGG	155
miR-138-1	CCCUGGCAUGGUGUGGUGGGGCAGCUGGUGUUGUGAAUCAGGCCGUUGCCAAUCAGAGAACGGCUACUUCACAACACCAGGGCCACACCACACUACAGG	156
miR-138-2	CGUUGCUGCAGCUGGUGUUGUGAAUCAGGCCGACGAGCAGCGCAUCCUCUUACCCGGCUAUUUCACGACACCAGGGUUGCAUCA	157
miR-138	CAGCUGGUGUUGUGAAUCAGGCCGACGAGCAGCGCAUCCUCUUACCCGGCUAUUUCACGACACCAGGGUUG	158
miR-139	GUGUAUUCUACAGUGCACGUGUCUCCAGUGUGGCUCGGAGGCUGGAGACGCGGCCCUGUUGGAGUAAC	159
miR-140	UGUGUCUCUCUCUGUGUCCUGCCAGUGGUUUUACCCUAUGGUAGGUUACGUCAUGCUGUUCUACCACAGGGUAGAACCACGGACAGGAUACCGGGGCACC	160
miR-140as	UCCUGCCAGUGGUUUUACCCUAUGGUAGGUUACGUCAUGCUGUUCUACCACAGGGUAGAACCACGGACAGGA	161

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-140s	CCUGCCAGUGGUUUUACCCUAUGGUAGGUUACGU CAUGCUGUUCUACCACAGGGUAGAACCACGGACA GG	162
miR-141-1	CGGCCGGCCCUGGGUCCAUCUUCCAGUACAGUGU UGGAUGGUCUAAUUGUGAAGCUCCUAACACUGUC UGGUAAAGAUGGCUCCCGGGUGGGUUC	163
miR-141-2	GGGUCCAUCUUCCAGUACAGUGUUGGAUGGUCUA AUUGUGAAGCUCCUAACACUGUCUGGUAAAGAUG GCCC	164
miR-142	ACCCAUAAAGUAGAAAGCACUACUAACAGCACUG GAGGGUGUAGUGUUUCCUACUUUAUGGAUG	165
miR-143-1	GCGCAGCGCCCUGUCUCCCAGCCUGAGGUGCAGUG CUGCAUCUCUGGUCAGUUGGGAGUCUGAGAUGAA GCACUGUAGCUCAGGAAGAGAGAAGUUGUUCUGC AGC	166
miR-143-2	CCUGAGGUGCAGUGCUGCAUCUCUGGUCAGUUGG GAGUCUGAGAUGAAGCACUGUAGCUCAGG	167
miR-144-1	UGGGGCCCUGGCUGGGAUAUCAUCAUAUACUGUA AGUUUGCGAUGAGACACUACAGUAUAGAUGAUGU ACUAGUCCGGGCACCCCC	168
miR-144-2	GGCUGGGAUAUCAUCAUAUACUGUAAGUUUGCGA UGAGACACUACAGUAUAGAUGAUGUACUAGUC	169
miR-145-1	CACCUUGUCCUCACGGUCCAGUUUUCCCAGGAAUC CCUUAGAUGCUAAGAUGGGGAUUCCUGGAAAUAC UGUUCUUGAGGUCAUGGUU	170
miR-145-2	CUCACGGUCCAGUUUUCCCAGGAAUCCCUUAGAU GCUAAGAUGGGGAUUCCUGGAAAUACUGUUCUUG AG	171
miR-146-1	CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUUC CAUGGGUUGUGUCAGUGUCAGACCUCUGAAAUUC AGUUCUUCAGCUGGGAUAUCUCUGUCAUCGU	172
miR-146-2	AGCUUUGAGAACUGAAUUCCAUGGGUUGUGUCAG UGUCAGACCUGUGAAAUUCAGUUCUUCAGCU	173
miR-147	AAUCUAAAGACAACAUUUCUGCACACACACCAGA CUAUGGAAGCCAGUGUGUGGAAAUGCUUCUGCUA GAUU	174
miR-148a (miR-148)	GAGGCAAAGUUCUGAGACACUCCGACUCUGAGUA UGAUAGAAGUCAGUGCACUACAGAACUUUGUCUC	175
miR-148b	CAAGCACGAUUAGCAUUUGAGGUGAAGUUCUGUU AUACACUCAGGCUGUGGCUCUCUGAAAGUCAGUG CAUCACAGAACUUUGUCUCGAAAGCUUUCUA	176
miR-148b-small	AAGCACGAUUAGCAUUUGAGGUGAAGUUCUGUUA UACACUCAGGCUGUGGCUCUCUGAAAGUCAGUGC AU	177

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-149-1	GCCGGCGCCCGAGCUCUGGCUCCGUGUCUUCACUCCCGUGCUUGUCCGAGGAGGGAGGGAGGGACGGGGGCUGUGCUGGGGCAGCUGGA	178
miR-149-2	GCUCUGGCUCCGUGUCUUCACUCCCGUGCUUGUCCGAGGAGGGAGGGAGGGAC	179
miR-150-1	CUCCCCAUGGCCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCCUGGUACAGGCCUGGGGGGACAGGGACCUGGGGAC	180
miR-150-2	CCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCCUGGUACAGGCCUGGGGGACAGGG	181
miR-151	UUUCCUGCCCUCGAGGAGCUCACAGUCUAGUAUGUCUCAUCCCCUACUAGACUGAAGCUCCUUGAGGACAGG	182
miR-151-2	CCUGUCCUCAAGGAGCUUCAGUCUAGUAGGGGAUGAGACAUACUAGACUGUGAGCUCCUCGAGGGCAGG	183
miR-152-1	UGUCCCCCCCGGCCCAGGUUCUGUGAUACACUCCGACUCGGGCUCUGGAGCAGUCAGUGCAUGACAGAACUUGGGCCCGGAAGGACC	184
miR-152-2	GGCCCAGGUUCUGUGAUACACUCCGACUCGGGCUCUGGAGCAGUCAGUGCAUGACAGAACUUGGGCCCCGG	185
miR-153-1-1	CUCACAGCUGCCAGUGUCAUUUUUGUGAUCUGCAGCUAGUAUUCUCACUCCAGUUGCAUAGUCACAAAAGUGAUCAUUGGCAGGUGUGGC	186
miR-153-1-2	UCUCUCUCUCCCUCACAGCUGCCAGUGUCAUUGUCACAAAAGUGAUCAUUGGCAGGUGUGGCUGCUGCAUG	187
miR-153-2-1	AGCGGUGGCCAGUGUCAUUUUUGUGAUGUUGCAGCUAGUAAUAUGAGCCCAGUUGCAUAGUCACAAAAGUGAUCAUUGGAAACUGUG	188
miR-153-2-2	CAGUGUCAUUUUUGUGAUGUUGCAGCUAGUAAUAUGAGCCCAGUUGCAUAGUCACAAAAGUGAUCAUUG	189
miR-154-1	GUGGUACUUGAAGAUAGGUUAUCCGUGUUGCCUUCGCUUUAUUUGUGACGAAUCAUACACGGUUGACCUAUUUUUCAGUACCAA	190
miR-154-2	GAAGAUAGGUUAUCCGUGUUGCCUUCGCUUUAUUUGUGACGAAUCAUACACGGUUGACCUAUUUUU	191
miR-155	CUGUUAAUGCUAAUCGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACAUAUUAGCAUUAACAG	192
miR-156 = miR-157=overlap miR-141	CCUAACACUGUCUGGUAAAGAUGGCUCCCGGGUGGGUUCUCUCGGCAGUAACCUUCAGGGAGCCCUGAAGACCAUGGAGGAC	193

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-158-small = miR-192	GCCGAGACCGAGUGCACAGGGCUCUGACCUAUGAAUUGACAGCCAGUGCUCUCGUCUCCCCUCUGGCUGCCAAUUCCAUAGGUCACAGGUAUGUUCGCCUCAAUGCCAGC	194
miR-159-1-small	UCCCGCCCCCUGUAACAGCAACUCCAUGUGGAAGUGCCCACUGGUUCCAGUGGGGCUGCUGUUAUCUGGGGCGAGGGCCA	195
miR-161-small	AAAGCUGGGUUGAGAGGGCGAAAAAGGAUGAGGUGACUGGUCUGGGCUACGCUAUGCUGCGGCGCUCGGG	196
miR-163-1b-small	CAUUGGCCUCCUAAGCCAGGGAUUGUGGGUUCGAGUCCCACCCGGGGUAAAGAAAGGCCGAAUU	197
miR-163-3-small	CCUAAGCCAGGGAUUGUGGGUUCGAGUCCCACCUGGGGUAGAGGUGAAAGUUCCUUUUACGGAAUUUUUU	198
miR-162	CAAUGUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUAUCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGACCAUACUCUACAGUUG	199
miR-175-small=miR-224	GGGCUUUCAAGUCACUAGUGGUUCCGUUUAGUAGAUGAUUGUGCAUUGUUUCAAAAUGGUGCCCUAGUGACUACAAAGCCC	200
miR-177-small	ACGCAAGUGUCCUAAGGUGAGCUCAGGGAGCACAGAAACCUCCAGUGGAACAGAAGGGCAAAAGCUCAUU	201
miR-180-small	CAUGUGUCACUUUCAGGUGGAGUUUCAAGAGUCCCUUCCUGGUUCACCGUCUCCUUUGCUCUUCCACAAC	202
miR-181a	AGAAGGGCUAUCAGGCCAGCCUUCAGAGGACUCCAAGGAACAUUCAACGCUGUCGGUGAGUUUGGGAUUUUGAAAAAACCACUGACCGUUGACUGUACCUUGGGGUCCUUA	203
miR-181b-1	CCUGUGCAGAGAUUAUUUUUUAAAAGGUCACAAUCAACAUUCAUUGCUGUCGGUGGGUUGAACUGUGUGGACAAGCUCACUGAACAAUGAAUGCAACUGUGGCCCCGCUU	204
miR-181b-2	CUGAUGGCUGCACUCAACAUUCAUUGCUGUCGGUGGGUUUGAGUCUGAAUCAACUCACUGAUCAAUGAAUGCAAACUGCGGACCAAACA	205
miR-181c	CGGAAAAUUUGCCAAGGGUUUGGGGGAACAUUCAACCUGUCGGUGAGUUUGGGCAGCUCAGGCAAACCAUCGACCGUUGAGUGGACCCUGAGGCCUGGAAUUGCCAUCCU	206

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-182-as	GAGCUGCUUGCCUCCCCCCGUUUUUGGCAAUGGU AGAACUCACACUGGUGAGGUAACAGGAUCCGGUG GUUCUAGACUUGCCAACUAUGGGGCGAGGACUCA GCCGGCAC	207
miR-182	UUUUUGGCAAUGGUAGAACUCACACUGGUGAGGU AACAGGAUCCGGUGGUUCUAGACUUGCCAACUAU GG	208
miR-183	CCGCAGAGUGUGACUCCUGUUCUGUGUAUGGCAC UGGUAGAAUUCACUGUGAACAGUCUCAGUCAGUG AAUUACCGAAGGGCCAUAAACAGAGCAGAGACAG AUCCACGA	209
miR-184-1	CCAGUCACGUCCCCUUAUCACUUUUCCAGCCCAGC UUUGUGACUGUAAGUGUUGGACGGAGAACUGAUA AGGGUAGGUGAUUGA	210
miR-184-2	CCUUAUCACUUUUCCAGCCCAGCUUUGUGACUGU AAGUGUUGGACGGAGAACUGAUAAGGGUAGG	211
miR-185-1	AGGGGGCGAGGGAUUGGAGAGAAAGGCAGUUCCU GAUGGUCCCCUCCCCAGGGGCUGGCUUUCCUCUGG UCCUUCCCUCCCA	212
miR-185-2	AGGGAUUGGAGAGAAAGGCAGUUCCUGAUGGUCC CCUCCCCAGGGGCUGGCUUUCCUCUGGUCCUU	213
miR-186-1	UGCUUGUAACUUUCCAAAGAAUUCUCCUUUUGGG CUUUCUGGUUUUAUUUUAAGCCCAAAGGUGAAUU UUUUGGGAAGUUUGAGCU	214
miR-186-2	ACUUUCCAAAGAAUUCUCCUUUUGGGCUUUCUGG UUUUAUUUUAAGCCCAAAGGUGAAUUUUUUGGGA AGU	215
miR-187	GGUCGGGCUCACCAUGACACAGUGUGAGACUCGG GCUACAACACAGGACCCGGGGCGCUGCUCUGACCC CUCGUGUCUUGUGUUGCAGCCGGAGGGACGCAGG UCCGCA	216
miR-188-1	UGCUCCCUCUCUCACAUCCCUUGCAUGGUGGAGG GUGAGCUUUCUGAAAACCCCUCCCACAUGCAGGG UUUGCAGGAUGGCGAGCC	217
miR-188-2	UCUCACAUCCCUUGCAUGGUGGAGGGUGAGCUUU CUGAAAACCCCUCCCACAUGCAGGGUUUGCAGGA	218
miR-189-1	CUGUCGAUUGGACCCGCCCUCCGGUGCCUACUGAG CUGAUAUCAGUUCUCAUUUUACACACUGGCUCAG UUCAGCAGGAACAGGAGUCGAGCCCUUGAGCAA	219
miR-189-2	CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAU UUUACACACUGGCUCAGUUCAGCAGGAACAGGAG	220
miR-190-1	UGCAGGCCUCUGUGUGAUAUGUUUGAUAUAUUAG GUUGUUAUUUAAUCCAACUAUAUAUCAAACAUAU UCCUACAGUGUCUUGCC	221

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-190-2	CUGUGUGAUAUGUUUGAUAUAUUAGGUUGUUAUU UAAUCCAACUAUAUAUCAAACAUAUUCCUACAG	222
miR-191-1	CGGCUGGACAGCGGGCAACGGAAUCCCAAAAGCA GCUGUUGUCUCCAGAGCAUUCCAGCUGCGCUUGG AUUUCGUCCCCUGCUCUCCUGCCU	223
miR-191-2	AGCGGGCAACGGAAUCCCAAAAGCAGCUGUUGUC UCCAGAGCAUUCCAGCUGCGCUUGGAUUUCGUCC CCUGCU	224
miR-192-2/3	CCGAGACCGAGUGCACAGGGCUCUGACCUAUGAA UUGACAGCCAGUGCUCUCGUCUCCCCUCUGGCUGC CAAUUCCAUAGGUCACAGGUAUGUUCGCCUCAAU GCCAG	225
miR-192	GCCGAGACCGAGUGCACAGGGCUCUGACCUAUGA AUUGACAGCCAGUGCUCUCGUCUCCCCUCUGGCUG CCAAUUCCAUAGGUCACAGGUAUGUUCGCCUCAA UGCCAGC	226
miR-193-1	CGAGGAUGGGAGCUGAGGGCUGGGUCUUUGCGGG CGAGAUGAGGGUGUCGGAUCAACUGGCCUACAAA GUCCCAGUUCUCGGCCCCCG	227
miR-193-2	GCUGGGUCUUUGCGGGCGAGAUGAGGGUGUCGGA UCAACUGGCCUACAAAGUCCCAGU	228
miR-194-1	AUGGUGUUAUCAAGUGUAACAGCAACUCCAUGUG GACUGUGUACCAAUUUCCAGUGGAGAUGCUGUUA CUUUUGAUGGUUACCAA	229
miR-194-2	GUGUAACAGCAACUCCAUGUGGACUGUGUACCAA UUUCCAGUGGAGAUGCUGUUACUUUUGAU	230
miR-195-1	AGCUUCCCUGGCUCUAGCAGCACAGAAAUAUUGG CACAGGGAAGCGAGUCUGCCAAUAUUGGCUGUGC UGCUCCAGGCAGGGUGGUG	231
miR-195-2	UAGCAGCACAGAAAUAUUGGCACAGGGAAGCGAG UCUGCCAAUAUUGGCUGUGCUGCU	232
miR-196-1	CUAGAGCUUGAAUUGGAACUGCUGAGUGAAUUAG GUAGUUUCAUGUUGUUGGGCCUGGGUUUCUGAAC ACAACAACAUUAAACCACCCGAUUCACGGCAGUU ACUGCUCC	233
miR-196a-1	GUGAAUUAGGUAGUUUCAUGUUGUUGGGCCUGGG UUUCUGAACACAACAACAUUAAACCACCCGAUUC AC	234
miR-196a-2 (miR-196-2)	UGCUCGCUCAGCUGAUCUGUGGCUUAGGUAGUUU CAUGUUGUUGGGAUUGAGUUUUGAACUCGGCAAC AAGAAACUGCCUGAGUUACAUCAGUCGGUUUUCG UCGAGGGC	235
miR-196	GUGAAUUAGGUAGUUUCAUGUUGUUGGGCCUGGG UUUCUGAACACAACAACAUUAAACCACCCGAUUC AC	236

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-196b	ACUGGUCGGUGAUUUAGGUAGUUUCCUGUUGUUG GGAUCCACCUUUCUCUCGACAGCACGACACUGCCU UCAUUACUUCAGUUG	237
miR-197	GGCUGUGCCGGGUAGAGAGGGCAGUGGGAGGUAA GAGCUCUUCACCCUUCACCACCUUCUCCACCCAGC AUGGCC	238
miR-197-2	GUGCAUGUGUAUGUAUGUGUGCAUGUGCAUGUGU AUGUGUAUGAGUGCAUGCGUGUGUGC	239
miR-198	UCAUUGGUCCAGAGGGGAGAUAGGUUCCUGUGAU UUUUCCUUCUUCUCUAUAGAAUAAAUGA	240
miR-199a-1	GCCAACCCAGUGUUCAGACUACCUGUUCAGGAGG CUCUCAAUGUGUACAGUAGUCUGCACAUUGGUUA GGC	241
miR-199a-2	AGGAAGCUUCUGGAGAUCCUGCUCCGUCGCCCCA GUGUUCAGACUACCUGUUCAGGACAAUGCCGUUG UACAGUAGUCUGCACAUUGGUUAGACUGGGCAAG GGAGAGCA	242
miR-199b	CCAGAGGACACCUCCACUCCGUCUACCCAGUGUUU AGACUAUCUGUUCAGGACUCCCAAAUUGUACAGU AGUCUGCACAUUGGUUAGGCUGGGCUGGGUUAGA CCCUCGG	243
miR-199s	GCCAACCCAGUGUUCAGACUACCUGUUCAGGAGG CUCUCAAUGUGUACAGUAGUCUGCACAUUGGUUA GGC	244
miR-200a	GCCGUGGCCAUCUUACUGGGCAGCAUUGGAUGGA GUCAGGUCUCUAAUACUGCCUGGUAAUGAUGACG GC	245
miR-200b	CCAGCUCGGGCAGCCGUGGCCAUCUUACUGGGCA GCAUUGGAUGGAGUCAGGUCUCUAAUACUGCCUG GUAAUGAUGACGGCGGAGCCCUGCACG	246
miR-200c	CCCUCGUCUUACCCAGCAGUGUUUGGGUGCGGUU GGGAGUCUCUAAUACUGCCGGGUAAUGAUGGAGG	247
miR-202	GUUCCUUUUUCCUAUGCAUAUACUUCUUUGAGGA UCUGGCCUAAAGAGGUAUAGGGCAUGGGAAGAUG GAGC	248
miR-203	GUGUUGGGGACUCGCGCGCUGGGUCCAGUGGUUC UUAACAGUUCAACAGUUCUGUAGCGCAAUUGUGA AAUGUUUAGGACCACUAGACCCGGCGGGCGCGGC GACAGCGA	249
miR-204	GGCUACAGUCUUUCUUCAUGUGACUCGUGGACUU CCCUUUGUCAUCCUAUGCCUGAGAAUAUAUGAAG GAGGCUGGGAAGGCAAAGGGACGUUCAAUUGUCA UCACUGGC	250

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-205	AAAGAUCCUCAGACAAUCCAUGUGCUUCUCUUGUCCUUCAUUCCACCGGAGUCUGUCUCAUACCCAACCAGAUUUCAGUGGAGUGAAGUUCAGGAGGCAUGGAGCUGACA	251
miR-206-1	UGCUUCCCGAGGCCACAUGCUUCUUUAUAUCCCCAUAUGGAUUACUUUGCUAUGGAAUGUAAGGAAGUGUGUGGUUUCGGCAAGUG	252
miR-206-2	AGGCCACAUGCUUCUUUAUAUCCCCAUAUGGAUUACUUUGCUAUGGAAUGUAAGGAAGUGUGUGGUUUU	253
miR-208	UGACGGGCGAGCUUUUGGCCCGGGUUAUACCUGAUGCUCACGUAUAAGACGAGCAAAAAGCUUGUUGGUCA	254
miR-210	ACCCGGCAGUGCCUCCAGGCGCAGGGCAGCCCCUGCCCACCGCACACUGCGCUGCCCCAGACCCACUGUGCGUGUGACAGCGGCUGAUCUGUGCCUGGGCAGCGCGACCC	255
miR-211	UCACCUGGCCAUGUGACUUGUGGGCUUCCCUUUGUCAUCCUUCGCCUAGGGCUCUGAGCAGGGCAGGGACAGCAAAGGGGUGCUCAGUUGUCACUUCCCACAGCACGGAG	256
miR-212	CGGGGCACCCCGCCCGGACAGCGCGCCGGCACCUUGGCUCUAGACUGCUUACUGCCCGGGCCGCCCUCAGUAACAGUCUCCAGUCACGGCCACCGACGCCUGGCCCCGCC	257
miR-213-2	CCUGUGCAGAGAUUAUUUUUUAAAAGGUCACAAUCAACAUUCAUUGCUGUCGGUGGGUUGAACUGUGUGGACAAGCUCACUGAACAAUGAAUGCAACUGUGGCCCCGCUU	258
miR-213	GAGUUUUGAGGUUGCUUCAGUGAACAUUCAACGCUGUCGGUGAGUUUGGAAUUAAAAUCAAAACCAUCGACCGUUGAUUGUACCCUAUGGCUAACCAUCAUCUACUCC	259
miR-214	GGCCUGGCUGGACAGAGUUGUCAUGUGUCUGCCUGUCUACACUUGCUGUGCAGAACAUCCGCUCACCUGUACAGCAGGCACAGACAGGCAGUCACAUGACAACCCAGCCU	260
miR-215	AUCAUUCAGAAAUGGUAUACAGGAAAAUGACCUAUGAAUUGACAGACAAUAUAGCUGAGUUUGUCUGUCAUUUCUUUAGGCCAAUAUUCUGUAUGACUGUGCUACUUCAA	261
miR-216	GAUGGCUGUGAGUUGGCUUAAUCUCAGCUGGCAACUGUGAGAUGUUCAUACAAUCCCUCACAGUGGUCUCUGGGAUUAUGCUAAACAGAGCAAUUUCCUAGCCCUCACGA	262

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-217	AGUAUAAUUAUUACAUAGUUUUUGAUGUCGCAGA UACUGCAUCAGGAACUGAUUGGAUAAGAAUCAGU CACCAUCAGUUCCUAAUGCAUUGCCUUCAGCAUC UAAACAAG	263
miR-218-1	GUGAUAAUGUAGCGAGAUUUUCUGUUGUGCUUGA UCUAACCAUGUGGUUGCGAGGUAUGAGUAAAACA UGGUUCCGUCAAGCACCAUGGAACGUCACGCAGC UUUCUACA	264
miR-218-2	GACCAGUCGCUGCGGGGCUUUCCUUUGUGCUUGA UCUAACCAUGUGGUGGAACGAUGGAAACGGAACA UGGUUCUGUCAAGCACCGCGGAAAGCACCGUGCU CUCCUGCA	265
miR-219	CCGCCCCGGGCCGCGGCUCCUGAUUGUCCAAACGC AAUUCUCGAGUCUAUGGCUCCGGCCGAGAGUUGA GUCUGGACGUCCCGAGCCGCCGCCCCCAAACCUCG AGCGGG	266
miR-219-1	CCGCCCCGGGCCGCGGCUCCUGAUUGUCCAAACGC AAUUCUCGAGUCUAUGGCUCCGGCCGAGAGUUGA GUCUGGACGUCCCGAGCCGCCGCCCCCAAACCUCG AGCGGG	267
miR-219-2	ACUCAGGGGCUUCGCCACUGAUUGUCCAAACGCA AUUCUUGUACGAGUCUGCGGCCAACCGAGAAUUG UGGCUGGACAUCUGUGGCUGAGCUCCGGG	268
miR-220	GACAGUGUGGCAUUGUAGGGCUCCACACCGUAUC UGACACUUUGGGCGAGGGCACCAUGCUGAAGGUG UUCAUGAUGCGGUCUGGGAACUCCUCACGGAUCU UACUGAUG	269
miR-221	UGAACAUCCAGGUCUGGGGCAUGAACCUGGCAUA CAAUGUAGAUUUCUGUGUUCGUUAGGCAACAGCU ACAUUGUCUGCUGGGUUUCAGGCUACCUGGAAAC AUGUUCUC	270
miR-222	GCUGCUGGAAGGUGUAGGUACCCUCAAUGGCUCA GUAGCCAGUGUAGAUCCUGUCUUUCGUAAUCAGC AGCUACAUCUGGCUACUGGGUCUCUGAUGGCAUC UUCUAGCU	271
miR-223	CCUGGCCUCCUGCAGUGCCACGCUCCGUGUAUUUG ACAAGCUGAGUUGGACACUCCAUGUGGUAGAGUG UCAGUUUGUCAAAUACCCCAAGUGCGGCACAUGC UUACCAG	272
miR-224	GGGCUUUCAAGUCACUAGUGGUUCCGUUUAGUAG AUGAUUGUGCAUUGUUUCAAAAUGGUGCCCUAGU GACUACAAAGCCC	273

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-294-1 (chr16)	CAAUCUUCCUUUAUCAUGGUAUUGAUUUUUCAGUGCU UCCCUUUUGUGUGAGAGAAGAUA	274
miR-296	AGGACCCUUCCAGAGGGCCCCCCCUCAAUCCUGUUGUG CCUAAUUCAGAGGGUUGGGUGGAGGCUCUCCUGAAGG GCUCU	275
miR-299	AAGAAAUGGUUUACCGUCCCACAUACAUUUUGAAUAU GUAUGUGGGAUGGUAAACCGCUUCUU	276
miR-301	ACUGCUAACGAAUGCUCUGACUUUAUUGCACUACUGU ACUUUACAGCUAGCAGUGCAAUAGUAUUGUCAAAGCA UCUGAAAGCAGG	277
miR-302a	CCACCACUUAAACGUGGAUGUACUUGCUUUGAAACUA AAGAAGUAAGUGCUUCCAUGUUUUGGUGAUGG	278
miR-302b	GCUCCCUUCAACUUUAACAUGGAAGUGCUUUCUGUGA CUUUAAAAGUAAGUGCUUCCAUGUUUUAGUAGGAGU	279
miR-302c	CCUUUGCUUUAACAUGGGGGUACCUGCUGUGUGAAAC AAAAGUAAGUGCUUCCAUGUUUCAGUGGAGG	280
miR-302d	CCUCUACUUUAACAUGGAGGCACUUGCUGUGACAUGA CAAAAAUAAGUGCUUCCAUGUUUGAGUGUGG	281
miR-320	GCUUCGCUCCCCUCCGCCUUCUCUUCCCGGUUCUUCCC GGAGUCGGGAAAAGCUGGGUUGAGAGGGCGAAAAAGG AUGAGGU	282
miR-321	UUGGCCUCCUAAGCCAGGGAUUGUGGGUUCGAGUCCC ACCCGGGGUAAAGAAAGGCCGA	283
miR-323	UUGGUACUUGGAGAGAGGUGGUCCGUGGCGCGUUCGC UUUAUUUAUGGCGCACAUUACACGGUCGACCUCUUUG CAGUAUCUAAUC	284
miR-324	CUGACUAUGCCUCCCCGCAUCCCCUAGGGCAUUGGUGU AAAGCUGGAGACCCACUGCCCCAGGUGCUGCUGGGGGU UGUAGUC	285
miR-325	AUACAGUGCUUGGUUCCUAGUAGGUGUCCAGUAAGUG UUUGUGACAUAAUUUGUUUAUUGAGGACCUCCUAUCA AUCAAGCACUGUGCUAGGCUCUGG	286
miR-326	CUCAUCUGUCUGUUGGGCUGGAGGCAGGGCCUUUGUG AAGGCGGGUGGUGCUCAGAUCGCCUCUGGGCCCUUCCU CCAGCCCCGAGGCGGAUUCA	287
miR-328	UGGAGUGGGGGGGCAGGAGGGGCUCAGGGAGAAAGUG CAUACAGCCCCUGGCCCUCUCUGCCCUUCCGUCCCCUG	288
miR-330	CUUUGGCGAUCACUGCCUCUCUGGGCCUGUGUCUUAGG CUCUGCAAGAUCAACCGAGCAAAGCACACGGCCUGCAG AGAGGCAGCGCUCUGCCC	289
miR-331	GAGUUUGGUUUUGUUUGGGUUUGUUCUAGGUAUGGUC CCAGGGAUCCCAGAUCAAACCAGGCCCCUGGGCCUAUC CUAGAACCAACCUAAGCUC	290

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-335	UGUUUUGAGCGGGGGUCAAGAGCAAUAACGAAAAAUG UUUGUCAUAAACCGUUUUUCAUUAUUGCUCCUGACCU CCUCUCAUUUGCUAUAUUCA	291
miR-337	GUAGUCAGUAGUUGGGGGGUGGGAACGGCUUCAUACA GGAGUUGAUGCACAGUUAUCCAGCUCCUAUAUGAUGC CUUUCUUCAUCCCCUUCAA	292
miR-338	UCUCCAACAAUAUCCUGGUGCUGAGUGAUGACUCAGG CGACUCCAGCAUCAGUGAUUUUGUUGAAGA	293
miR-339	CGGGGCGGCCGCUCUCCCUGUCCUCCAGGAGCUCACGU GUGCCUGCCUGUGAGCGCCUCGACGACAGAGCCGGCGC CUGCCCCAGUGUCUGCGC	294
miR-340	UUGUACCUGGUGUGAUUAUAAAGCAAUGAGACUGAUU GUCAUAUGUCGUUUGUGGGAUCCGUCUCAGUUACUUU AUAGCCAUACCUGGUAUCUUA	295
miR-342	GAAACUGGGCUCAAGGUGAGGGGUGCUAUCUGUGAUU GAGGGACAUGGUUAAUGGAAUUGUCUCACACAGAAAU CGCACCCGUCACCUUGGCCUACUUA	296
miR-345	ACCCAAACCCUAGGUCUGCUGACUCCUAGUCCAGGGCU CGUGAUGGCUGGUGGGCCCUGAACGAGGGGUCUGGAG GCCUGGGUUUGAAUAUCGACAGC	297
miR-346	GUCUGUCUGCCCGCAUGCCUGCCUCUCUGUUGCUCUGA AGGAGGCAGGGGCUGGGCCUGCAGCUGCCUGGGCAGA GCGGCUCCUGC	298
miR-367	CCAUUACUGUUGCUAAUAUGCAACUCUGUUGAAUAUA AAUUGGAAUUGCACUUUAGCAAUGGUGAUGG	299
miR-368	AAAAGGUGGAUAUUCCUUCUAUGUUUAUGUUAUUUAU GGUUAAACAUAGAGGAAAUUCCACGUUUU	300
miR-369	UUGAAGGGAGAUCGACCGUGUUAUAUUCGCUUUAUUG ACUUCGAAUAAUACAUGGUUGAUCUUUUCUCAG	301
miR-370	AGACAGAGAAGCCAGGUCACGUCUCUGCAGUUACACA GCUCACGAGUGCCUGCUGGGGUGGAACCUGGUCUGUC U	302
miR-371	GUGGCACUCAAACUGUGGGGGCACUUUCUGCUCUCUG GUGAAAGUGCCGCCAUCUUUUGAGUGUUAC	303
miR-372	GUGGGCCUCAAAUGUGGAGCACUAUUCUGAUGUCCAA GUGGAAAGUGCUGCGACAUUUGAGCGUCAC	304
miR-373	GGGAUACUCAAAAUGGGGGCGCUUUCCUUUUUGUCUG UACUGGGAAGUGCUUCGAUUUUGGGGUGUCCC	305
miR-374	UACAUCGGCCAUUAUAAUACAACCUGAUAAGUGUUAU AGCACUUAUCAGAUUGUAUUGUAAUUGUCUGUGUA	306
miR-hes1	AUGGAGCUGCUCACCCUGUGGGCCUCAAAUGUGGAGG AACUAUUCUGAUGUCCAAGUGGAAAGUGCUGCGACAU UUGAGCGUCACCGGUGACGCCCAUAUCA	307

Precursor Name	Sequence (5' To 3')*	SEQ ID NO.
miR-hes2	GCAUCCCCUCAGCCUGUGGCACUCAAACUGUGGGGGCA CUUUCUGCUCUCUGGUGAAAGUGCCGCCAUCUUUUGA GUGUUACCGCUUGAGAAGACUCAACC	308
miR-hes3	CGAGGAGCUCAUACUGGGAUACUCAAAAUGGGGGCGC UUUCCUUUUUGUCUGUUACUGGGAAGUGCUUCGAUUU UGGGGUGUCCCUGUUUGAGUAGGGCAUC	309

* An underlined sequence within a precursor sequence corresponds to a mature processed miR transcript (see Table 1b). Some precursor sequences have two underlined sequences denoting two different mature miRs that are derived from the same precursor. All sequences are human.

Table 1b- Human Mature microRNA Sequences.

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
let-7a	ugagguaguagguuguauaguu	310	*let-7a-1; let-7a-2; let-7a-3; let-7a-4*
let-7b	ugagguaguagguuguguggguu	311	*let-7b*
let-7c	ugagguaguagguuguaugguu	312	*let-7c*
let-7d	agagguaguagguugcauagu	313	*let-7d; let-7d-v1*
let-7e	ugagguaggagguuguauagu	314	*let-7e*
let-7f	ugagguaguagauuguauaguu	315	*let-7f-1; let-7f-2-1; let-7f-2-2*
let-7g	ugagguaguaguuuguacagu	316	*let-7g*
let-7i	ugagguaguaguuugugcu	317	*let-7i*
miR-1	uggaauguaaagaaguaugua	318	*miR-1b; miR-1b-1; miR-1b-2*
miR-7	uggaagacuagugauuuuguu	319	*miR-7-1; miR-7-1a; miR-7-2; miR-7-3*
miR-9	ucuuugguuaucuagcuguaug a	320	*miR-9-1; miR-9-2; miR-9-3*
*miR-9**	uaaagcuagauaaccgaaagu	321	*miR-9-1; miR-9-2; miR-9-3*
miR-10a	uacccuguagauccgaauuugug	322	*miR-10a*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-10b	uacccuguagaaccgaauuugu	323	*miR-10b*
miR-15a	uagcagcacauaaugguuugug	324	*miR-15a; miR-15a-2*
miR-15b	uagcagcacaucaugguuuaca	325	*miR-15b*
miR-16	uagcagcacguaaauauuggcg	326	*miR-16-1; miR-16-2; miR-16-13*
miR-17-5p	caaagugcuuacagugcagguag u	327	*miR-17*
miR-17-3p	acugcagugaaggcacuugu	328	*miR-17*
miR-18	uaaggugcaucuagugcagaua	329	*miR-18; miR-18-13*
miR-19a	ugugcaaaucuaugcaaaacuga	330	*miR-19a; miR-19a-13*
miR-19b	ugugcaaauccaugcaaaacuga	331	*miR-19b-1; miR-19b-2*
miR-20	uaaagugcuuauagugcaggua	332	*miR-20 (miR-20a)*
miR-21	uagcuuaucagacugauguuga	333	*miR-21; miR-21-17*
miR-22	aagcugccaguugaagaacugu	334	*miR-22*
miR-23a	aucacauugccagggauuucc	335	*miR-23a*
miR-23b	aucacauugccagggauuaccac	336	*miR-23b*
miR-24	uggcucaguucagcaggaacag	337	*miR-24-1; miR-24-2; miR-24-19; miR-24-9*
miR-25	cauugcacuugucucggucuga	338	*miR-25*
miR-26a	uucaaguaauccaggauaggcu	339	*miR-26a; miR-26a-1; miR-26a-2*
miR-26b	uucaaguaauucaggauaggu	340	*miR-26b*
miR-27a	uucacaguggcuaaguuccgcc	341	*miR-27a*
miR-27b	uucacaguggcuaaguucug	342	*miR-27b-1; miR-27b-2*
miR-28	aaggagcucacagucuauugag	343	*miR-28*
miR-29a	cuagcaccaucugaaaucgguu	344	*miR-29a-2; miR-29a*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-29b	uagcaccauuugaaaucagu	345	*miR-29b-1; miR-29b-2*
miR-29c	uagcaccauuugaaaucgguua	346	*miR-29c*
miR-30a-5p	uguaaacauccucgacuggaagc	347	*miR-30a*
miR-30a-3p	cuuucagucggauguuugcagc	348	*miR-30a*
miR-30b	uguaaacauccuacacucagc	349	*miR-30b-1; miR-30b-2*
miR-30c	uguaaacauccuacacucucagc	350	*miR-30c*
miR-30d	uguaaacaucccccgacuggaag	351	*miR-30d*
miR-30e	uguaaacauccuugacugga	352	*miR-30e*
miR-31	ggcaagaugcuggcauagcug	353	*miR-31*
miR-32	uauugcacauuacuaaguugc	354	*miR-32*
miR-33	gugcauuguaguugcauug	355	*miR-33; miR-33b*
miR-34a	uggcagugucuuagcugguugu	356	*miR-34a*
miR-34b	aggcagugucauuagcugauug	357	*miR-34b*
miR-34c	aggcaguguaguuagcugauug	358	*miR-34c*
miR-92	uauugcacuugucccggccugu	359	*miR-92-2; miR-92-1*
miR-93	aaagugcuguucgugcagguag	360	*miR-93-1; miR-93-2*
miR-95	uucaacggguauuuauugagca	361	*miR-95*
miR-96	uuuggcacuagcacauuuuugc	362	*miR-96*
miR-98	ugagguaguaaguuguauuguu	363	*miR-98*
miR-99a	aacccguagauccgaucuugug	364	*miR-99a*
miR-99b	cacccguagaaccgaccuugcg	365	*miR-99b*
miR-100	uacaguacugugauaacugaag	366	*miR-100*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-101	uacaguacugugauaacugaag	367	*miR-101-1; miR-101-2*
miR-103	agcagcauuguacagggcuauga	368	*miR-103-1*
miR-105	ucaaaugcucagacuccugu	369	*miR-105*
miR-106-a	aaaagugcuuacagugcagguag c	370	*miR-106-a*
miR-106-b	uaaagugcugacagugcagau	371	*miR-106-b*
miR-107	agcagcauuguacagggcuauca	372	*miR-107*
miR-122a	uggagugugacaaugguguuug u	373	*miR-122a-1; miR-122a-2*
miR-124a	uuaaggcacgcggugaaugcca	374	*miR-124a-1; miR-124a-2; miR-124a-3*
miR-125a	ucccugagacccuuuaaccugug	375	*miR-125a-1; miR-125a-2*
miR-125b	ucccugagacccuaacuuguga	376	*miR-125b-1; miR-125b-2*
*miR-126**	cauuauuacuuuugguacgcg	377	*miR-126-1; miR-126-2*
miR-126	ucguaccgugaguaauaaugc	378	*miR-126-1; miR-126-2*
miR-127	ucggauccgucugagcuuggcu	379	*miR-127-1; miR-127-2*
miR-128a	ucacagugaaccggucucuuuu	380	*miR-128; miR-128a*
miR-128b	ucacagugaaccggucucuuuc	381	*miR-128b*
miR-129	cuuuuugcggucugggcuugc	382	*miR-129-1; miR-129-2*
miR-130a	cagugcaauguuaaaagggc	383	*miR-130a*
miR-130b	cagugcaaugaugaaagggcau	384	*miR-130b*
miR-132	uaacagucuacagccauggucg	385	*miR-132-1*
miR-133a	uuggucccuucaaccagcugu	386	*miR-133a-1; miR-133a-2*
miR-133b	uuggucccuucaaccagcua	387	*miR-133b*
miR-134	ugugacugguugaccagaggg	388	*miR-134-1; miR-134-2*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-135a	uauggcuuuuuauuccuaugug a	389	*miR-135a; miR-135a-2 (miR-135-2)*
miR-135b	uauggcuuuucauuccuaugug	390	*miR-135b*
miR-136	acuccauuuguuuugaugaugga	391	*miR-136-1; miR-136-2*
miR-137	uauugcuuaagaauacgcguag	392	*miR-137*
miR-138	agcuggugugugaauc	393	*miR-138-1; miR-138-2*
miR-139	ucuacagugcacgugucu	394	*miR-139*
miR-140	agugguuuuacccuaugguag	395	*miR-140; miR-140as; miR-140s*
miR-141	aacacugucugguaaagaugg	396	*miR-141-1; miR-141-2*
miR-142-3p	uguaguguuuccuacuuuaugg a	397	*miR-142*
miR-142-5p	cauaaaguagaaagcacuac	398	*miR-142*
miR-143	ugagaugaagcacuguagcuca	399	*miR-143-1*
miR-144	uacaguauagaugauguacuag	400	*miR-144-1; miR-144-2*
miR-145	guccaguuuucccaggaaucccu u	401	*miR-145-1; miR-145-2*
miR-146	ugagaacugaauuccauggguu	402	*miR-146-1; miR-146-2*
miR-147	guguguggaaaugcuucugc	403	*miR-147*
miR-148a	ucagugcacuacagaacuuugu	404	*miR-148a (miR-148)*
miR-148b	ucagugcaucacagaacuuugu	405	*miR-148b*
miR-149	ucuggcuccgugucuucacucc	406	*miR-149*
miR-150	ucucccaacccuuguaccagug	407	*miR-150-1; miR-150-2*
miR-151	acuagacugaagcuccuugagg	408	*miR-151*
miR-152	ucagugcaugacagaacuugg	409	*miR-152-1; miR-152-2*
miR-153	uugcauagucacaaaaguga	410	*miR-153-1-1; miR-153-1-2; miR-153-2-1; miR-153-2-2*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-154	uagguuauccguguugccuucg	411	*miR-154-1; miR-154-2*
*miR-154**	aaucauacacgguugaccuauu	412	*miR-154-1; miR-154-2*
miR-155	uuaaugcuaaucgugauagggg	413	*miR-155*
miR-181a	aacauucaacgcugucggugagu	414	*miR-181a*
miR-181b	aacauucauugcugucgguggguu	415	*miR-181b-1; miR-181b-2*
miR-181c	aacauucaaccugucggugagu	416	*miR-181c*
miR-182	uuuggcaaugguagaacucaca	417	*miR-182; miR-182as*
*miR-182**	ugguucuagacuugccaacua	418	*miR-182; miR-182as*
miR-183	uauggcacugguagaauucacug	419	*miR-183*
miR-184	uggacggagaacugauaagggu	420	*miR-184-1; miR-184-2*
miR-185	uggagagaaaggcaguuc	421	*miR-185-1; miR-185-2*
miR-186	caaagaauucuccuuuugggcuu	422	*miR-186-1; miR-186-2*
miR-187	ucgugucuuguguugcagccg	423	*miR-187*
miR-188	caucccuugcaugguggagggu	424	*miR-188*
miR-189	gugccuacugagcugauaucagu	425	*miR-189-1; miR-189-2*
miR-190	ugauauguuugauauauuaggu	426	*miR-190-1; miR-190-2*
miR-191	caacggaaucccaaaagcagcu	427	*miR-191-1; miR-191-2*
miR-192	cugaccuaugaauugacagcc	428	*miR-192*
miR-193	aacuggccuacaaagucccag	429	*miR-193-1; miR-193-2*
miR-194	uguaacagcaacuccaugugga	430	*miR-194-1; miR-194-2*
miR-195	uagcagcacagaaauauuggc	431	*miR-195-1; miR-195-2*
miR-196a	uagguaguuucauguuguugg	432	*miR-196a; miR-196a-2 (miR196-2)*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-196b	uagguaguuuccuguuguugg	433	*miR-196b*
miR-197	uucaccaccuucuccacccagc	434	*miR-197*
miR-198	gguccagaggggagauagg	435	*miR-198*
miR-199a	cccaguguucagacuaccuguuc	436	*miR-199a-1; miR-199a-2*
*miR-199a**	uacaguagucugcacauugguu	437	*miR-199a-1; miR-199a-2; miR-199s; miR-199b*
miR-199b	cccaguguuuagacuaucuguuc	438	*miR-199b*
miR-200a	uaacacugucugguaacgaugu	439	*miR-200a*
miR-200b	cucuaauacugccugguaaugaug	440	*miR-200b*
miR-200c	aauacugccggguaaugaugga	441	*miR-200c*
miR-202	agagguauagggcaugggaaga	442	*miR-202*
miR-203	gugaaauguuuaggaccacuag	443	*miR-203*
miR-204	uucccuuugucauccuaugccu	444	*miR-204*
miR-205	uccuucauuccaccggagucug	445	*miR-205*
miR-206	uggaauguaaggaagugugugg	446	*miR-206-1; miR-206-2*
miR-208	auaagacgagcaaaaagcuugu	447	*miR-208*
miR-210	cugugcgugugacagcggcug	448	*miR-210*
miR-211	uucccuuugucauccuucgccu	449	*miR-211*
miR-212	uaacagucuccagucacggcc	450	*miR-212*
miR-213	accaucgaccguugauuguacc	451	*miR-213*
miR-214	acagcaggcacagacaggcag	452	*miR-214*
miR-215	augaccuaugaauugacagac	453	*miR-215*
miR-216	uaaucucagcuggcaacugug	454	*miR-216*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-217	uacugcaucaggaacugauugga u	455	*miR-217*
miR-218	uugugcuugaucuaaccaugu	456	*miR-218-1; miR-218-2*
miR-219	ugauuguccaaacgcaauucu	457	*miR-219; miR-219-1; miR-219-2*
miR-220	ccacaccguaucugacacuuu	458	*miR-220*
miR-221	agcuacauugucugcuggguuuc	459	*miR-221*
miR-222	agcuacaucuggcuacugggucu c	460	*miR-222*
miR-223	ugucaguuugucaaauacccc	461	*miR-223*
miR-224	caagucacuagugguuccguuua	462	*miR-224*
miR-296	agggccccccucaauccugu	463	*miR-296*
miR-299	uggauuaccgucccacauacau	464	*miR-299*
miR-301	cagugcaauaguauugucaaagc	465	*miR-301*
miR-302a	uaagugcuuccauguuuuggug a	466	*miR-302a*
*miR-302b**	acuuuaacauggaagugcuuucu	467	*miR-302b*
miR-302b	uaagugcuuccauguuuuaguag	468	*miR-302b*
*miR-302c**	uuuaacaugggguaccugcug	469	*miR-302c*
miR-302c	uaagugcuuccauguuucagugg	470	*miR-302c*
miR-302d	uaagugcuuccauguuugagug u	471	*miR-302d*
miR-320	aaaagcuggguugagagggcgaa	472	*miR-320*
miR-321	uaagccagggauuguggguuc	473	*miR-321*
miR-323	gcacauuacacggucgaccucu	474	*miR-323*
miR-324-5p	cgcauccccuagggcauuggugu	475	*miR-324*
miR-324-3p	ccacugccccaggugcugcugg	476	*miR-324*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-325	ccuaguagguguccaguaagu	477	*miR-325*
miR-326	ccucugggcccuuccuccag	478	*miR-326*
miR-328	cuggcccucucugcccuuccgu	479	*miR-328*
miR-330	gcaaagcacacggccugcagaga	480	*miR-330*
miR-331	gccccugggccuauccuagaa	481	*miR-331*
miR-335	ucaagagcaauaacgaaaaaugu	482	*miR-335*
miR-337	uccagcuccuauaugaugccuuu	483	*miR-337*
miR-338	uccagcaucagugauuuuguuga	484	*miR-338*
miR-339	ucccuguccuccaggagcuca	485	*miR-339*
miR-340	uccgucucaguuacuuuauagcc	486	*miR-340*
miR-342	ucucacacagaaaucgcacccguc	487	*miR-342*
miR-345	ugcugacuccuaguccagggc	488	*miR-345*
miR-346	ugucugcccgcaugccugccucu	489	*miR-346*
miR-367	aauugcacuuuagcaaugguga	490	*miR-367*
miR-368	acauagaggaaauuccacguuu	491	*miR-368*
miR-369	aauaauacaugguugaucuuu	492	*miR-369*
miR-370	gccugcuggguggaaccugg	493	*miR-370*
miR-371	gugccgccaucuuuugagugu	494	*miR-371*
miR-372	aaagugcugcgacauuugagcgu	495	*miR-372*
*miR-373**	acucaaaaugggggcgcuuucc	496	*miR-373*
miR-373	gaagugcuucgauuuuggggug u	497	*miR-373*

Mature miRNA Name	Mature miRNA Sequence (5' to 3')	SEQ ID NO.	Corresponding precursor microRNA(s); see Table 1a
miR-374	uuauaauacaaccugauaagug	498	*miR-374*

The level of at least one miR gene product can be measured in cells of a biological sample obtained from the subject. For example, a tissue sample can be removed from a subject suspected of having lung cancer by conventional biopsy techniques. In another embodiment, a blood sample can be removed from the subject, and white blood cells can be isolated for DNA extraction by standard techniques. The blood or tissue sample is preferably obtained from the subject prior to initiation of radiotherapy, chemotherapy or other therapeutic treatment. A corresponding control tissue or blood sample, or a control reference sample, can be obtained from unaffected tissues of the subject, from a normal human individual or population of normal individuals, or from cultured cells corresponding to the majority of cells in the subject's sample. The control tissue or blood sample is then processed along with the sample from the subject, so that the levels of miR gene product produced from a given miR gene in cells from the subject's sample can be compared to the corresponding miR gene product levels from cells of the control sample. Alternatively, a reference sample can be obtained and processed separately (e.g., at a different time) from the test sample and the level of a miR gene product produced from a given miR gene in cells from the test sample can be compared to the corresponding miR gene product level from the reference sample.

In one embodiment, the level of the at least one miR gene product in the test sample is greater than the level of the corresponding miR gene product in the control sample (i.e., expression of the miR gene product is "up-regulated"). As used herein, expression of a miR gene product is "up-regulated" when the amount of miR gene product in a cell or tissue sample from a subject is greater than the amount of the same gene product in a control cell or tissue sample. In another embodiment, the level of the at least one miR gene product in the test sample is less than the level of the corresponding miR gene product in the control sample (i.e., expression of the miR gene product is "down-regulated"). As used herein, expression of a miR gene is "down-regulated" when the amount of miR gene product produced from that gene in a cell or

tissue sample from a subject is less than the amount produced from the same gene in a control cell or tissue sample. The relative miR gene expression in the control and normal samples can be determined with respect to one or more RNA expression standards. The standards can comprise, for example, a zero miR gene expression level, the miR gene expression level in a standard cell line, the miR gene expression level in unaffected tissues of the subject, or the average level of miR gene expression previously obtained for a population of normal human controls.

An alteration (*i.e.*, an increase or decrease) in the level of a miR gene product in the sample obtained from the subject, relative to the level of a corresponding miR gene product in a control sample, is indicative of the presence of lung cancer in the subject. In one embodiment, the level of at least one miR gene product in the test sample is greater than the level of the corresponding miR gene product in the control sample. In another embodiment, the level of at least one miR gene product in the test sample is less than the level of the corresponding miR gene product in the control sample. In a certain embodiment, the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1. In a particular embodiment, the at least one miR gene product is selected from the group consisting of miR-21, miR-205 and miR-216. In another embodiment, the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126* and miR-224.

In a particular embodiment, the miR gene product is not one or more of let7a-2, let-7c, let-7g, let-7i, miR-7-2, miR-7-3, miR-9, miR-9-1, miR-10a, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-17-5p, miR-20a, miR-21, miR-24-1, miR-24-2, miR-25, miR-29b-2, miR-30, miR-30a-5p, miR-30c, miR-30d, miR-31, miR-32, miR-34, miR-34a, miR-34a prec, miR-34a-1, miR-34a-2, miR-92-2, miR-96, miR-99a, miR-99b prec, miR-100, miR-103, miR-106a, miR-107, miR-123, miR-124a-1, miR-125b-1, miR-125b-2, miR-126*, miR-127, miR-128b, miR-129, miR-129-1/2 prec, miR-132,

miR-135-1, miR-136, miR-137, miR-141, miR-142-as, miR-143, miR-146, miR-148, miR-149, miR-153, miR-155, miR 159-1, miR-181, miR-181b-1, miR-182, miR-186, miR-191, miR-192, miR-195, miR-196-1, miR-196-1 prec, miR-196-2, miR-199a-1, miR-199a-2, miR-199b, miR-200b, miR-202, miR-203, miR-204, miR-205, miR-210, miR-211, miR-212, miR-214, miR-215, miR-217, miR-221 and/or miR-223.

The level of a miR gene product in a sample can be measured using any technique that is suitable for detecting RNA expression levels in a biological sample. Suitable techniques (e.g., Northern blot analysis, RT-PCR, *in situ* hybridization) for determining RNA expression levels in a biological sample (e.g., cells, tissues) are well known to those of skill in the art. In a particular embodiment, the level of at least one miR gene product is detected using Northern blot analysis. For example, total cellular RNA can be purified from cells by homogenization in the presence of nucleic acid extraction buffer, followed by centrifugation. Nucleic acids are precipitated, and DNA is removed by treatment with DNase and precipitation. The RNA molecules are then separated by gel electrophoresis on agarose gels according to standard techniques, and transferred to nitrocellulose filters. The RNA is then immobilized on the filters by heating. Detection and quantification of specific RNA is accomplished using appropriately labeled DNA or RNA probes complementary to the RNA in question. See, for example, Molecular Cloning: A Laboratory Manual, J. Sambrook *et al.*, eds., 2nd edition, Cold Spring Harbor Laboratory Press, 1989, Chapter 7, the entire disclosure of which is incorporated by reference.

Suitable probes (e.g., DNA probes, RNA probes) for Northern blot hybridization of a given miR gene product can be produced from the nucleic acid sequences provided in Table 1a and Table 1b and include, but are not limited to, probes having at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% complementarity to a miR gene product of interest, as well as probes that have complete complementarity to a miR gene product of interest. Methods for preparation of labeled DNA and RNA probes, and the conditions for hybridization thereof to target nucleotide sequences, are described in Molecular Cloning: A Laboratory Manual, J. Sambrook *et al.*, eds., 2nd edition, Cold Spring Harbor Laboratory Press, 1989, Chapters 10 and 11, the disclosures of which are incorporated herein by reference.

For example, the nucleic acid probe can be labeled with, *e.g.*, a radionuclide, such as ^{3}H, ^{32}P, ^{33}P, ^{14}C, or ^{35}S; a heavy metal; a ligand capable of functioning as a specific binding pair member for a labeled ligand (*e.g.*, biotin, avidin or an antibody); a fluorescent molecule; a chemiluminescent molecule; an enzyme or the like.

Probes can be labeled to high specific activity by either the nick translation method of Rigby *et al.* (1977), *J. Mol. Biol.* 113:237-251 or by the random priming method of Fienberg *et al.* (1983), *Anal. Biochem.* 132:6-13, the entire disclosures of which are incorporated herein by reference. The latter is the method of choice for synthesizing ^{32}P-labeled probes of high specific activity from single-stranded DNA or from RNA templates. For example, by replacing preexisting nucleotides with highly radioactive nucleotides according to the nick translation method, it is possible to prepare ^{32}P-labeled nucleic acid probes with a specific activity well in excess of 10^{8} cpm/microgram. Autoradiographic detection of hybridization can then be performed by exposing hybridized filters to photographic film. Densitometric scanning of the photographic films exposed by the hybridized filters provides an accurate measurement of miR gene transcript levels. Using another approach, miR gene transcript levels can be quantified by computerized imaging systems, such as the Molecular Dynamics 400-B 2D Phosphorimager available from Amersham Biosciences, Piscataway, NJ.

Where radionuclide labeling of DNA or RNA probes is not practical, the random-primer method can be used to incorporate an analogue, for example, the dTTP analogue 5-(N-(N-biotinyl-epsilon-aminocaproyl)-3-aminoallyl)deoxyuridine triphosphate, into the probe molecule. The biotinylated probe oligonucleotide can be detected by reaction with biotin-binding proteins, such as avidin, streptavidin and antibodies (e.g., anti-biotin antibodies) coupled to fluorescent dyes or enzymes that produce color reactions.

In addition to Northern and other RNA hybridization techniques, determining the levels of RNA transcripts can be accomplished using the technique of *in situ* hybridization. This technique requires fewer cells than the Northern blotting technique and involves depositing whole cells onto a microscope cover slip and probing the nucleic acid content of the cell with a solution containing radioactive or otherwise labeled nucleic acid (e.g., cDNA or RNA) probes. This technique is particularly well-suited for analyzing tissue biopsy samples from subjects. The practice of the *in situ*

hybridization technique is described in more detail in U.S. Patent No. 5,427,916, the entire disclosure of which is incorporated herein by reference. Suitable probes for *in situ* hybridization of a given miR gene product can be produced from the nucleic acid sequences provided in Table 1a and Table 1b, and include, but are not limited to, probes having at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% complementarity to a miR gene product of interest, as well as probes that have complete complementarity to a miR gene product of interest, as described above.

The relative number of miR gene transcripts in cells can also be determined by reverse transcription of miR gene transcripts, followed by amplification of the reverse-transcribed transcripts by polymerase chain reaction (RT-PCR). The levels of miR gene transcripts can be quantified in comparison with an internal standard, for example, the level of mRNA from a "housekeeping" gene present in the same sample. A suitable "housekeeping" gene for use as an internal standard includes, e.g., myosin or glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Methods for performing quantitative and semi-quantitative RT-PCR, and variations thereof, are well known to those of skill in the art.

In some instances, it may be desirable to simultaneously determine the expression level of a plurality of different miR gene products in a sample. In other instances, it may be desirable to determine the expression level of the transcripts of all known miR genes correlated with a cancer. Assessing cancer-specific expression levels for hundreds of miR genes or gene products is time consuming and requires a large amount of total RNA (e.g., at least 20 μg for each Northern blot) and autoradiographic techniques that require radioactive isotopes.

To overcome these limitations, an oligolibrary, in microchip format (i.e., a microarray), may be constructed containing a set of oligonucleotide (e.g., oligodeoxynucleotide) probes that are specific for a set of miR genes. Using such a microarray, the expression level of multiple microRNAs in a biological sample can be determined by reverse transcribing the RNAs to generate a set of target oligodeoxynucleotides, and hybridizing them to probe the oligonucleotides on the microarray to generate a hybridization, or expression, profile. The hybridization profile of the test sample can then be compared to that of a control sample to determine which microRNAs have an altered expression level in lung cancer cells. As used herein,

"probe oligonucleotide" or "probe oligodeoxynucleotide" refers to an oligonucleotide that is capable of hybridizing to a target oligonucleotide. "Target oligonucleotide" or "target oligodeoxynucleotide" refers to a molecule to be detected (e.g., via hybridization). By "miR-specific probe oligonucleotide" or "probe oligonucleotide specific for a miR" is meant a probe oligonucleotide that has a sequence selected to hybridize to a specific miR gene product, or to a reverse transcript of the specific miR gene product.

An "expression profile" or "hybridization profile" of a particular sample is essentially a fingerprint of the state of the sample; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. That is, normal tissue may be distinguished from lung cancer tissue, and within lung cancer tissue, different prognosis states (for example, good or poor long term survival prospects) may be determined. By comparing expression profiles of lung cancer tissue in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. The identification of sequences that are differentially expressed in lung cancer tissue or normal lung tissue, as well as differential expression resulting in different prognostic outcomes, allows the use of this information in a number of ways. For example, a particular treatment regime may be evaluated (e.g., to determine whether a chemotherapeutic drug acts to improve the long-term prognosis in a particular patient). Similarly, diagnosis may be done or confirmed by comparing patient samples with known expression profiles. Furthermore, these gene expression profiles (or individual genes) allow screening of drug candidates that suppress the lung cancer expression profile or convert a poor prognosis profile to a better prognosis profile.

Accordingly, the invention provides methods of diagnosing whether a subject has, or is at risk for developing, lung cancer, comprising reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides, hybridizing the target oligodeoxynucleotides to a microarray comprising miRNA-specific probe oligonucleotides to provide a hybridization profile for the test sample, and comparing the test sample hybridization profile to a hybridization profile generated from a control sample, wherein an alteration in the

signal of at least one miRNA is indicative of the subject either having, or being at risk for developing, lung cancer. In one embodiment, the microarray comprises miRNA-specific probe oligonucleotides for a substantial portion of all known human miRNAs. In a particular embodiment, the microarray comprises miRNA-specific probe oligonucleotides for one or more miRNAs selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a, let-7f-1 and a combination thereof.

The microarray can be prepared from gene-specific oligonucleotide probes generated from known miRNA sequences. The array may contain two different oligonucleotide probes for each miRNA, one containing the active, mature sequence and the other being specific for the precursor of the miRNA. The array may also contain controls, such as one or more mouse sequences differing from human orthologs by only a few bases, which can serve as controls for hybridization stringency conditions. tRNAs and other RNAs (e.g., rRNAs, mRNAs) from both species may also be printed on the microchip, providing an internal, relatively stable, positive control for specific hybridization. One or more appropriate controls for non-specific hybridization may also be included on the microchip. For this purpose, sequences are selected based upon the absence of any homology with any known miRNAs.

The microarray may be fabricated using techniques known in the art. For example, probe oligonucleotides of an appropriate length, e.g., 40 nucleotides, are 5'-amine modified at position C6 and printed using commercially available microarray systems, e.g., the GeneMachine OmniGrid™ 100 Microarrayer and Amersham CodeLink™ activated slides. Labeled cDNA oligomer corresponding to the target RNAs is prepared by reverse transcribing the target RNA with labeled primer. Following first strand synthesis, the RNA/DNA hybrids are denatured to degrade the RNA templates. The labeled target cDNAs thus prepared are then hybridized to the microarray chip under hybridizing conditions, e.g., 6X SSPE/30% formamide at 25°C

for 18 hours, followed by washing in 0.75X TNT at 37°C for 40 minutes. At positions on the array where the immobilized probe DNA recognizes a complementary target cDNA in the sample, hybridization occurs. The labeled target cDNA marks the exact position on the array where binding occurs, allowing automatic detection and quantification. The output consists of a list of hybridization events, indicating the relative abundance of specific cDNA sequences, and therefore the relative abundance of the corresponding complementary miRs, in the patient sample. According to one embodiment, the labeled cDNA oligomer is a biotin-labeled cDNA, prepared from a biotin-labeled primer. The microarray is then processed by direct detection of the biotin-containing transcripts using, e.g., Streptavidin-Alexa647 conjugate, and scanned utilizing conventional scanning methods. Image intensities of each spot on the array are proportional to the abundance of the corresponding miR in the patient sample.

The use of the array has several advantages for miRNA expression detection. First, the global expression of several hundred genes can be identified in the same sample at one time point. Second, through careful design of the oligonucleotide probes, expression of both mature and precursor molecules can be identified. Third, in comparison with Northern blot analysis, the chip requires a small amount of RNA, and provides reproducible results using 2.5 μg of total RNA. The relatively limited number of miRNAs (a few hundred per species) allows the construction of a common microarray for several species, with distinct oligonucleotide probes for each. Such a tool would allow for analysis of trans-species expression for each known miR under various conditions.

In addition to use for quantitative expression level assays of specific miRs, a microchip containing miRNA-specific probe oligonucleotides corresponding to a substantial portion of the miRNome, preferably the entire miRNome, may be employed to carry out miR gene expression profiling, for analysis of miR expression patterns. Distinct miR signatures can be associated with established disease markers, or directly with a disease state.

According to the expression profiling methods described herein, total RNA from a sample from a subject suspected of having a cancer (e.g., lung cancer) is quantitatively reverse transcribed to provide a set of labeled target oligodeoxynucleotides complementary to the RNA in the sample. The target

oligodeoxynucleotides are then hybridized to a microarray comprising miRNA-specific probe oligonucleotides to provide a hybridization profile for the sample. The result is a hybridization profile for the sample representing the expression pattern of miRNA in the sample. The hybridization profile comprises the signal from the binding of the target oligodeoxynucleotides from the sample to the miRNA-specific probe oligonucleotides in the microarray. The profile may be recorded as the presence or absence of binding (signal vs. zero signal). More preferably, the profile recorded includes the intensity of the signal from each hybridization. The profile is compared to the hybridization profile generated from a normal, e.g., noncancerous, control sample. An alteration in the signal is indicative of the presence of, or propensity to develop, cancer in the subject.

Other techniques for measuring miR gene expression are also within the skill in the art, and include various techniques for measuring rates of RNA transcription and degradation.

The invention also provides methods of determining the prognosis of a subject with lung cancer, comprising measuring the level of at least one miR gene product, which is associated with a particular prognosis in lung cancer (e.g., a good or positive prognosis, a poor or adverse prognosis), in a test sample from the subject. According to these methods, an alteration in the level of a miR gene product that is associated with a particular prognosis, in the test sample, as compared to the level of a corresponding miR gene product in a control sample, is indicative of the subject having a lung cancer with a particular prognosis. In one embodiment, the miR gene product is associated with an adverse (i.e., poor) prognosis. Examples of an adverse prognosis include, but are not limited to, low survival rate and rapid disease progression. In certain embodiments, the at least one miR gene product associated with a particular prognosis is selected from the group consisting of miR-155, miR-17-3p, miR-106a, miR-93, let-7a-2, miR-145, let-7b, miR-20 and miR-21. In a particular embodiment, the lung cancer is a lung adenocarcinoma and the at least one miR gene product associated with a particular prognosis is selected from the group consisting of miR-155 and let-7a-2. In certain embodiments, the level of the at least one miR gene product is measured by reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides, hybridizing the target oligodeoxynucleotides to a

microarray that comprises miRNA-specific probe oligonucleotides to provide a hybridization profile for the test sample, and comparing the test sample hybridization profile to a hybridization profile generated from a control sample.

Without wishing to be bound by any one theory, it is believed that alterations in the level of one or more miR gene products in cells can result in the deregulation of one or more intended targets for these miRs, which can lead to the formation of lung cancer. Therefore, altering the level of the miR gene product (e.g., by decreasing the level of a miR that is up-regulated in lung cancer cells, by increasing the level of a miR that is down-regulated in lung cancer cells) may successfully treat the lung cancer.

Accordingly, the present invention encompasses methods of treating lung cancer in a subject, wherein at least one miR gene product is deregulated (e.g., down-regulated, up-regulated) in the cells (e.g., lung cancer cells) of the subject. In one embodiment, the level of at least one miR gene product in a test sample (e.g., a lung cancer sample) is greater than the level of the corresponding miR gene product in a control sample. In another embodiment, the level of at least one miR gene product in a test sample (e.g., a lung cancer sample) is less than the level of the corresponding miR gene product in a control sample. When the at least one isolated miR gene product is down-regulated in the lung cancer cells, the method comprises administering an effective amount of the at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, such that proliferation of cancer cells in the subject is inhibited. For example, when a miR gene product is down-regulated in a cancer cell in a subject, administering an effective amount of an isolated miR gene product to the subject can inhibit proliferation of the cancer cell. The isolated miR gene product that is administered to the subject can be identical to an endogenous wild-type miR gene product (e.g., a miR gene product shown in Table 1a or Table 1b) that is down-regulated in the cancer cell or it can be a variant or biologically-active fragment thereof. As defined herein, a "variant" of a miR gene product refers to a miRNA that has less than 100% identity to a corresponding wild-type miR gene product and possesses one or more biological activities of the corresponding wild-type miR gene product. Examples of such biological activities include, but are not limited to, inhibition of expression of a target RNA molecule (e.g., inhibiting translation of a target RNA molecule, modulating the stability of a target RNA molecule, inhibiting

processing of a target RNA molecule) and inhibition of a cellular process associated with lung cancer (e.g., cell differentiation, cell growth, cell death). These variants include species variants and variants that are the consequence of one or more mutations (e.g., a substitution, a deletion, an insertion) in a miR gene. In certain embodiments, the variant is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to a corresponding wild-type miR gene product.

As defined herein, a "biologically-active fragment" of a miR gene product refers to an RNA fragment of a miR gene product that possesses one or more biological activities of a corresponding wild-type miR gene product. As described above, examples of such biological activities include, but are not limited to, inhibition of expression of a target RNA molecule and inhibition of a cellular process associated with lung cancer. In certain embodiments, the biologically-active fragment is at least about 5, 7, 10, 12, 15, or 17 nucleotides in length. In a particular embodiment, an isolated miR gene product can be administered to a subject in combination with one or more additional anti-cancer treatments. Suitable anti-cancer treatments include, but are not limited to, chemotherapy, radiation therapy and combinations thereof (e.g., chemoradiation).

When the at least one isolated miR gene product is up-regulated in the cancer cells, the method comprises administering to the subject an effective amount of a compound that inhibits expression of the at least one miR gene product, such that proliferation of lung cancer cells is inhibited. Such compounds are referred to herein as miR gene expression-inhibition compounds. Examples of suitable miR gene expression-inhibition compounds include, but are not limited to, those described herein (e.g., double-stranded RNA, antisense nucleic acids and enzymatic RNA molecules). In a particular embodiment, a miR gene expression-inhibiting compound can be administered to a subject in combination with one or more additional anti-cancer treatments. Suitable anti-cancer treatments include, but are not limited to, chemotherapy, radiation therapy and combinations thereof (e.g., chemoradiation).

In a certain embodiment, the isolated miR gene product that is deregulated in lung cancer is selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2,

miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1. In a particular embodiment, the at least one miR gene product is selected from the group consisting of miR-21, miR-205 and miR-216. In another embodiment, the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126* and miR-224.

In a particular embodiment, the miR gene product is not one or more of let7a-2, let-7c, let-7g, let-7i, miR-7-2, miR-7-3, miR-9, miR-9-1, miR-10a, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-17-5p, miR-20a, miR-21, miR-24-1, miR-24-2, miR-25, miR-29b-2, miR-30, miR-30a-5p, miR-30c, miR-30d, miR-31, miR-32, miR-34, miR-34a, miR-34a prec, miR-34a-1, miR-34a-2, miR-92-2, miR-96, miR-99a, miR-99b prec, miR-100, miR-103, miR-106a, miR-107, miR-123, miR-124a-1, miR-125b-1, miR-125b-2, miR-126*, miR-127, miR-128b, miR-129, miR-129-1/2 prec, miR-132, miR-135-1, miR-136, miR-137, miR-141, miR-142-as, miR-143, miR-146, miR-148, miR-149, miR-153, miR-155, miR 159-1, miR-181, miR-181b-1, miR-182, miR-186, miR-191, miR-192, miR-195, miR-196-1, miR-196-1 prec, miR-196-2, miR-199a-1, miR-199a-2, miR-199b, miR-200b, miR-202, miR-203, miR-204, miR-205, miR-210, miR-211, miR-212, miR-214, miR-215, miR-217, miR-221 and/or miR-223.

The terms "treat", "treating" and "treatment", as used herein, refer to ameliorating symptoms associated with a disease or condition, for example, lung cancer, including preventing or delaying the onset of the disease symptoms, and/or lessening the severity or frequency of symptoms of the disease or condition. The terms "subject" and "individual" are defined herein to include animals, such as mammals, including, but not limited to, primates, cows, sheep, goats, horses, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent, or murine species. In a preferred embodiment, the animal is a human.

As used herein, an "effective amount" of an isolated miR gene product is an amount sufficient to inhibit proliferation of a cancer cell in a subject suffering from lung cancer. One skilled in the art can readily determine an effective amount of a miR gene product to be administered to a given subject, by taking into account factors, such as

the size and weight of the subject; the extent of disease penetration; the age, health and sex of the subject; the route of administration; and whether the administration is regional or systemic.

For example, an effective amount of an isolated miR gene product can be based on the approximate weight of a tumor mass to be treated. The approximate weight of a tumor mass can be determined by calculating the approximate volume of the mass, wherein one cubic centimeter of volume is roughly equivalent to one gram. An effective amount of the isolated miR gene product based on the weight of a tumor mass can be in the range of about 10-500 micrograms/gram of tumor mass. In certain embodiments, the tumor mass can be at least about 10 micrograms/gram of tumor mass, at least about 60 micrograms/gram of tumor mass or at least about 100 micrograms/gram of tumor mass.

An effective amount of an isolated miR gene product can also be based on the approximate or estimated body weight of a subject to be treated. Preferably, such effective amounts are administered parenterally or enterally, as described herein. For example, an effective amount of the isolated miR gene product that is administered to a subject can range from about 5 – 3000 micrograms/kg of body weight, from about 700 - 1000 micrograms/kg of body weight, or greater than about 1000 micrograms/kg of body weight.

One skilled in the art can also readily determine an appropriate dosage regimen for the administration of an isolated miR gene product to a given subject. For example, a miR gene product can be administered to the subject once (*e.g.*, as a single injection or deposition). Alternatively, a miR gene product can be administered once or twice daily to a subject for a period of from about three to about twenty-eight days, more particularly from about seven to about ten days. In a particular dosage regimen, a miR gene product is administered once a day for seven days. Where a dosage regimen comprises multiple administrations, it is understood that the effective amount of the miR gene product administered to the subject can comprise the total amount of gene product administered over the entire dosage regimen.

As used herein, an "isolated" miR gene product is one that is synthesized, or altered or removed from the natural state through human intervention. For example, a synthetic miR gene product, or a miR gene product partially or completely separated

from the coexisting materials of its natural state, is considered to be "isolated." An isolated miR gene product can exist in a substantially-purified form, or can exist in a cell into which the miR gene product has been delivered. Thus, a miR gene product that is deliberately delivered to, or expressed in, a cell is considered an "isolated" miR gene product. A miR gene product produced inside a cell from a miR precursor molecule is also considered to be an "isolated" molecule. According to the invention, the isolated miR gene products described herein can be used for the manufacture of a medicament for treating lung cancer in a subject (e.g., a human).

Isolated miR gene products can be obtained using a number of standard techniques. For example, the miR gene products can be chemically synthesized or recombinantly produced using methods known in the art. In one embodiment, miR gene products are chemically synthesized using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer. Commercial suppliers of synthetic RNA molecules or synthesis reagents include, e.g., Proligo (Hamburg, Germany), Dharmacon Research (Lafayette, CO, U.S.A.), Pierce Chemical (part of Perbio Science, Rockford, IL, U.S.A.), Glen Research (Sterling, VA, U.S.A.), ChemGenes (Ashland, MA, U.S.A.) and Cruachem (Glasgow, UK).

Alternatively, the miR gene products can be expressed from recombinant circular or linear DNA plasmids using any suitable promoter. Suitable promoters for expressing RNA from a plasmid include, e.g., the U6 or H1 RNA pol III promoter sequences, or the cytomegalovirus promoters. Selection of other suitable promoters is within the skill in the art. The recombinant plasmids of the invention can also comprise inducible or regulatable promoters for expression of the miR gene products in cancer cells.

The miR gene products that are expressed from recombinant plasmids can be isolated from cultured cell expression systems by standard techniques. The miR gene products that are expressed from recombinant plasmids can also be delivered to, and expressed directly in, the cancer cells. The use of recombinant plasmids to deliver the miR gene products to cancer cells is discussed in more detail below.

The miR gene products can be expressed from a separate recombinant plasmid, or they can be expressed from the same recombinant plasmid. In one embodiment, the miR gene products are expressed as RNA precursor molecules from a single plasmid,

and the precursor molecules are processed into the functional miR gene product by a suitable processing system, including, but not limited to, processing systems extant within a cancer cell. Other suitable processing systems include, e.g., the *in vitro* Drosophila cell lysate system (e.g., as described in U.S. Published Patent Application No. 2002/0086356 to Tuschl *et al.*, the entire disclosure of which is incorporated herein by reference) and the *E. coli* RNAse III system (e.g., as described in U.S. Published Patent Application No. 2004/0014113 to Yang *et al.*, the entire disclosure of which is incorporated herein by reference).

Selection of plasmids suitable for expressing the miR gene products, methods for inserting nucleic acid sequences into the plasmid to express the gene products, and methods of delivering the recombinant plasmid to the cells of interest are within the skill in the art. See, for example, Zeng *et al.* (2002), *Molecular Cell* 9:1327-1333; Tuschl (2002), *Nat. Biotechnol*, 20:446-448; Brummelkamp *et al.* (2002), *Science* 296:550-553; Miyagishi *et al.* (2002), *Nat. Biotechnol.* 20:497-500; Paddison *et al.* (2002), *Genes Dev.* 16:948-958; Lee *et al.* (2002), *Nat. Biotechnol.* 20:500-505; and Paul *et al.* (2002), *Nat. Biotechnol.* 20:505-508, the entire disclosures of which are incorporated herein by reference.

In one embodiment, a plasmid expressing the miR gene products comprises a sequence encoding a miR precursor RNA under the control of the CMV intermediate-early promoter. As used herein, "under the control" of a promoter means that the nucleic acid sequences encoding the miR gene product are located 3' of the promoter, so that the promoter can initiate transcription of the miR gene product coding sequences.

The miR gene products can also be expressed from recombinant viral vectors. It is contemplated that the miR gene products can be expressed from two separate recombinant viral vectors, or from the same viral vector. The RNA expressed from the recombinant viral vectors can either be isolated from cultured cell expression systems by standard techniques, or can be expressed directly in cancer cells. The use of recombinant viral vectors to deliver the miR gene products to cancer cells is discussed in more detail below.

The recombinant viral vectors of the invention comprise sequences encoding the miR gene products and any suitable promoter for expressing the RNA sequences.

Suitable promoters include, but are not limited to, the U6 or H1 RNA pol III promoter sequences, or the cytomegalovirus promoters. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the miR gene products in a cancer cell.

Any viral vector capable of accepting the coding sequences for the miR gene products can be used; for example, vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (*e.g.*, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of the viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors of the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors of the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors that express different capsid protein serotypes are within the skill in the art; *see, e.g.*, Rabinowitz, J.E., *et al.* (2002), *J. Virol.* 76:791-801, the entire disclosure of which is incorporated herein by reference.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing RNA into the vector, methods of delivering the viral vector to the cells of interest, and recovery of the expressed RNA products are within the skill in the art. See, for example, Dornburg (1995), *Gene Therap.* 2:301-310; Eglitis (1988), *Biotechniques* 6:608-614; Miller (1990), *Hum. Gene Therap.* 1:5-14; and Anderson (1998), *Nature* 392:25-30, the entire disclosures of which are incorporated herein by reference.

Particularly suitable viral vectors are those derived from AV and AAV. A suitable AV vector for expressing the miR gene products, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia *et al.* (2002), *Nat. Biotech.* 20:1006-1010, the entire disclosure of

which is incorporated herein by reference. Suitable AAV vectors for expressing the miR gene products, methods for constructing the recombinant AAV vector, and methods for delivering the vectors into target cells are described in Samulski *et al.* (1987), *J. Virol.* 61:3096-3101; Fisher *et al.* (1996), *J. Virol.*, 70:520-532; Samulski *et al.* (1989), *J. Virol.* 63:3822-3826; U.S. Patent No. 5,252,479; U.S. Patent No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are incorporated herein by reference. In one embodiment, the miR gene products are expressed from a single recombinant AAV vector comprising the CMV intermediate early promoter.

In a certain embodiment, a recombinant AAV viral vector of the invention comprises a nucleic acid sequence encoding a miR precursor RNA in operable connection with a polyT termination sequence under the control of a human U6 RNA promoter. As used herein, "in operable connection with a polyT termination sequence" means that the nucleic acid sequences encoding the sense or antisense strands are immediately adjacent to the polyT termination signal in the 5' direction. During transcription of the miR sequences from the vector, the polyT termination signals act to terminate transcription.

In other embodiments of the treatment methods of the invention, an effective amount of at least one compound that inhibits miR expression can be administered to the subject. As used herein, "inhibiting miR expression" means that the production of the precursor and/or active, mature form of miR gene product after treatment is less than the amount produced prior to treatment. One skilled in the art can readily determine whether miR expression has been inhibited in a cancer cell, using, for example, the techniques for determining miR transcript level discussed herein. Inhibition can occur at the level of gene expression (i.e., by inhibiting transcription of a miR gene encoding the miR gene product) or at the level of processing (e.g., by inhibiting processing of a miR precursor into a mature, active miR).

As used herein, an "effective amount" of a compound that inhibits miR expression is an amount sufficient to inhibit proliferation of a cancer cell in a subject suffering from a cancer (e.g., lung cancer). One skilled in the art can readily determine an effective amount of a miR expression-inhibiting compound to be administered to a given subject, by taking into account factors, such as the size and weight of the subject;

the extent of disease penetration; the age, health and sex of the subject; the route of administration; and whether the administration is regional or systemic.

For example, an effective amount of the expression-inhibiting compound can be based on the approximate weight of a tumor mass to be treated, as described herein. An effective amount of a compound that inhibits miR expression can also be based on the approximate or estimated body weight of a subject to be treated, as described herein.

One skilled in the art can also readily determine an appropriate dosage regimen for administering a compound that inhibits miR expression to a given subject, as described herein. Suitable compounds for inhibiting miR gene expression include double-stranded RNA (such as short- or small-interfering RNA or "siRNA"), antisense nucleic acids, and enzymatic RNA molecules, such as ribozymes. Each of these compounds can be targeted to a given miR gene product and interfere with the expression (e.g., by inhibiting translation, by inducing cleavage and/or degradation) of the target miR gene product.

For example, expression of a given miR gene can be inhibited by inducing RNA interference of the miR gene with an isolated double-stranded RNA ("dsRNA") molecule which has at least 90%, for example at least 95%, at least 98%, at least 99%, or 100%, sequence homology with at least a portion of the miR gene product. In a particular embodiment, the dsRNA molecule is a "short or small interfering RNA" or "siRNA."

siRNA useful in the present methods comprise short double-stranded RNA from about 17 nucleotides to about 29 nucleotides in length, preferably from about 19 to about 25 nucleotides in length. The siRNA comprise a sense RNA strand and a complementary antisense RNA strand annealed together by standard Watson-Crick base-pairing interactions (hereinafter "base-paired"). The sense strand comprises a nucleic acid sequence that is substantially identical to a nucleic acid sequence contained within the target miR gene product.

As used herein, a nucleic acid sequence in an siRNA that is "substantially identical" to a target sequence contained within the target mRNA is a nucleic acid sequence that is identical to the target sequence, or that differs from the target sequence by one or two nucleotides. The sense and antisense strands of the siRNA can comprise two complementary, single-stranded RNA molecules, or can comprise a single

molecule in which two complementary portions are base-paired and are covalently linked by a single-stranded "hairpin" area.

The siRNA can also be altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or to one or more internal nucleotides of the siRNA, or modifications that make the siRNA resistant to nuclease digestion, or the substitution of one or more nucleotides in the siRNA with deoxyribonucleotides.

One or both strands of the siRNA can also comprise a 3' overhang. As used herein, a "3' overhang" refers to at least one unpaired nucleotide extending from the 3'-end of a duplexed RNA strand. Thus, in certain embodiments, the siRNA comprises at least one 3' overhang of from 1 to about 6 nucleotides (which includes ribonucleotides or deoxyribonucleotides) in length, from 1 to about 5 nucleotides in length, from 1 to about 4 nucleotides in length, or from about 2 to about 4 nucleotides in length. In a particular embodiment, the 3' overhang is present on both strands of the siRNA, and is 2 nucleotides in length. For example, each strand of the siRNA can comprise 3' overhangs of dithymidylic acid ("TT") or diuridylic acid ("uu").

The siRNA can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing dsRNA or siRNA molecules are described in U.S. Published Patent Application No. 2002/0173478 to Gewirtz and in U.S. Published Patent Application No. 2004/0018176 to Reich *et al.*, the entire disclosures of both of which are incorporated herein by reference.

Expression of a given miR gene can also be inhibited by an antisense nucleic acid. As used herein, an "antisense nucleic acid" refers to a nucleic acid molecule that binds to target RNA by means of RNA-RNA, RNA-DNA or RNA-peptide nucleic acid interactions, which alters the activity of the target RNA. Antisense nucleic acids suitable for use in the present methods are single-stranded nucleic acids (*e.g.*, RNA, DNA, RNA-DNA chimeras, peptide nucleic acids (PNA)) that generally comprise a nucleic acid sequence complementary to a contiguous nucleic acid sequence in a miR gene product. The antisense nucleic acid can comprise a nucleic acid sequence that is 50-100% complementary, 75-100% complementary, or 95-100% complementary to a

contiguous nucleic acid sequence in a miR gene product. Nucleic acid sequences of particular human miR gene products are provided in Table 1a and Table 1b. Without wishing to be bound by any theory, it is believed that the antisense nucleic acids activate RNase H or another cellular nuclease that digests the miR gene product/antisense nucleic acid duplex.

Antisense nucleic acids can also contain modifications to the nucleic acid backbone or to the sugar and base moieties (or their equivalent) to enhance target specificity, nuclease resistance, delivery or other properties related to efficacy of the molecule. Such modifications include cholesterol moieties, duplex intercalators, such as acridine, or one or more nuclease-resistant groups.

Antisense nucleic acids can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing are within the skill in the art; *see, e.g.*, Stein and Cheng (1993), *Science* 261:1004 and U.S. Patent No. 5,849,902 to Woolf *et al.*, the entire disclosures of which are incorporated herein by reference.

Expression of a given miR gene can also be inhibited by an enzymatic nucleic acid. As used herein, an "enzymatic nucleic acid" refers to a nucleic acid comprising a substrate binding region that has complementarity to a contiguous nucleic acid sequence of a miR gene product, and which is able to specifically cleave the miR gene product. The enzymatic nucleic acid substrate binding region can be, for example, 50-100% complementary, 75-100% complementary, or 95-100% complementary to a contiguous nucleic acid sequence in a miR gene product. The enzymatic nucleic acids can also comprise modifications at the base, sugar, and/or phosphate groups. An exemplary enzymatic nucleic acid for use in the present methods is a ribozyme.

The enzymatic nucleic acids can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing dsRNA or siRNA molecules are described in Werner and Uhlenbeck (1995), *Nucl. Acids Res.* 23:2092-96; Hammann *et al.* (1999), *Antisense and Nucleic Acid Drug Dev.* 9:25-31; and U.S. Patent No. 4,987,071 to Cech *et al*, the entire disclosures of which are incorporated herein by reference.

Administration of at least one miR gene product, or at least one compound for inhibiting miR expression, will inhibit the proliferation of cancer cells in a subject who has a cancer (e.g., lung cancer). As used herein, to "inhibit the proliferation of a cancer cell" means to kill the cell, or permanently or temporarily arrest or slow the growth of the cell. Inhibition of cancer cell proliferation can be inferred if the number of such cells in the subject remains constant or decreases after administration of the miR gene products or miR gene expression-inhibiting compounds. An inhibition of cancer cell proliferation can also be inferred if the absolute number of such cells increases, but the rate of tumor growth decreases.

The number of cancer cells in the body of a subject can be determined by direct measurement, or by estimation from the size of primary or metastatic tumor masses. For example, the number of cancer cells in a subject can be measured by immunohistological methods, flow cytometry, or other techniques designed to detect characteristic surface markers of cancer cells.

The size of a tumor mass can be ascertained by direct visual observation, or by diagnostic imaging methods, such as X-ray, magnetic resonance imaging, ultrasound, and scintigraphy. Diagnostic imaging methods used to ascertain size of the tumor mass can be employed with or without contrast agents, as is known in the art. The size of a tumor mass can also be ascertained by physical means, such as palpation of the tissue mass or measurement of the tissue mass with a measuring instrument, such as a caliper.

The miR gene products or miR gene expression-inhibiting compounds can be administered to a subject by any means suitable for delivering these compounds to cancer cells of the subject. For example, the miR gene products or miR expression-inhibiting compounds can be administered by methods suitable to transfect cells of the subject with these compounds, or with nucleic acids comprising sequences encoding these compounds. In one embodiment, the cells are transfected with a plasmid or viral vector comprising sequences encoding at least one miR gene product or miR gene expression-inhibiting compound.

Transfection methods for eukaryotic cells are well known in the art, and include, e.g., direct injection of the nucleic acid into the nucleus or pronucleus of a cell; electroporation; liposome transfer or transfer mediated by lipophilic materials;

receptor-mediated nucleic acid delivery, bioballistic or particle acceleration; calcium phosphate precipitation, and transfection mediated by viral vectors.

For example, cells can be transfected with a liposomal transfer compound, e.g., DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammonium methylsulfate, Boehringer-Mannheim) or an equivalent, such as LIPOFECTIN. The amount of nucleic acid used is not critical to the practice of the invention; acceptable results may be achieved with 0.1-100 micrograms of nucleic acid/10^5 cells. For example, a ratio of about 0.5 micrograms of plasmid vector in 3 micrograms of DOTAP per 10^5 cells can be used.

A miR gene product or miR gene expression-inhibiting compound can also be administered to a subject by any suitable enteral or parenteral administration route. Suitable enteral administration routes for the present methods include, e.g., oral, rectal, or intranasal delivery. Suitable parenteral administration routes include, e.g., intravascular administration (e.g., intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (e.g., peri-tumoral and intra-tumoral injection, intra-retinal injection, or subretinal injection); subcutaneous injection or deposition, including subcutaneous infusion (such as by osmotic pumps); direct application to the tissue of interest, for example by a catheter or other placement device (e.g., a retinal pellet or a suppository or an implant comprising a porous, non-porous, or gelatinous material); and inhalation. Particularly suitable administration routes are injection, infusion and direct injection into the tumor.

In the present methods, a miR gene product or miR gene product expression-inhibiting compound can be administered to the subject either as naked RNA, in combination with a delivery reagent, or as a nucleic acid (*e.g.*, a recombinant plasmid or viral vector) comprising sequences that express the miR gene product or miR gene expression-inhibiting compound. Suitable delivery reagents include, e.g., the Mirus Transit TKO lipophilic reagent; LIPOFECTIN; lipofectamine; cellfectin; polycations (*e.g.*, polylysine) and liposomes.

Recombinant plasmids and viral vectors comprising sequences that express the miR gene products or miR gene expression-inhibiting compounds, and techniques for

delivering such plasmids and vectors to cancer cells, are discussed herein and/or are well known in the art.

In a particular embodiment, liposomes are used to deliver a miR gene product or miR gene expression-inhibiting compound (or nucleic acids comprising sequences encoding them) to a subject. Liposomes can also increase the blood half-life of the gene products or nucleic acids. Suitable liposomes for use in the invention can be formed from standard vesicle-forming lipids, which generally include neutral or negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of factors, such as the desired liposome size and half-life of the liposomes in the blood stream. A variety of methods are known for preparing liposomes, for example, as described in Szoka *et al.* (1980), *Ann. Rev. Biophys. Bioeng.* 9:467; and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, the entire disclosures of which are incorporated herein by reference.

The liposomes for use in the present methods can comprise a ligand molecule that targets the liposome to cancer cells. Ligands that bind to receptors prevalent in cancer cells, such as monoclonal antibodies that bind to tumor cell antigens, are preferred.

The liposomes for use in the present methods can also be modified so as to avoid clearance by the mononuclear macrophage system ("MMS") and reticuloendothelial system ("RES"). Such modified liposomes have opsonization-inhibition moieties on the surface or incorporated into the liposome structure. In a particularly preferred embodiment, a liposome of the invention can comprise both an opsonization-inhibition moiety and a ligand.

Opsonization-inhibiting moieties for use in preparing the liposomes of the invention are typically large hydrophilic polymers that are bound to the liposome membrane. As used herein, an opsonization-inhibiting moiety is "bound" to a liposome membrane when it is chemically or physically attached to the membrane, e.g., by the intercalation of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids. These opsonization-inhibiting hydrophilic polymers form a protective surface layer that significantly decreases the uptake of the liposomes by the MMS and RES; e.g., as described in U.S. Patent No. 4,920,016, the entire disclosure of which is incorporated herein by reference.

Opsonization-inhibiting moieties suitable for modifying liposomes are preferably water-soluble polymers with a number-average molecular weight from about 500 to about 40,000 daltons, and more preferably from about 2,000 to about 20,000 daltons. Such polymers include polyethylene glycol (PEG) or polypropylene glycol (PPG) or derivatives thereof; e.g., methoxy PEG or PPG, and PEG or PPG stearate; synthetic polymers, such as polyacrylamide or poly N-vinyl pyrrolidone; linear, branched, or dendrimeric polyamidoamines; polyacrylic acids; polyalcohols, e.g., polyvinylalcohol and polyxylitol to which carboxylic or amino groups are chemically linked, as well as gangliosides, such as ganglioside GM1. Copolymers of PEG, methoxy PEG, or methoxy PPG, or derivatives thereof, are also suitable. In addition, the opsonization-inhibiting polymer can be a block copolymer of PEG and either a polyamino acid, polysaccharide, polyamidoamine, polyethyleneamine, or polynucleotide. The opsonization-inhibiting polymers can also be natural polysaccharides containing amino acids or carboxylic acids, e.g., galacturonic acid, glucuronic acid, mannuronic acid, hyaluronic acid, pectic acid, neuraminic acid, alginic acid, carrageenan; aminated polysaccharides or oligosaccharides (linear or branched); or carboxylated polysaccharides or oligosaccharides, e.g., reacted with derivatives of carbonic acids with resultant linking of carboxylic groups. Preferably, the opsonization-inhibiting moiety is a PEG, PPG, or a derivative thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called "PEGylated liposomes."

The opsonization-inhibiting moiety can be bound to the liposome membrane by any one of numerous well-known techniques. For example, an N-hydroxysuccinimide ester of PEG can be bound to a phosphatidyl-ethanolamine lipid-soluble anchor, and then bound to a membrane. Similarly, a dextran polymer can be derivatized with a stearylamine lipid-soluble anchor via reductive amination using $Na(CN)BH_3$ and a solvent mixture, such as tetrahydrofuran and water in a 30:12 ratio at 60°C.

Liposomes modified with opsonization-inhibition moieties remain in the circulation much longer than unmodified liposomes. For this reason, such liposomes are sometimes called "stealth" liposomes. Stealth liposomes are known to accumulate in tissues fed by porous or "leaky" microvasculature. Thus, tissue characterized by such microvasculature defects, for example, solid tumors (e.g., lung cancers), will efficiently accumulate these liposomes; see Gabizon, *et al.* (1988), Proc. Natl. Acad.

Sci., U.S.A., 18:6949-53. In addition, the reduced uptake by the RES lowers the toxicity of stealth liposomes by preventing significant accumulation of the liposomes in the liver and spleen. Thus, liposomes that are modified with opsonization-inhibition moieties are particularly suited to deliver the miR gene products or miR gene expression-inhibition compounds (or nucleic acids comprising sequences encoding them) to tumor cells.

The miR gene products or miR gene expression-inhibition compounds can be formulated as pharmaceutical compositions, sometimes called "medicaments," prior to administering them to a subject, according to techniques known in the art. Accordingly, the invention encompasses pharmaceutical compositions for treating lung cancer. In one embodiment, the pharmaceutical composition comprises at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, and a pharmaceutically-acceptable carrier. In a particular embodiment, the at least one miR gene product corresponds to a miR gene product that has a decreased level of expression in lung cancer cells relative to suitable control cells. In certain embodiments the isolated miR gene product is selected from the group consisting of miR-126*, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, miR-125b-1, let-7f-1 and a combination thereof. In one embodiment, the isolated miR gene product is not miR-15a or miR-16-1. In an additional embodiment, the miR gene product is not miR-210 or miR-212. In another embodiment, the miR gene product is not miR-21, miR-143, miR-205 or miR-9. In yet another embodiment, the miR gene product is not miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-126, miR-30a-5p, miR-140, miR-214, miR-218-2, miR-145, miR-106a, miR-192, miR-203, miR-150, miR-220, miR-212 or miR-9.

In other embodiments, the pharmaceutical compositions of the invention comprise at least one miR expression-inhibition compound. In a particular embodiment, the at least one miR gene expression-inhibition compound is specific for a miR gene whose expression is greater in lung cancer cells than control cells. In certain embodiments, the miR gene expression-inhibition compound is specific for one or more

miR gene products selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof. In one embodiment, the isolated miR gene product is not specific for miR-15a or miR-16-1. In an additional embodiment, the miR gene product is not specific for miR-210 or miR-212. In another embodiment, the miR gene product is not specific for miR-21, miR-143, miR-205 or miR-9. In yet another embodiment, the miR gene product is not specific for miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-126, miR-30a-5p, miR-140, miR-214, miR-218-2, miR-145, miR-106a, miR-192, miR-203, miR-150, miR-220, miR-212 or miR-9. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. As used herein, "pharmaceutical compositions" include formulations for human and veterinary use. Methods for preparing pharmaceutical compositions of the invention are within the skill in the art, for example, as described in Remington's Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, PA. (1985), the entire disclosure of which is incorporated herein by reference.

The present pharmaceutical compositions comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) (e.g., 0.1 to 90% by weight), or a physiologically-acceptable salt thereof, mixed with a pharmaceutically-acceptable carrier. In certain embodiments, the pharmaceutical composition of the invention additionally comprises one or more anti-cancer agents (e.g., chemotherapeutic agents). The pharmaceutical formulations of the invention can also comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound), which are encapsulated by liposomes and a pharmaceutically-acceptable carrier. In one embodiment, the pharmaceutical composition comprises a miR gene or gene product that is not miR-15, miR-16, miR-143 and/or miR-145.

Especially suitable pharmaceutically-acceptable carriers are water, buffered water, normal saline, 0.4% saline, 0.3% glycine, hyaluronic acid and the like.

In a particular embodiment, the pharmaceutical compositions of the invention comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) that is resistant to degradation by nucleases. One skilled in the art can readily synthesize nucleic acids that are nuclease resistant, for example by incorporating one or more ribonucleotides that is modified at the 2'-position into the miR gene product. Suitable 2'-modified ribonucleotides include those modified at the 2'-position with fluoro, amino, alkyl, alkoxy and O-allyl.

Pharmaceutical compositions of the invention can also comprise conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include, e.g., physiologically biocompatible buffers (e.g., tromethamine hydrochloride), additions of chelants (such as, for example, DTPA or DTPA-bisamide) or calcium chelate complexes (such as, for example, calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). Pharmaceutical compositions of the invention can be packaged for use in liquid form, or can be lyophilized.

For solid pharmaceutical compositions of the invention, conventional nontoxic solid pharmaceutically-acceptable carriers can be used; for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

For example, a solid pharmaceutical composition for oral administration can comprise any of the carriers and excipients listed above and 10-95%, preferably 25%-75%, of the at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising sequences encoding them). A pharmaceutical composition for aerosol (inhalational) administration can comprise 0.01-20% by weight, preferably 1%-10% by weight, of the at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) encapsulated in a liposome as described above, and a propellant. A carrier can also be included as desired; e.g., lecithin for intranasal delivery.

The pharmaceutical compositions of the invention can further comprise one or more anti-cancer agents. In a particular embodiment, the compositions comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) and at least one chemotherapeutic agent. Chemotherapeutic agents that are suitable for the methods of the invention include, but are not limited to, DNA-alkylating agents, anti-tumor antibiotic agents, anti-metabolic agents, tubulin stabilizing agents, tubulin destabilizing agents, hormone antagonist agents, topoisomerase inhibitors, protein kinase inhibitors, HMG-CoA inhibitors, CDK inhibitors, cyclin inhibitors, caspase inhibitors, metalloproteinase inhibitors, antisense nucleic acids, triple-helix DNAs, nucleic acids aptamers, and molecularly-modified viral, bacterial and exotoxic agents. Examples of suitable agents for the compositions of the present invention include, but are not limited to, cytidine arabinoside, methotrexate, vincristine, etoposide (VP-16), doxorubicin (adriamycin), cisplatin (CDDP), dexamethasone, arglabin, cyclophosphamide, sarcolysin, methylnitrosourea, fluorouracil, 5-fluorouracil (5FU), vinblastine, camptothecin, actinomycin-D, mitomycin C, hydrogen peroxide, oxaliplatin, irinotecan, topotecan, leucovorin, carmustine, streptozocin, CPT-11, taxol, tamoxifen, dacarbazine, rituximab, daunorubicin, 1-β-D-arabinofuranosylcytosine, imatinib, fludarabine, docetaxel and FOLFOX4.

The invention also encompasses methods of identifying an anti-lung cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR gene product in the cell. In one embodiment, the method comprises providing a test agent to a cell and measuring the level of at least one miR gene product associated with decreased expression levels in lung cancer cells. An increase in the level of the miR gene product in the cell, relative to a suitable control (e.g., the level of the miR gene product in a control cell), is indicative of the test agent being an anti-lung cancer agent. In a particular embodiment, the at least one miR gene product associated with decreased expression levels in lung cancer cells is selected from the group consisting of miR-126*, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-

101-1, miR-124a-3, miR-125b-1, let-7f-1 and a combination thereof. In one embodiment, the miR gene product is not one or more of let7a-2, let-7c, let-7g, let-7i, miR-7-2, miR-7-3, miR-9, miR-9-1, miR-10a, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-17-5p, miR-20a, miR-21, miR-24-1, miR-24-2, miR-25, miR-29b-2, miR-30, miR-30a-5p, miR-30c, miR-30d, miR-31, miR-32, miR-34, miR-34a, miR-34a prec, miR-34a-1, miR-34a-2, miR-92-2, miR-96, miR-99a, miR-99b prec, miR-100, miR-103, miR-106a, miR-107, miR-123, miR-124a-1, miR-125b-1, miR-125b-2, miR-126*, miR-127, miR-128b, miR-129, miR-129-1/2 prec, miR-132, miR-135-1, miR-136, miR-137, miR-141, miR-142-as, miR-143, miR-146, miR-148, miR-149, miR-153, miR-155, miR 159-1, miR-181, miR-181b-1, miR-182, miR-186, miR-191, miR-192, miR-195, miR-196-1, miR-196-1 prec, miR-196-2, miR-199a-1, miR-199a-2, miR-199b, miR-200b, miR-202, miR-203, miR-204, miR-205, miR-210, miR-211, miR-212, miR-214, miR-215, miR-217, miR-221 and/or miR-223.

In other embodiments the method comprises providing a test agent to a cell and measuring the level of at least one miR gene product associated with increased expression levels in lung cancer cells. A decrease in the level of the miR gene product in the cell, relative to a suitable control (e.g., the level of the miR gene product in a control cell), is indicative of the test agent being an anti-lung cancer agent. In a particular embodiment, at least one miR gene product associated with increased expression levels in lung cancer cells is selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof. In one embodiment, the miR gene product is not one or more of let7a-2, let-7c, let-7g, let-7i, miR-7-2, miR-7-3, miR-9, miR-9-1, miR-10a, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-17-5p, miR-20a, miR-21, miR-24-1, miR-24-2, miR-25, miR-29b-2, miR-30, miR-30a-5p, miR-30c, miR-30d, miR-31, miR-32, miR-34, miR-34a, miR-34a prec, miR-34a-1, miR-34a-2, miR-92-2, miR-96, miR-99a, miR-99b prec, miR-100, miR-103, miR-106a, miR-107, miR-123, miR-124a-1, miR-125b-1, miR-125b-2, miR-126*, miR-127, miR-128b, miR-129, miR-129-1/2 prec, miR-132, miR-135-1, miR-136, miR-137, miR-141, miR-142-as, miR-143, miR-146, miR-148, miR-149, miR-153, miR-155, miR 159-1, miR-181, miR-181b-1, miR-182, miR-186, miR-191, miR-192, miR-195, miR-196-1, miR-196-1 prec, miR-196-2, miR-199a-1, miR-

199a-2, miR-199b, miR-200b, miR-202, miR-203, miR-204, miR-205, miR-210, miR-211, miR-212, miR-214, miR-215, miR-217, miR-221 and/or miR-223.

Suitable agents include, but are not limited to drugs (e.g., small molecules, peptides), and biological macromolecules (e.g., proteins, nucleic acids). The agent can be produced recombinantly, synthetically, or it may be isolated (i.e., purified) from a natural source. Various methods for providing such agents to a cell (e.g., transfection) are well known in the art, and several of such methods are described hereinabove. Methods for detecting the expression of at least one miR gene product (e.g., Northern blotting, *in situ* hybridization, RT-PCR, expression profiling) are also well known in the art. Several of these methods are also described herein.

The invention will now be illustrated by the following non-limiting examples.

EXEMPLIFICATION

Example 1: Altered miRNA expression in primary lung cancers

Materials and methods

Samples

104 pairs of primary lung cancer and corresponding noncancerous lung tissues were used in this study. An additional 32 cases, which could be followed up until 5 years, were used for an independent validation dataset. These tissues were obtained between 1990 and 1999 as surgical specimens from patients in the Baltimore metropolitan area, with informed consent and in agreement with the Institutional Review Board. Lung cancer tissues were obtained from 65 lung adenocarcinoma patients and 39 lung squamous cell carcinoma patients. 65 male and 39 female patients, having a median age of 65 (range 38–84), comprised the set. 65 tumors were classified as stage I, 17 as stage II, and 22 as stage III or IV tumors. For the majority of samples, clinical and biological information was available. Total RNA from tissues was isolated by TRIzol® Reagent (Invitrogen), according to the manufacturer's instructions.

Microarray analysis

Microarray analysis was performed as previously described (Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004)). Briefly, 5 μg of total RNA was hybridized with miRNA microarray chips containing 352 probes in triplicate. Specifically, these chips contain gene-specific 40-mer oligonucleotide probes, spotted

by contacting technologies and covalently attached to a polymeric matrix, which were generated from 161 human miRNAs, 84 mouse miRNAs, miRNAs from three other species and tRNA. The microarrays were hybridized in 6X SSPE (0.9 M NaCl/60 mM $NaH_2PO_4 \cdot H_2O$/8 mM EDTA, pH 7.4)/30% formamide at 25°C for 18 hr, washed in 0.75X TNT (Tris·HCl/NaCl/Tween 20) at 37°C for 40 min, and processed using a method of direct detection of biotin-containing transcripts by streptavidin-Alexa647 conjugate (Molecular Probes, Carlsbad, CA). Processed slides were scanned using a PerkinElmer ScanArray XL5K Scanner, with the laser set to 635 nm, at Power 80 and PMT 70 setting, and a scan resolution of 10 μm. An average value of the three spot replicates for each miRNA was normalized and analyzed in BRB-ArrayTools version 3.2.3. After excluding negative values with hybridization intensity below background, normalization was performed by using a per chip on median normalization method and normalization to median array as reference. Finally, 147 miRNAs with consistent log values present in more than 50% of the samples were selected. Genes that were differently expressed among groups were identified using *t*- or *F*-test and genes were considered statistically significant if their *p* value was less than 0.001. A global test of whether the expression profiles differed between the groups was also performed by permutating the labels of which arrays corresponded to which groups. For each permutation, the *p* values were re-computed and the number of genes significant at the 0.001 level was noted. The proportion of the permutations that gave at least as many significant genes as with the actual data was the significance level of the global test.

Solution hybridization detection analysis and Real-Time RT-PCR analysis

The expression levels of mature miRNAs were measured by solution hybridization detection using the *mir*Vana™ miRNA Detection Kit (Ambion Inc., TX). Briefly, 1 μg total RNA was incubated with radiolabeled probes corresponding to these miRNAs. Following digestion to remove any probe that was not bound by target miRNA, the radiolabeled products were fractionated by denaturing polyacrylamide gel electrophoresis. Probes were prepared by 5' end labeling using T4 Polynucleotide Kinase with *mir*Vana™ Probe & Marker Kit (Ambion Inc., TX), according to the manufacturer's instructions. Quantitative real-time PCR was performed as described (Schmittgen *et al.*, *Nucl. Acids Res. 32*:e43 (2004)) on an Applied Biosystem's Sequence Detection System, and all reactions were run in triplicate. Briefly, RNA was

reverse-transcribed to cDNA with gene-specific primers and Thermoscript, and the relative amount of each miRNA to tRNA for initiator methionine was determined, using the equation: 2^{-dCT}, where $dC_T = (C_{TmiRNA} - C_{TU6})$.

Survival analysis

Genes whose expression was significantly related to survival of the patient were identified. A statistical significance level for each gene was computed based on univariate Cox proportional hazard regression model in BRB-ArrayTools version 3.2.3. These *p* values were then used in a multivariate permutation test in which the survival times and censoring indicators were randomly permuted among arrays. Genes were considered statistically significant if their *p* value was less than 0.05.

Survival curves were estimated by the Kaplan-Meier method (SAS Institute, Cary, NC), and the resulting curves were compared using the log-rank test. The joint effect of co-variables was examined using the Cox proportional hazard regression model. Statistical analysis was performed using StatMate (ATMS Co. Ltd., Tokyo, Japan).

Results

miRNA expression in 104 pairs of primary lung cancer and corresponding noncancerous lung tissues was analyzed to investigate the involvement of miRNAs in lung cancer. Comparisons of miRNA expression for several specific group pairs are listed in the Table 2. miRNAs, which were expressed differently in 5 phenotypical and histological classifications (Table 2), were identified.

Upon comparison of miRNA expression in lung cancer tissues and corresponding noncancerous lung tissues, 43 miRNAs were identified that displayed statistically-significant differences in expression between groups (Table 3). In class comparison analysis using our microarray analysis tool, the multivariate permutation test, was performed to control for multiple comparisons. The test provides a specific confidence level for ensuring that the number of false discoveries does not exceed a target level, or for ensuring that the proportion of the gene list representing false discoveries does not exceed a target level. Thus, the probability of getting at least 43 differentially-expressed miRNAs that are statistically significant by chance at the < 0.001 level, if there are no real differences between the classes, was 0 as estimated by the multivariate permutation test. Furthermore, 91% of 104 lung cancers were correctly

classified using the leave-one-out cross-validated class prediction method based on the compound covariate predictor. Based on 2000 random permutations, the *p* value, which is defined as the proportion of the random permutations that gave a cross-validated error rate no greater than the cross-validated error rate with the real data, was < 0.0005.

Several of these miRNAs were associated with FRAs (Table 3). In particular, three miRNAs are located inside fragile sites (*hsa-mir-21* at FRA17B, *hsa-mir-27b* at FRA9D, and *hsa-mir-32* at FRA9E). Furthermore, many of these identified miRNAs are located at frequently deleted or amplified regions in several malignancies (Table 3). For example, *hsa-mir-21* and *hsa-mir-205* are located at the region amplified in lung cancer, whereas *hsa-mir-126** and *hsa-mir-126* are at 9q34.3, a region deleted in lung cancer. Reduced expression of precursor *let-7a-2* and *let-7f-1* was also found in adenocarcinoma and squamous cell carcinoma at a *p* value cutoff of 0.05. In the same way, comparison analyses between lung adenocarcinoma *vs.* noncancerous tissues and squamous cell carcinoma *vs.* noncancerous tissues revealed 17 and 16 miRNAs with statistically different expression, respectively (Table 4). Six miRNAs (*hsa-mir-21*, *hsa-mir-191*, *hsa-mir-155*, *hsa-mir-210*, *hsa-mir-126**, and *hsa-mir-224*) were shared in both histological types of non-small cell lung carcinoma (NSCLC).

Table 2. Comparison analysis of clinicopathological classifications

Classification (Number)	Total	No. of genes[a]	FDR[b]	% correctly classified[c] (*p*-value)
Phenotypical classification				
All tumor (104) *vs.* All normal (104)	208	43	0	91 (< 0.0005)
Adeno[d] tumor (65) *vs.* Adeno normal(65)	130	17	0.001	80 (< 0.0005)
SCC[e] tumor (39) *vs.* SCC normal (39)	78	16	0	92 (< 0.0005)
Histological classification				
Adeno tumor (65) *vs.* SCC tumor (39)	104	6	0.001	81 (< 0.0005)
Age classification				
All; Age<67 (56) *vs.* Age≥67 (48)	104	0		
Adeno; Age<67 (37) *vs.* Age≥67 (28)	65	0		
SCC; Age<67 (19) *vs.* Age≥67 (20)	39	0		
Sex classification				
All; Male (65) *vs.* Female (39)	104	0		
Adeno; Male (39) *vs.* Female (26)	65	0		
SCC; Male (26) *vs.* Female (13)	39	0		
Race classification				
All; African American (21) *vs.* White American (83)	104	0		
Adeno; African American (13) *vs.* White American (52)	65	0		
SCC; African American (8) *vs.* White American (31)	39	0		
Stage classification				
All; Stage I (65) *vs.* stage II (17) *vs.* stage III, IV (22)	104	0		
Adeno; Stage I (41) *vs.* stage II (8) *vs.* stage III, IV (16)	65	1		
SCC; Stage I (24) *vs.* stage II (9) *vs.* stage III, IV (6)	39	0		

[a]No. of genes, Number of genes significant at 0.001.

[b]FDR, False discovery rate which is probability of significant genes by chance.
[c]% correctly classified (*p*-value). The leave-one-out cross-validated class prediction method based on the compound covariate predictor. The *p*-value is the proportion of the random permutations that gave a cross-validated error rate no greater than the cross-validated error rate with the real data.
[d]Adeno, Adenocarcinoma.
[e]SCC, Squamous cell carcinoma.

Table 3. 43 miRNAs differentially expressed in lung cancer tissues *vs.* noncancerous lung tissues.

miRNA	Location	*p*-value	Type	FRA association[a]	Cancer-associated genomic regions[a]	Host gene[b]
hsa-mir-21	17q23.2	p < 1e-07	Up	FRA17B	Amp[c]-neuroblastoma; lung ca	*TMEM49*
hsa-mir-191	3p21.31	p < 1e-07	Up			Novel protein
hsa-mir-126*	9q34.3	p < 1e-07	Down		Del[d]-NSCLC[e]; HCC[f]	*EGFL-7*
hsa-mir-210	11p15.5	1.00E-07	Up		Del-ovarian: lung ca	Novel protein
hsa-mir-155	21q21.3	1.00E-07	Up		Amp-colon ca	*BIC*
hsa-mir-143	5q32	4.00E-07	Down		Del-prostate ca	mlncRNA[g]
hsa-mir-205	1q32.2	4.00E-07	Up		Amp-lung ca	mlncRNA
hsa-mir-192-prec	11q13.1	5.00E-07	Down	FRA11A	Del-thyroid ca	mlncRNA
hsa-mir-224	Xq28	5.00E-07	Down	FRAXF		*GABRE*
hsa-mir-126	9q34.3	7.00E-07	Down		Del-NSCLC; HCC	*EGFL-7*
hsa-mir-24-2	19p13.1	1.30E-06	Up			ND[h]
hsa-mir-30a-5p	6q13	4.80E-06	Down			mlncRNA
hsa-mir-212	17p13.3	5.00E-06	Up			ND
hsa-mir-140	16q22.1	5.10E-06	Down			*ATROPIN-1*
hsa-mir-9	15q26.1	6.50E-06	Down			Novel protein
hsa-mir-214	1q24.3	8.60E-06	Up			ND
hsa-mir-17-3p	13q31.3	9.40E-06	Up			Novel protein
hsa-mir-124a-1	8p23.1	1.23E-05	Down		Amp-MFHs[i]	Novel protein
hsa-mir-218-2	5q34	1.34E-05	Down			*SLIT3*
hsa-mir-95	4p16.1	1.48E-05	Down			*ABLIM2*
hsa-mir-145	5q32	1.90E-05	Down		Del-prostate ca	mlncRNA
hsa-mir-198	3q13.33	2.43E-05	Down			*FSTL1*
hsa-mir-216-prec	2p16.1	3.05E-05	Down			ND
hsa-mir-219-1	6p21.32	5.56E-05	Down			ND
hsa-mir-106a	Xq26.2	6.20E-05	Up		Del-ovarian ca	ND
hsa-mir-197	1p13.3	7.23E-05	Up			ND
hsa-mir-192	11q13.1	0.000119	Up	FRA11A	Del-thyroid ca	ND
hsa-mir-125a-prec	19q13.41	0.000143	Down			mlncRNA
hsa-mir-26a-1-prec	3p22.3	0.000148	Down		Del-epithelial ca	*NIF1*
hsa-mir-146	5q33.3	0.000163	Up			mlncRNA
hsa-mir-203	14q32.33	0.000267	Up			ND
hsa-mir-199b-prec	9q34.11	0.000304	Down		Del-bladder ca	*GOLGA2*
hsa-let-7a-2-prec	11q24.1	0.000398	Down	FRA11B	Del-lung ca	mlncRNA
hsa-mir-27b	9q22.32	0.000454	Down	FRA9D	Del-bladder ca	Novel protein
hsa-mir-32	9q31.3	0.000458	Down	FRA9E	Del-lung ca	Novel protein
hsa-mir-29b-2	1q32.2	0.000466	Down			mlncRNA
hsa-mir-220	Xq25	0.000630	Down			ND
hsa-mir-33	22q13.2	0.000683	Down		Del-colon ca	*SREBF2*
hsa-mir-181c-prec	19p13.12	0.000736	Down			*NANOS3*
hsa-mir-150	19q13.33	0.000784	Up			ND
hsa-mir-101-1	1p31.3	0.000844	Down	FRA1C	Del-ovarian; breast ca	ND
hsa-mir-124a-3	20q13.33	0.000968	Down			ND
hsa-mir-125a	19q13.41	0.000993	Down			ND

[a]Information was obtained from previous report (Calin, G.A., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*: 2999-3004 (2004)). [b]Information was obtained from previous report (Rodriguez, A., *et al.*, Genome Res. *14*: 1902-1910 (2004)).

[c]Amp, Amplification; [d]Del, Deletion; [e]NSCLC, Non-small cell lung carcinoma; [f]HCC, hepatocellular carcinoma; [g]mlncRNA, mRNA-like noncoding RNA; [h]ND, not defined; [i]MFHs, Malignant fibrous histocytomas.

Real-time RT-PCR analysis of select precursor miRNAs was performed to validate the results from the microarray analysis. First, cDNA from 16 pairs of lung adenocarcinoma, and 16 pairs of lung squamous cell carcinoma, were prepared using gene-specific primers for *hsa-mir-21*, *hsa-mir-126**, *hsa-mir-205* and *U6* (as a control). Subsequently, real-time RT-PCR analyses were performed to determine the expression levels of these miRNAs in the different samples. At least a two-fold up-regulation of *hsa-mir-21* and *hsa-mir-205* precursor miRNA expression was found in 66% and 56% of 32 cases, respectively, when compared with the expression levels of these miRNAs in corresponding noncancerous tissues (FIG. 1). The differences were statistically significant at $p<0.001$ by paired t-test. In contrast, 31% of 32 lung cancer cases examined exhibited a greater than 50% reduction in precursor *hsa-mir-126** expression, although these results were not statistically significant (FIG. 1). These findings show that specific precursor miRNAs are frequently upregulated or reduced in lung cancers, consistent with the expression patterns of their mature miRNAs, as determined using microarray analysis.

Table 4. miRNAs differentially-expressed in adenocarcinoma tissues/squamous cell lung
carcinoma tissues *vs.* noncancerous lung tissues.

miRNA	Location	*p*-value	Type
Adenocarcinoma			
hsa-mir-21	17q23.2	p < 1e-07	Up
hsa-mir-191	3p21.31	1.20E-06	Up
hsa-mir-155	21q21.3	4.10E-06	Up
hsa-mir-210	11p15.5	9.90E-06	Up
hsa-mir-126*	9q34.3	1.92E-05	Down
hsa-mir-126	9q34.3	4.13E-05	Down
hsa-mir-24-2	19p13.1	0.000228	Up
hsa-mir-219-1	6p21.32	0.000251	Down
hsa-mir-95	4p16.1	0.000303	Down
hsa-mir-192-prec	11q13.1	0.000307	Down
hsa-mir-220	Xq25	0.000309	Down
hsa-mir-216-prec	2p16.1	0.00042	Down
hsa-mir-204-prec	9q21.11	0.000449	Down
hsa-mir-188	Xp11.23	0.000475	Down
hsa-mir-198	3q13.33	0.000494	Down
hsa-mir-145	5q32	0.000579	Down
hsa-mir-224	Xq28	0.000925	Down
Squamous cell carcinoma			
hsa-mir-205	1q32.2	p < 1e-07	Up
hsa-mir-224	Xq28	4.14E-05	Down
hsa-mir-191	3p21.31	5.18E-05	Up
hsa-mir-126*	9q34.3	9.74E-05	Down
hsa-mir-140	16q22.1	0.000132	Down
hsa-mir-210	11p15.5	0.0001383	Up
hsa-mir-17-3p	13q31.3	0.0001772	Up
hsa-mir-29b	1q32.2	0.0002046	Down
hsa-mir-143	5q32	0.0003141	Down
hsa-mir-203	14q32.33	0.0003293	Up
hsa-mir-155	21q21.3	0.0003688	Up
hsa-mir-21	17q23.2	0.0003904	Up
hsa-mir-214	1q24.3	0.0004546	Up
hsa-mir-212	17p13.3	0.0005426	Up
hsa-mir-30a-5p	6q13	0.0006165	Down
hsa-mir-197	1p13.3	0.0008507	Up

In addition, the microarray data for the three precursor miRNAs, *hsa-mir-21*, *hsa-mir-126**, and *hsa-mir-205*, were confirmed for their mature miRNAs by solution hybridization detection method. Specifically, seven pairs of primary lung cancer tissues and corresponding noncancerous lung tissues, for which sufficient amounts of RNA were available, were analyzed. The mature forms of *hsa-mir-21* and *hsa-mir-205* were clearly up-regulated in lung cancer tissues when compared with the corresponding noncancerous lung tissues (FIG. 2), while *hsa-mir-126** was down-regulated in most of

the lung cancer tissues examined. Therefore, like the RT-PCR results, these analyses confirmed the microarray expression data for these three miRNAs.

Example 2: Distinct miRNA expression signatures in human lung cancer cell lines

Materials and methods

Samples

Thirteen lung cancer cell lines, consisting of five small cell lung carcinoma (SCLCs) cell lines and eight non-small cell lung carcinoma (NSCLCs) cell lines, were used in this study. The 5 SCLC cell lines were DMS 92, NCI-H82, NCI-H146, NCI-H446, and NCI-H417 (American Tissue Culture Collection). The eight NSCLC cell lines were NCI-H157, Calu-1, Calu-6, NCI-H292, NCI-H596, A-427, A549, and A2182 (American Tissue Culture Collection, Manassas, VA). Total RNA from tissues and cultured cells was isolated by TRIzol® Reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

Microarray analysis

Microarray analysis was performed as previously described (Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004), see also, Example 1).

Statistical analysis

Statistical analyses were performed as described hereinabove (see, e.g., Example 1).

Results

miRNA expression profiles of five small cell lung carcinoma (SCLCs) cell lines, and eight non-small cell lung carcinoma (NSCLCs) cell lines, were generated by microarray analysis. Comparison of miRNA expression profiles of NSCLCs and SCLCs revealed statistically-significant differences ($p<0.001$ by t-test) in the expression level of 3 miRNAs (*hsa-mir-24-1*, *hsa-mir-29a*, and *hsa-mir-29c*). Furthermore, when hierarchical clustering analysis was applied to the 18 most differentially-expressed miRNAs for each sample type, distinct clusters were revealed, with all NSCLC cell lines falling into a cluster that was distinct from that of SCLC cell lines (FIG. 3A, FIG. 3B). These results indicate that miRNA expression profiles may differ in cells with different origins and/or types, as was found in previous studies (see, e.g., Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004); Bhattacharjee, A., *et al.*, *Proc. Natl.*

Acad. Sci. U.S.A. 98:13790-13795 (2001); Garber, M.E., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 98*:13784-13789 (2001)).

Example 3: Identification of miRNAs associated with clinicopathological features of lung cancer

Materials and methods

Microarray analysis

Microarray analysis was performed as previously described (Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004), see also, Example 1).

Statistical analysis

Statistical analyses were performed as described hereinabove (see, e.g., Example 1).

Results

Whether the microarray data revealed specific molecular signatures for subsets of lung cancer that differ in clinical behavior was analyzed. For this analysis, the relationship of five types of clinical and pathological information were examined (Table 2). In the histological classification, six miRNAs (*hsa-mir-205*, *hsa-mir-99b*, *hsa-mir-203*, *hsa-mir-202*, *hsa-mir-102*, and *hsa-mir-204-prec*) that were expressed differently in the two most common histological types of NSCLC, adenocarcinoma and squamous cell carcinoma, were identified. The expression levels of *hsa-mir-99b* and *hsa-mir-102* were higher in adenocarcinoma. No differentially-expressed miRNAs were identified for groups that were differentiated by age, gender, or race.

Example 4: Correlation between hsa-mir-155 and hsa-let-7a-2 expression and prognosis of patients with lung adenocarcinoma.

Materials and methods

Microarray analysis

Microarray analysis was performed as previously described (Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004), see also, Example 1).

Statistical analysis

Statistical analyses were performed as described hereinabove (see, e.g., Example 1).

Gene Ontology analysis

Predicted targets of *hsa-mir-155* and *hsa-let-7a* were determined by the methods of Lewis *et al.*, (Lewis, B.P., *et al.*, *Cell 120*: 15-20 (2005)) and PicTar (Krek, A., *et al.*, *Nat. Genet. 37*: 495-500 (2005)) and were analyzed with respect to the over-representation within particular Gene Ontology (GO) biological groupings. GO term lists were subjected to analysis using the Whole Pathway Scope (WPS) application and those terms with Fisher Exact scores of less than 0.005 were listed.

Results

The correlation of miRNA expression with patient survival was assessed. Univariate Cox proportional hazard regression model with global permutation test in BRB-ArrayTools indicated eight miRNAs (*hsa-mir-155*, *hsa-mir-17-3p*, *hsa-mir-106a*, *hsa-mir-93*, *hsa-let-7a-2*, *hsa-mir-145*, *hsa-let-7b* and *hsa-mir-21*) were related to adenocarcinoma patient survival. High expression of either *hsa-mir-155*, *hsa-mir-17-3p*, *hsa-mir-106a*, *hsa-mir-93*, or *hsa-mir-21* and low expression of either *hsa-let-7a-2*, *hsa-let-7b* or *hsa-mir-145* were found to have a significantly worse prognosis. In addition, the survival analysis among 41 stage I adenocarcinoma patients revealed that three miRNAs (*hsa-mir-155*, *hsa-mir-17-3p*, and *hsa-mir-20*) were associated with patient outcome. These results demonstrate the important relationship between miRNA expression profiles and patient survival, independent of disease stage.

Because five of these miRNAs (*hsa-mir-155*, *hsa-mir-17-3p*, *hsa-let-7a-2*, *hsa-mir-145*, and *hsa-mir-21*) were expressed differently among lung cancer tissues *vs.* corresponding noncancerous lung tissues, these miRNAs were used for further survival analysis. The ratio of lung cancer expression to corresponding noncancerous lung tissue expression for each of these five miRNAs was calculated and the cases were classified according to the expression ratio. Using these groupings for each miRNA, Kaplan-Meier survival analysis was performed. Kaplan-Meier survival estimates showed that lung adenocarcinoma patients with either high *hsa-mir-155* expression or reduced *hsa-let-7a-2* expression had poorer survival prospects than patients with low *hsa-mir-155* or high *hsa-let-7a-2* expression (FIG. 4 and FIG. 5). The difference in prognosis of these two groups was highly significant for *hsa-mir-155* (p=0.006; log-rank test), but less significant for *hsa-let-7a-2* (p=0.033; log-rank test). Survival analysis of the clinicopathological factors showed that stage was significantly associated with survival (p=0.01; log-rank test), while age, race, sex, and smoking

history did not account for poor prognosis (Tables 5A and 5B). To adjust for multiple comparisons, we used the method by Storey *et al.*, (Storey, J.D. and Tibshirani, R., *Proc. Natl. Acad. Sci. U.S.A. 100*: 9440-9445 (2003)) limiting the false discovery rates to 0.05. Using this rate, *hsa-mir-155* and disease stage were still statistically significant. Subsequently, a multivariate Cox proportional hazard regression analysis was performed using all of these clinicopathological and molecular factors. High *hsa-mir-155* expression was determined to be an unfavorable prognosis factor, independent of other clinicopathological factors (p=0.027; risk ratio 3.03; 95% CI, 1.13−8.14), in addition to disease stage (p=0.013; risk ratio 3.27; 95% CI, 1.31-8.37; Table 5A).

Table 5A. Postoperative survival of patients with lung adenocarcinoma in relation to molecular and clinicopathological characteristics and miRNA expression analyzed by microarray analysis.

Variable	Subset	Hazard ratio (95% CI[a])	p
Univariate analysis (n=65)			
Age	Age≥67/Age<67	1.41 (0.67-3.06)	0.348
Sex	Male/female	1.36 (0.64-2.93)	0.413
Stage	II-IV/I	2.51 (1.29-6.82)	0.010
Smoking history	Current/former	1.32 (0.63-2.79)	0.456
hsa-mir-155 (n=55)	High/low	3.42 (1.42-8.19)	0.006
hsa-let-7a-2 (n=52)	Low/high	2.35 (1.08-6.86)	0.033
Multivariate analysis (n=55)[b, c]			
Age	Age≥67/Age<67	1.92 (0.71-5.17)	0.195
Sex	Male/female	1.23 (0.47-3.22)	0.669
Stage	II-IV/I	3.27 (1.31-8.37)	0.013
Smoking history	Current/former	1.49 (0.51-4.34)	0.457
hsa-mir-155	High/low	3.03 (1.13-8.14)	0.027

[a]95% CI, 95% confidence interval.
[b]Multivariate analysis, Cox proportional hazard regression model.
[c] *hsa-let-7a-2* low/high was not statistically significant (p=0.089).

Table 5B. Postoperative survival of patients with lung adenocarcinoma in relation to clinicopathological characteristics and precursor miRNA expression analyzed by real-time RT-PCR analysis.

Variable	Subset	Original cohort (n=32)		Additional cohort (n=32)		All cases (n=64)	
		Hazard ratio (95% CI[a])	*p*	Hazard ratio (95% CI)	*p*	Hazard ratio (95% CI)	*p*
Univariate analysis							
Age	Age≥67/Age<67	1.89 (0.62-5.34)	0.274	1.21 (0.46-3.21)	0.679	1.28 (0.64-2.58)	0.482
Sex	Male/female	0.53 (0.14-1.56)	0.232	1.37 (0.54-3.63)	0.479	0.99 (0.49-1.98)	0.975
Stage	II-IV/I	**4.22** (1.91-23.6)	0.003	**2.37** (1.01-7.83)	0.048	**3.07** (1.82-8.84)	<0.00
Smoking history	Current/former	0.92 (0.31-2.66)	0.921	1.22 (0.47-3.16)	0.674	1.12 (0.56-2.25)	0.757
precursor *hsa-mir-155*	High/low	**2.75** (1.05-12.1)	0.047	**2.52** (1.10-7.45)	0.033	**2.74** (1.53-6.91)	0.002
precursor *hsa-let-7a-2*	Low/high	**3.01** (1.09-9.86)	0.037	2.22 (0.91-5.71)	0.084	**2.73** (1.42-5.88)	0.003
Multivariate analysis[b]							
Age	Age≥67/Age<67	0.91 (0.22-3.68)	0.899	0.93 (0.30-2.91)	0.914	1.22 (0.58-2.53)	0.593
Sex	Male/female	0.35 (0.11-1.17)	0.089	0.92 (0.32-2.66)	0.885	0.85 (0.41-1.74)	0.659
Stage	II-IV/I	**8.99** (1.95-41.2)	0.004	**4.91** (1.51-15.9)	0.008	**5.58** (2.42-12.8)	<0.001
Smoking history	Current/former	1.01 (0.30-3.38)	0.980	2.27 (0.70-7.34)	0.170	1.89 (0.85-4.21)	0.117
precursor *hsa-mir-155*	High/low	**13.3** (2.59-69.0)	0.002	**3.77** (1.32-10.6)	0.013	**4.98** (2.29-10.8)	<0.001
precursor *hsa-let-7a-2*	Low/high	**3.93** (1.06-14.5)	0.040	**2.97** (1.07-8.23)	0.036	**3.55** (1.64-7.69)	0.001

[a]95% CI, 95% confidence interval.
[b]Multivariate analysis, Cox proportional hazard regression model.

To investigate the biological consequences of altered *hsa-mir-155* and *hsa-let-7a-2* expression, a bioinformatic analysis was conducted to group the predicted targets of these miRNAs according to Gene Ontology (GO) terms (Table 6). In addition to associations with more general functional GO terms, a significant enrichment for targets associated with transcription was seen for *hsa-mir-155*. *hsa-let-7a* showed an over-representation of gene targets linked with protein kinase and intracellular signaling cascades, a finding consistent with the reported functional interaction between *let-7* and RAS (Johnson, S.M., *et al.*, *Cell 120*:635-647 (2005)).

Table 6. Gene ontology analysis (biological process) for the predicted transcript targets of *hsa-mir-155* and *hsa-let-7a*.

Biological process	Gene Ontology	*p* -value
hsa-mir-155		
regulation of biological process	GO:0050789	3.44343E-05
regulation of nucleobase \ nucleoside \ nucleotide and nucleic acid metabolism	GO:0019219	0.000149553
regulation of physiological process	GO:0050791	0.000192938
regulation of transcription \ DNA-dependent	GO:0006355	0.000244233
regulation of metabolism	GO:0019222	0.000310887
regulation of transcription	GO:0045449	0.000367426
transcription\, DNA-dependent	GO:0006351	0.000373583
transcription	GO:0006350	0.000749403
NLS-bearing substrate-nucleus import	GO:0006607	0.000871079
B-cell differentiation	GO:0030183	0.00142995
nucleobase \ nucleoside \ nucleotide and nucleic acid metabolism	GO:0006139	0.0021327
protein targeting	GO:0006605	0.00238267
hemopoiesis	GO:0030097	0.00243434
cellular process	GO:0009987	0.00270393
uridine metabolism	GO:0046108	0.0040568
B-cell activation	GO:0042113	0.00458041
hsa-let-7a		
protein modification	GO:0006464	9.02643E-05
cell growth and/or maintenance	GO:0008151	9.99217E-05
cellular physiological process	GO:0050875	0.000128316
protein kinase cascade	GO:0007243	0.000703203
cellular process	GO:0009987	0.000870863
intracellular signaling cascade	GO:0007242	0.001290613
transport	GO:0006810	0.004305096
chromatin modification	GO:0016568	0.004414505
localization	GO:0051179	0.004492152
phosphorus metabolism	GO:0006793	0.00481218
phosphate metabolism	GO:0006796	0.00481218

Real-time RT-PCR analysis was performed for *hsa-mir-155* and *hsa-let-7a-2* to determine whether the precursor miRNAs expression also had prognostic impact on adenocarcinoma patients. First, 32 pairs of adenocarcinoma from the original set, in which RNA was available, were subjected to real-time RT-PCR analysis. The ratio of lung cancer expression to corresponding noncancerous lung tissue expression was calculated and the cases were classified according to the expression ratio. Kaplan-Meier survival analysis (FIG. 6, FIG. 7) demonstrated a significantly worse survival for patients with either high precursor *hsa-mir-155* expression (p=0.047; log-rank test) or reduced precursor *hsa-let-7a-2* expression (p=0.037; log-rank test) (Table 5B). To further validate the prognosis classifiers described here, an additional independent set of 32 adenocarcinomas was analyzed using real-time RT-PCR analysis. Kaplan-Meier survival curves (FIG. 8, FIG. 9) showed a clear relationship in precursor *hsa-mir-155* expression (p=0.033; log-rank test) and approaching significance in *hsa-let-7a-2* expression (p=0.084; log-rank test) in this cohort as well (Table 5B). In addition, high precursor *hsa-mir-155* expression was found to be an independent predictor of poor prognosis by a multivariate Cox proportional hazard regression analysis (Table 5B). To further confirm whether there was any grouping bias in the original set (32 cases) and the additional set (32 cases), univariate and multivariate survival analyses were performed for all 64 cases. Consistent with previous results, these analyses showed the significance of precursor *hsa-mir-155* expression (Table 5B; FIG. 10). Of note, reduced precursor *hsa-let-7a-2* expression also had similar prognostic impact on adenocarcinoma patients (Table 5B; FIG. 11), consistent with a previous report (Takamizawa, J., *et al.*, *Cancer Res. 64*, 3753-3756 (2004)).

Example 5: Lack of epigenetic regulation of miRNA expression in NSCLC cell lines.

Materials and methods

Microarray analysis

Microarray analysis was performed as previously described (Liu, C.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 101*:9740-9744 (2004), see also, Example 1).

Statistical analysis

Statistical analyses were performed as described hereinabove (see, e.g., Example 1).

5-aza-dC and/or TSA treatment

A549 and NCI-H157 lung cancer cells (available from the American Tissue Culture Collection) were incubated with medium containing 1.0 μM 5-aza-dC (Sigma, St. Louis, MO) for 48 hr, then were incubated for an additional 24 hr in the presence of 1.0 μM TSA (Sigma, St. Louis, MO). Total RNA was isolated with TRIzol® Reagent (Invitrogen), and microarray analysis was performed as described above. Each treatment was performed in triplicate.

Results

miRNA microarrays were used to analyze the expression of various miRNAs upon treatment with 5-aza-2'-deoxycytidine (5-aza-dC), a DNA methylation inhibitor, and/or Trichostatin A (TSA), a potent histone deacteylase inhibitor, in two lung cancer cell lines (A549 and NCI-H157). Although increased expression of a gene that is known to be transcriptionally-silenced (*MYO18B*) was confirmed following treatment with 5-aza-dC or TSA (FIG. 12), no miRNAs from the microarray displayed statistically-significant changes in expression after treatment with either compound, suggesting that hypermethylation and histone deacetylation were not responsible for reduced levels of miRNA expression in at least these two cell lines.

The relevant teachings of all publications cited herein that have not explicitly been incorporated by reference, are incorporated herein by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A method of diagnosing whether a subject has, or is at risk for developing, lung cancer, comprising measuring the level of at least one miR gene product in a test sample from said subject, wherein an alteration in the level of the miR gene product in the test sample, relative to the level of a corresponding miR gene product in a control sample, is indicative of the subject either having, or being at risk for developing, lung cancer.

2. The method of Claim 1, wherein the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a and let-7f-1.

3. The method of Claim 1, wherein the at least one miR gene product is selected from the group consisting of miR-21, miR-205 and miR-216.

4. The method of Claim 1, wherein the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126* and miR-224.

5. The method of Claim 1, wherein the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-21, miR-191, miR-155, miR-210, miR-126*, miR-126, miR-24-2, miR-219-1, miR-95, miR-192-prec, miR-220, miR-216-prec, miR-204-prec, miR-188, miR-198, miR-145 and miR-224.

6. The method of Claim 1, wherein the lung cancer is a lung squamous cell carcinoma and the at least one miR gene product is selected from the group consisting of miR-205, miR-224, miR-191, miR-126*, miR-140, miR-210, miR-17-3p, miR-29b, miR-143, miR-203, miR-155, miR-21, miR-214, miR-212, miR-30a-5p and miR-197.

7. The method of Claim 1, wherein the level of the at least one miR gene product in the test sample is less than the level of the corresponding miR gene product in the control sample.

8. The method of Claim 1, wherein the level of the at least one miR gene product in the test sample is greater than the level of the corresponding miR gene product in the control sample.

9. A method of determining the prognosis of a subject with lung cancer, comprising measuring the level of at least one miR gene product in a test sample from said subject, wherein:

the miR gene product is associated with an adverse prognosis in lung cancer; and

an alteration in the level of the at least one miR gene product in the lung test sample, relative to the level of a corresponding miR gene product in a control sample, is indicative of an adverse prognosis.

10. The method of Claim 9, wherein the at least one miR gene product is selected from the group consisting of miR-155, miR-17-3p, miR-106a, miR-93, let-7a-2, miR-145, let-7b, miR-20 and miR-21.

11. The method of Claim 9, wherein the lung cancer is a lung adenocarcinoma and the at least one miR gene product is selected from the group consisting of miR-155 and let-7a-2.

12. A method of diagnosing whether a subject has, or is at risk for developing, lung cancer, comprising:

(1) reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides;

(2) hybridizing the target oligodeoxynucleotides to a microarray comprising miRNA-specific probe oligonucleotides to provide a hybridization profile for the test sample; and

(3) comparing the test sample hybridization profile to a hybridization profile generated from a control sample,

wherein an alteration in the signal of at least one miRNA is indicative of the subject either having, or being at risk for developing, lung cancer.

13. The method of Claim 13 wherein the signal of at least one miRNA, relative to the signal generated from the control sample, is down-regulated.

14. The method of Claim 13 wherein the signal of at least one miRNA, relative to the signal generated from the control sample, is up-regulated.

15. The method of Claim 13 wherein the microarray comprises miRNA-specific probe oligonucleotides for one or more miRNAs selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214, miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a, let-7f-1 and a combination thereof.

16. A method of diagnosing whether a subject has, or is at risk for developing, a lung cancer with an adverse prognosis in a subject, comprising:

(1) reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides;

(2) hybridizing the target oligodeoxynucleotides to a microarray comprising miRNA-specific probe oligonucleotides to provide a hybridization profile for said test sample; and

(3) comparing the test sample hybridization profile to a hybridization profile generated from a control sample,

wherein an alteration in the signal is indicative of the subject either having, or being at risk for developing, a lung cancer with an adverse prognosis.

17. The method of Claim 16, wherein an alteration in the signal of at least one miR gene product selected from the group consisting of miR-155, miR-17-3p, miR-106a, miR-93, let-7a-2, miR-145, let-7b, miR-20 and miR-21 is indicative of the subject either having, or being at risk for developing, a lung cancer with an adverse prognosis.

18. The method of Claim 16, wherein the lung cancer is a lung adenocarcinoma and an alteration in the signal of at least one miR gene product selected from the group consisting of miR-155 and let-7a-2 is indicative of the subject either having, or being at risk for developing, a lung cancer with an adverse prognosis.

19. The method of Claim 16, wherein the microarray comprises at least one miRNA-specific probe oligonucleotide for a miRNA selected from the group consisting of miR-21, miR-191, miR-126*, miR-210, miR-155, miR-143, miR-205, miR-192-prec, miR-224, miR-126, miR-24-2, miR-30a-5p, miR-212, miR-140, miR-9, miR-214; miR-17-3p, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216-prec, miR-219-1, miR-106a, miR-197, miR-192, miR-125a-prec, miR-26a-1-prec, miR-146, miR-203, miR-199b-prec, let-7a-2-prec, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c-prec, miR-150, miR-101-1, miR-124a-3, miR-125a, let-7f-1 and a combination thereof.

20. A method of treating lung cancer in a subject who has a lung cancer in which at least one miR gene product is down-regulated or up-regulated in the cancer cells of the subject relative to control cells, comprising:

(1) when the at least one miR gene product is down-regulated in the cancer cells, administering to the subject an effective amount of at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, provided that the miR gene product is not miR-15a or miR-16-1, such that proliferation of cancer cells in the subject is inhibited; or

(2) when the at least one miR gene product is up-regulated in the cancer cells, administering to the subject an effective amount of at least one compound for inhibiting expression of the at least one miR gene product, such that proliferation of cancer cells in the subject is inhibited.

21. The method of Claim 20, wherein the at least one isolated miR gene product in step (1) is selected from the group consisting of miR-126*, miR-143, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, let-7f-1 and a combination thereof.

22. The method of Claim 20, wherein the at least one miR gene product in step (2) is selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

23. A method of treating lung cancer in a subject, comprising:

(1) determining the amount of at least one miR gene product in lung cancer cells, relative to control cells; and

(2) altering the amount of miR gene product expressed in the lung cancer cells by:

(i) administering to the subject an effective amount of at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, provided that the miR gene product is not miR-15a or miR-16-1, if the amount of the miR gene product expressed in the cancer cells is less than the amount of the miR gene product expressed in control cells; or

(ii) administering to the subject an effective amount of at least one compound for inhibiting expression of the at least one miR gene product, if the amount of the miR gene product expressed in the cancer cells is greater than the amount of the miR gene product expressed in control cells.

24. The method of Claim 23, wherein the at least one isolated miR gene product in step (i) is selected from the group consisting of miR-126*, miR-143, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, let-7f-1 and a combination thereof.

25. The method of Claim 23, wherein the at least one miR gene product in step (ii) is selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

26. A pharmaceutical composition for treating lung cancer, comprising at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, and a pharmaceutically-acceptable carrier.

27. The pharmaceutical composition of Claim 26, wherein the at least one isolated miR gene product corresponds to a miR gene product that is down-regulated in lung cancer cells relative to control cells.

28. The pharmaceutical composition of Claim 26, wherein the isolated miR gene product is selected from the group consisting of miR-126*, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, miR-125b-1, let-7f-1 and a combination thereof.

29. A pharmaceutical composition for treating lung cancer, comprising at least one miR expression-inhibitor compound and a pharmaceutically-acceptable carrier.

30. The pharmaceutical composition of Claim 29, wherein the at least one miR expression-inhibitor compound is specific for a miR gene product that is up-regulated in lung cancer cells relative to control cells.

31. The pharmaceutical composition of Claim 29, wherein the at least one miR expression-inhibitor compound is specific for a miR gene product selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

32. A method of identifying an anti-lung cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR gene product associated with decreased expression levels in lung cancer cells, wherein an increase in the level of the miR gene product in the cell, relative to a control cell, is indicative of the test agent being an anti-lung cancer agent.

33. The method of Claim 32, wherein the miR gene product is selected from the group consisting of miR-126*, miR-143, miR-192, miR-224, miR-126, miR-30a-5p, miR-140, miR-9, miR-124a-1, miR-218-2, miR-95, miR-145, miR-198, miR-216, miR-219-1, miR-125a, miR-26a-1, miR-199b, let-7a-2, miR-27b, miR-32, miR-29b-2, miR-220, miR-33, miR-181c, miR-101-1, miR-124a-3, let-7f-1 and a combination thereof.

34. A method of identifying an anti-lung cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR gene product associated with increased expression levels in lung cancer cells, wherein a decrease in the level of the miR gene product in the cell, relative to a control cell, is indicative of the test agent being an anti-lung cancer agent.

35. The method of Claim 34, wherein the miR gene product is selected from the group consisting of miR-21, miR-191, miR-210, miR-155, miR-205, miR-24-2, miR-212, miR-214, miR-17-3p, miR-106a, miR-197, miR-192, miR-146, miR-203, miR-150 and a combination thereof.

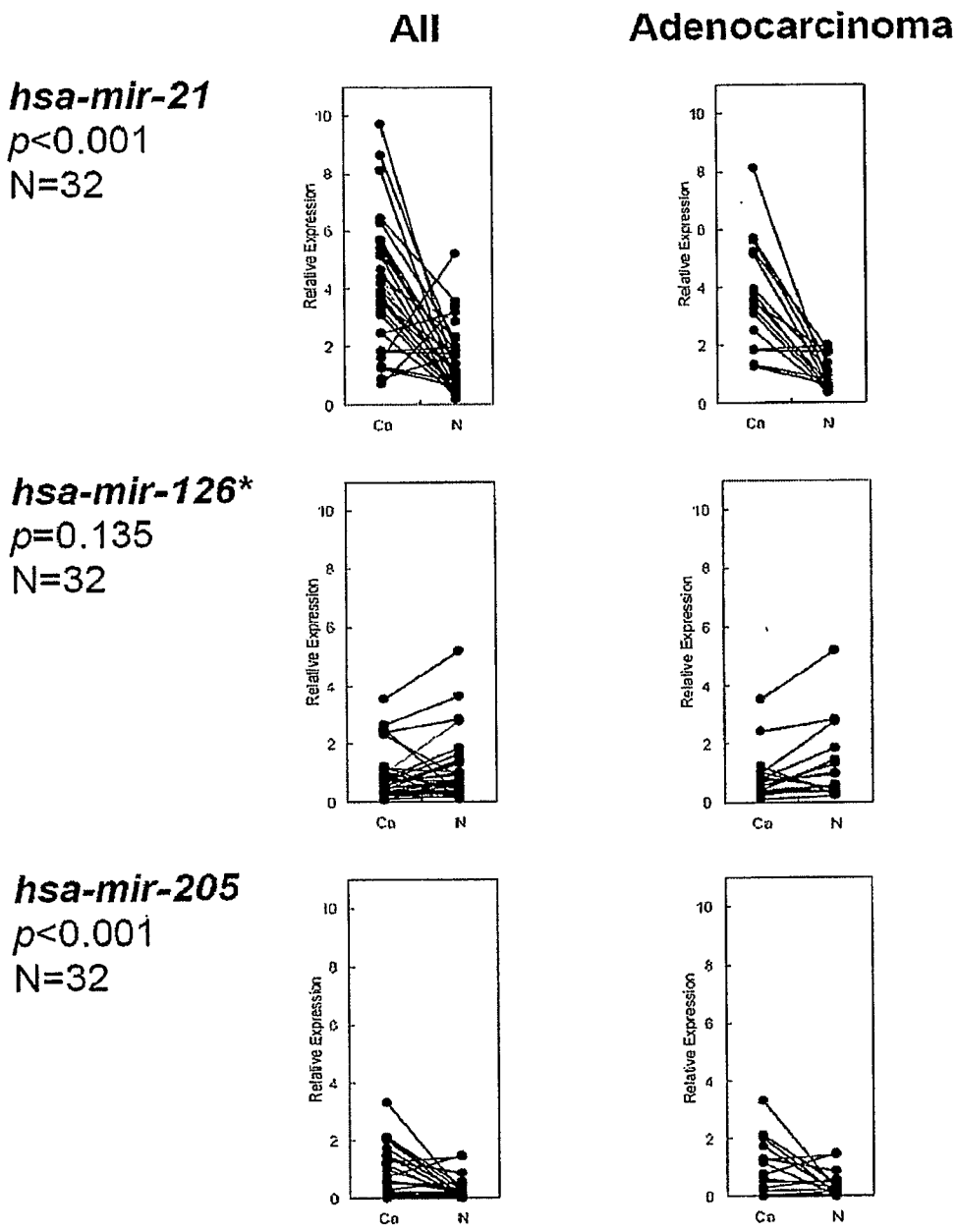
All
Adenocarcinoma
hsa-mir-21
p<0.001
N=32
hsa-mir-126*
p=0.135
N=32
hsa-mir-205
p<0.001
N=32
Relative Expression
Ca
N

FIG. 1

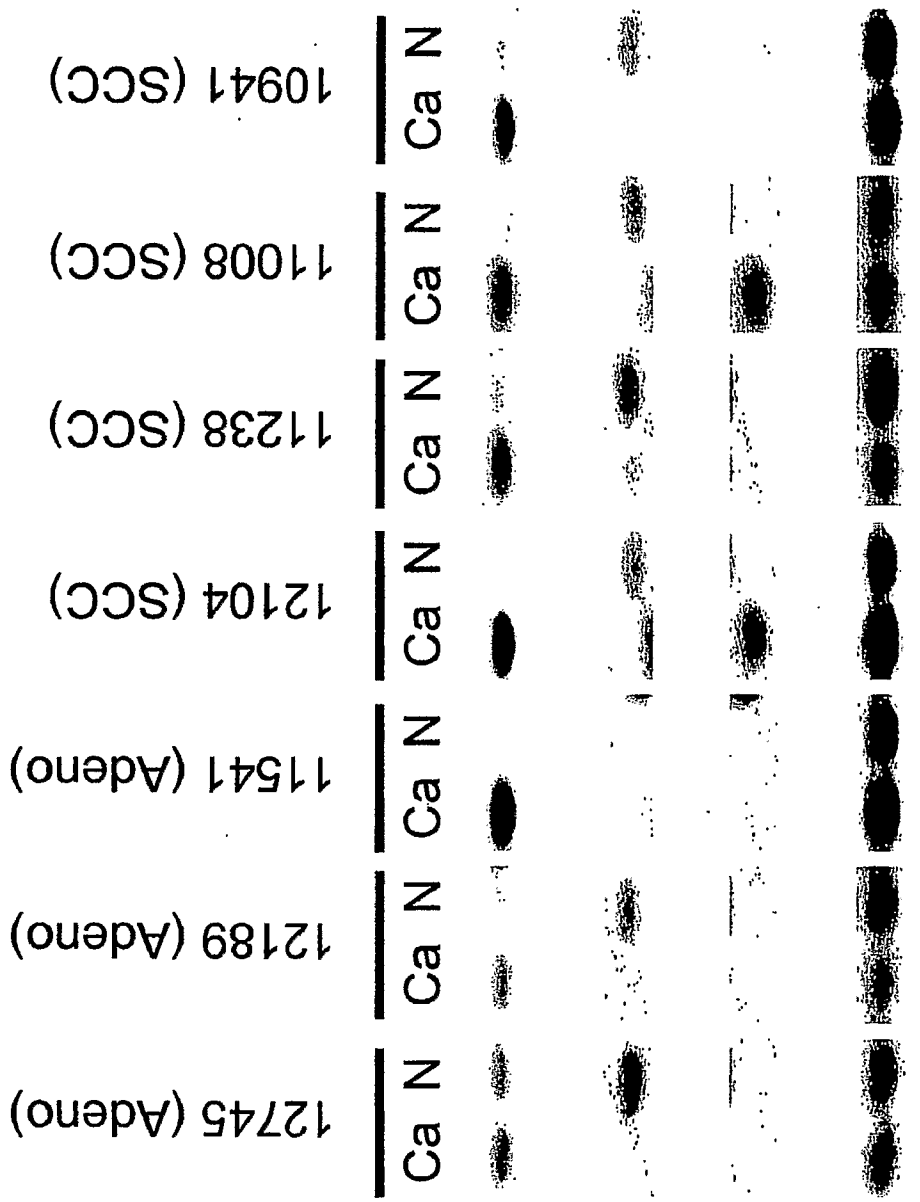
Ca N
12745 (Adeno)
12189 (Adeno)
11541 (Adeno)
12104 (SCC)
11238 (SCC)
11008 (SCC)
10941 (SCC)
hsa-mir-21
hsa-mir-126*
hsa-mir-205
5S rRNA

FIG. 2

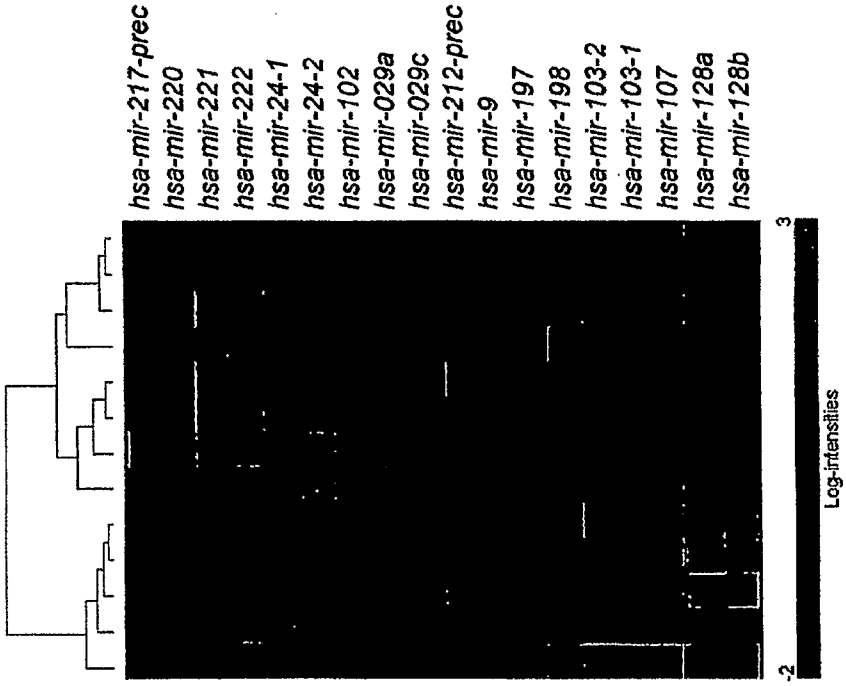
hsa-mir-217-prec
hsa-mir-220
hsa-mir-221
hsa-mir-222
hsa-mir-24-1
hsa-mir-24-2
hsa-mir-102
hsa-mir-029a
hsa-mir-029c
hsa-mir-212-prec
hsa-mir-9
hsa-mir-197
hsa-mir-198
hsa-mir-103-2
hsa-mir-103-1
hsa-mir-107
hsa-mir-128a
hsa-mir-128b
3
-2
Log-intensities

FIG. 3B

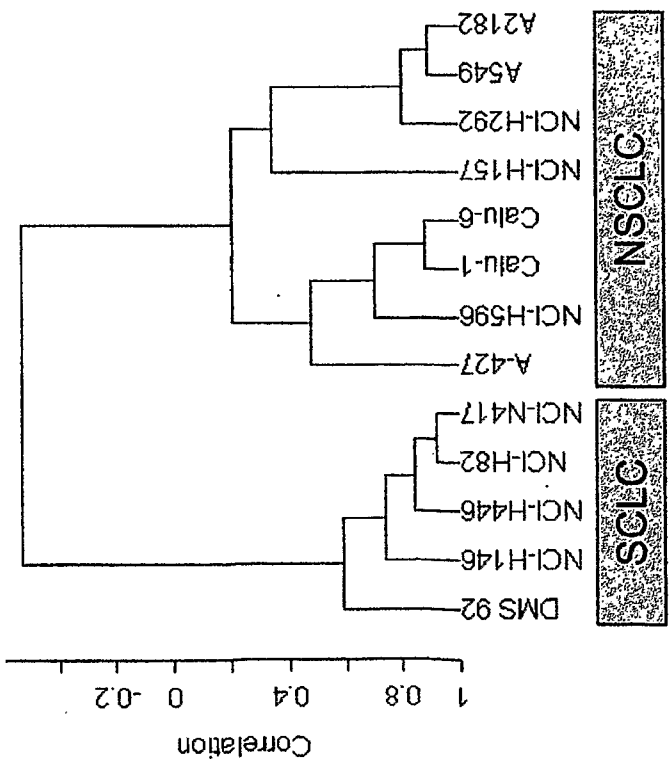
A2182
A549
NCI-H292
NCI-H157
Calu-6
Calu-1
NCI-H596
A-427
NCI-N417
NCI-H82
NCI-H446
NCI-H146
DMS 92
NSCLC
SCLC
1
0.8
0.4
0
-0.2
Correlation

FIG. 3A

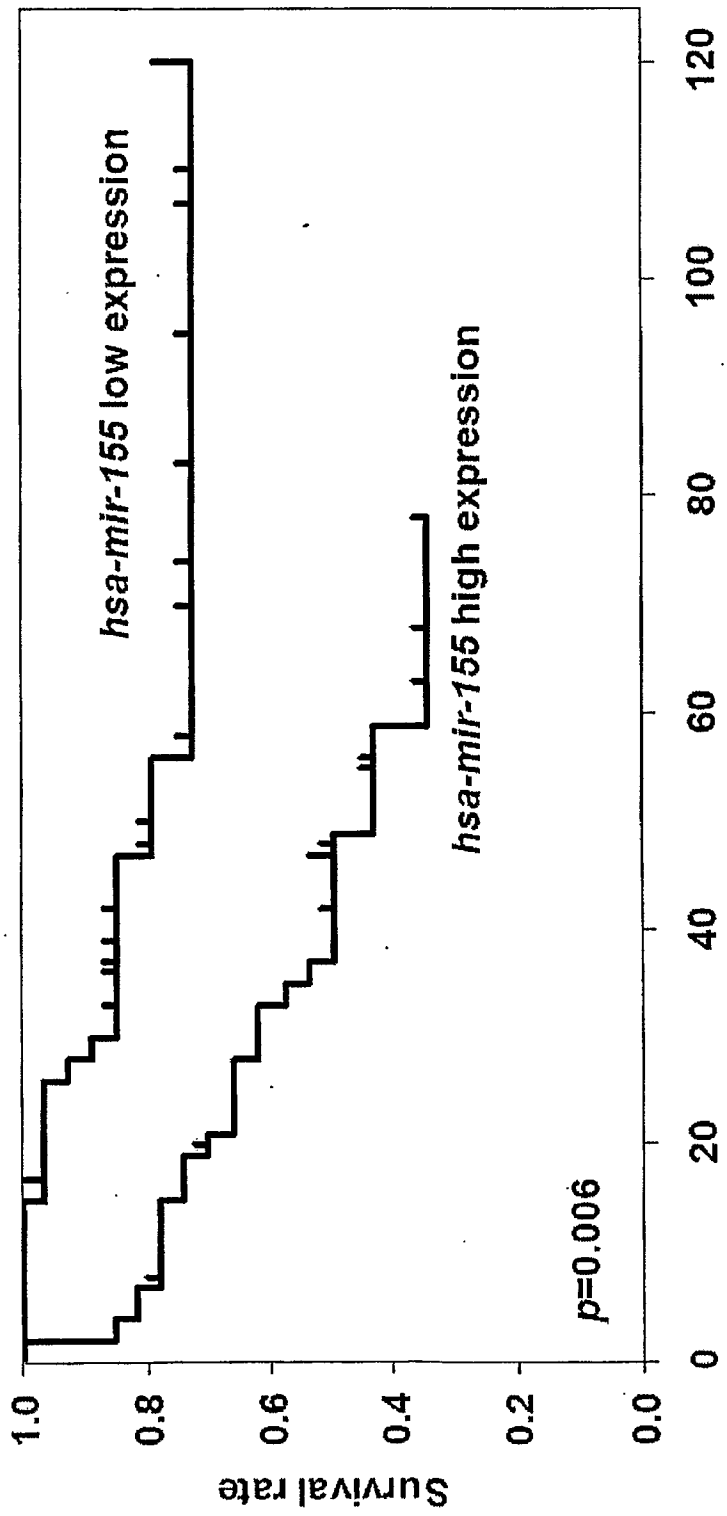
hsa-mir-155 low expression
hsa-mir-155 high expression
p=0.006
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Months

FIG. 4

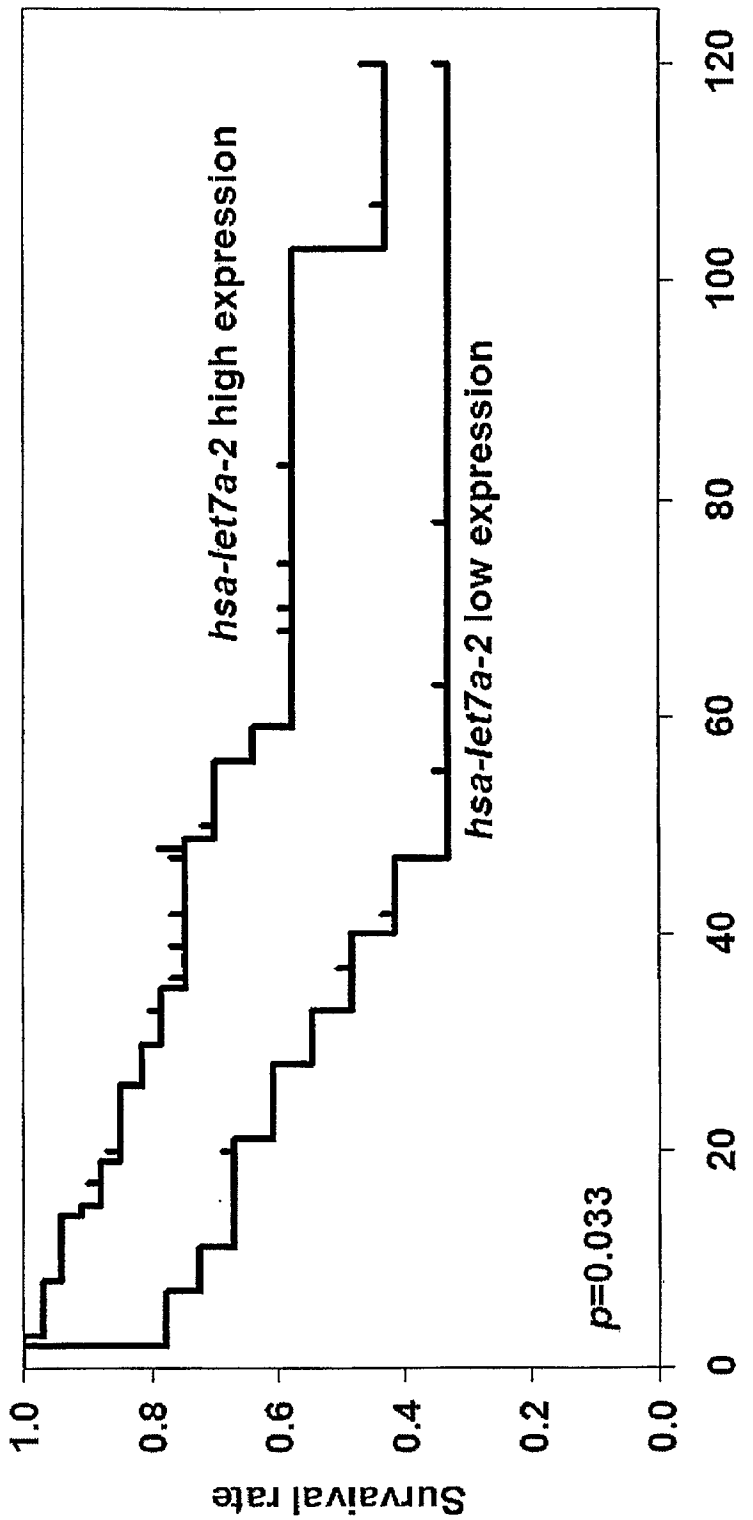
hsa-let7a-2 high expression
hsa-let7a-2 low expression
p=0.033
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Months

FIG. 5

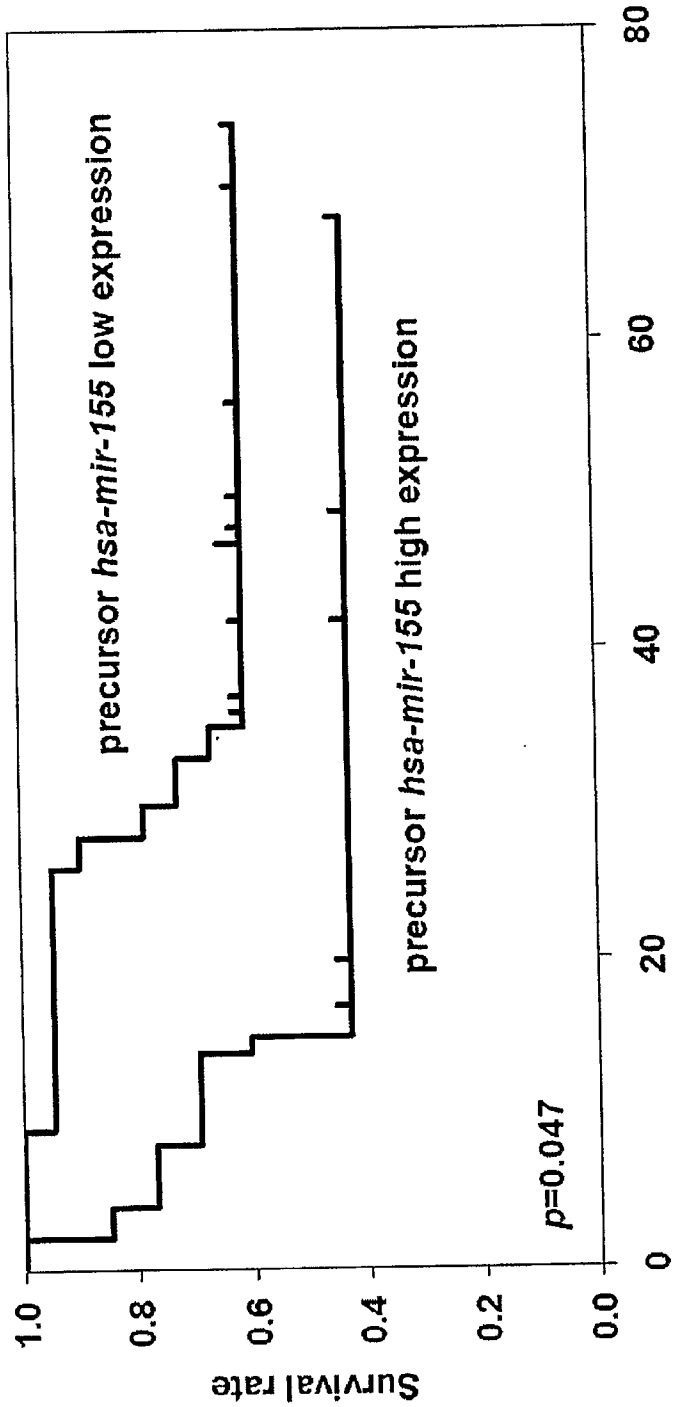
precursor hsa-mir-155 low expression
precursor hsa-mir-155 high expression
p=0.047
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Months

FIG. 6

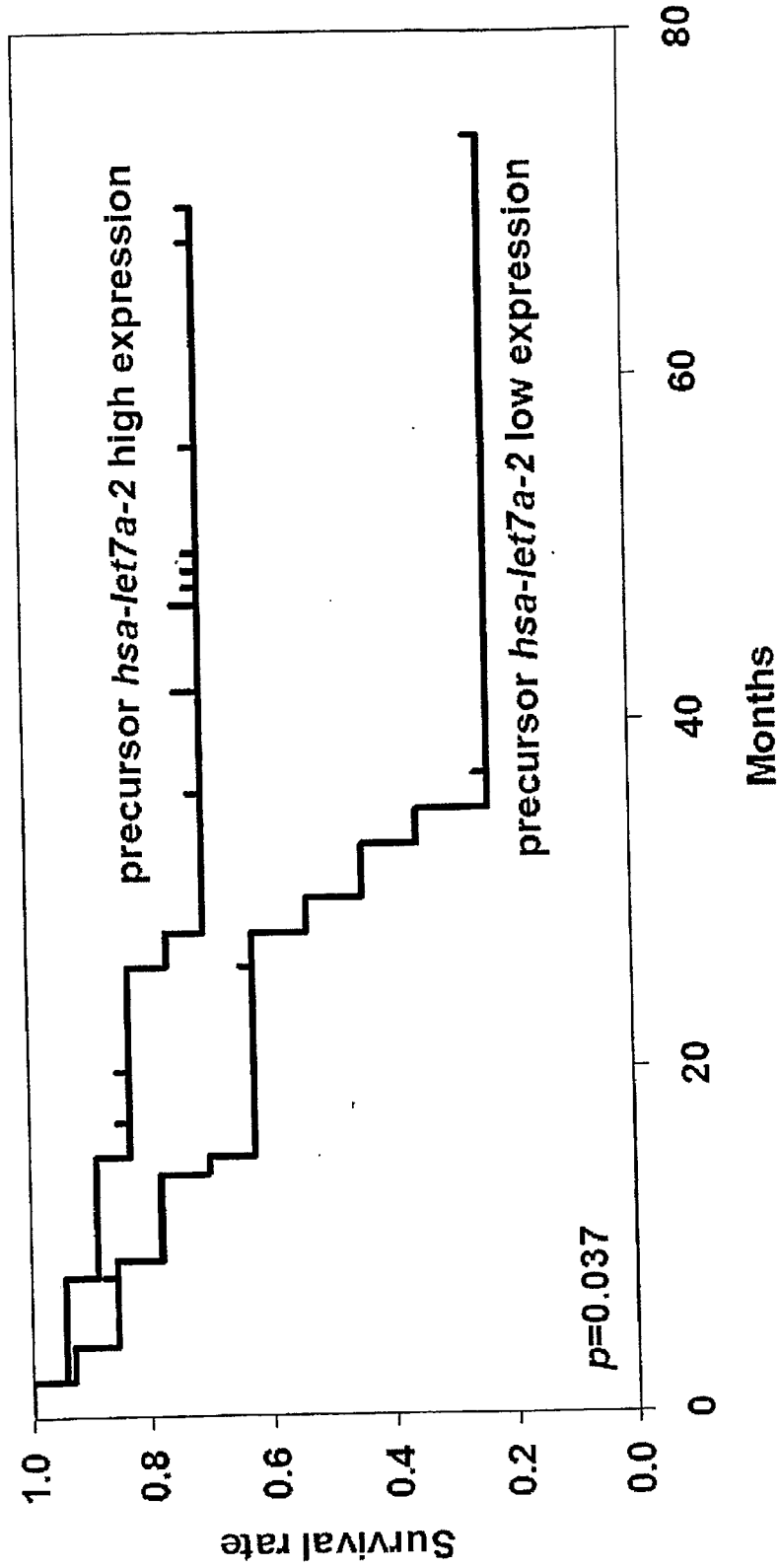
precursor hsa-let7a-2 high expression
precursor hsa-let7a-2 low expression
p=0.037
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Months

FIG. 7

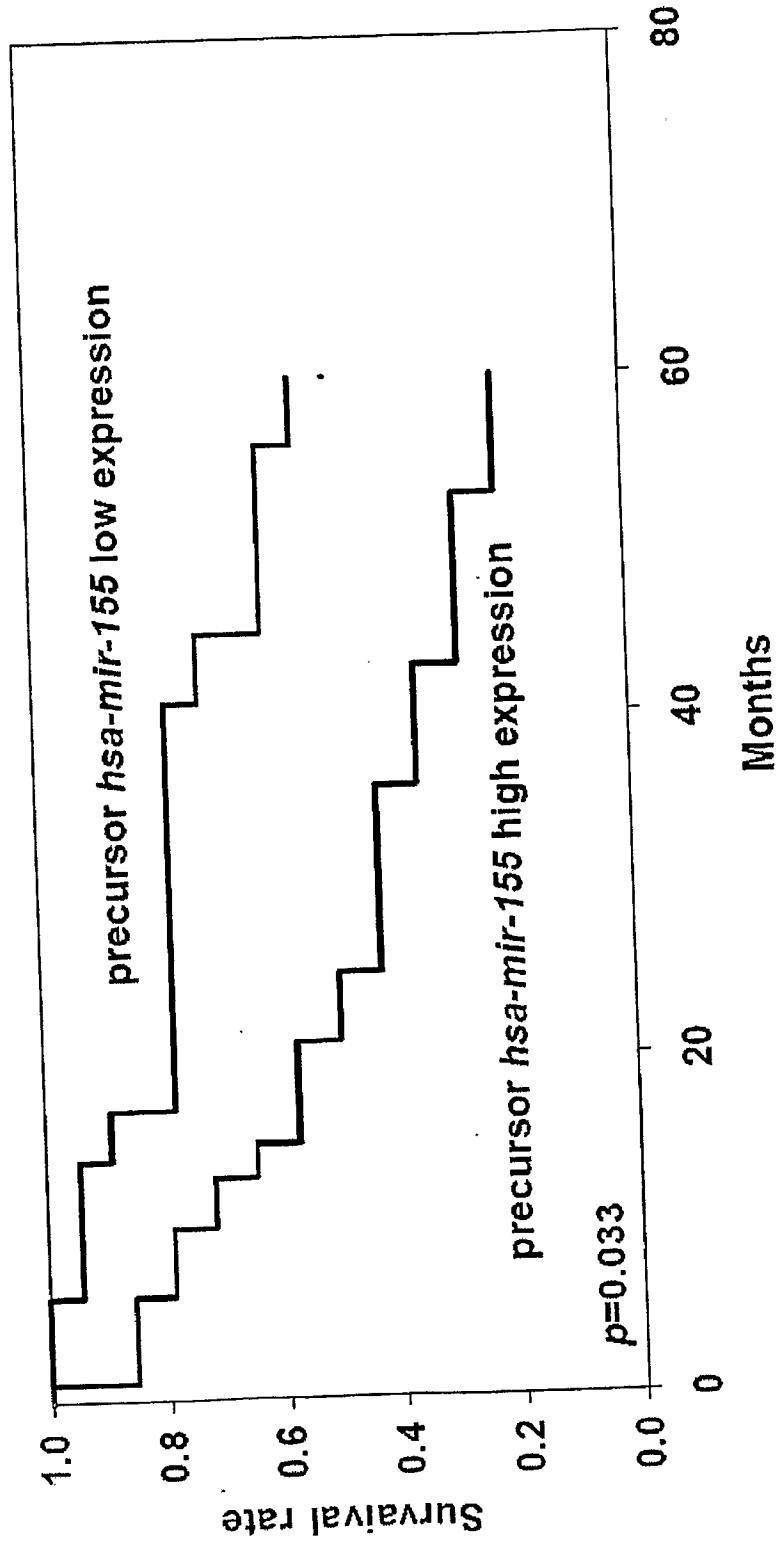
precursor hsa-mir-155 low expression
precursor hsa-mir-155 high expression
p=0.033
Survaival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Months

FIG. 8

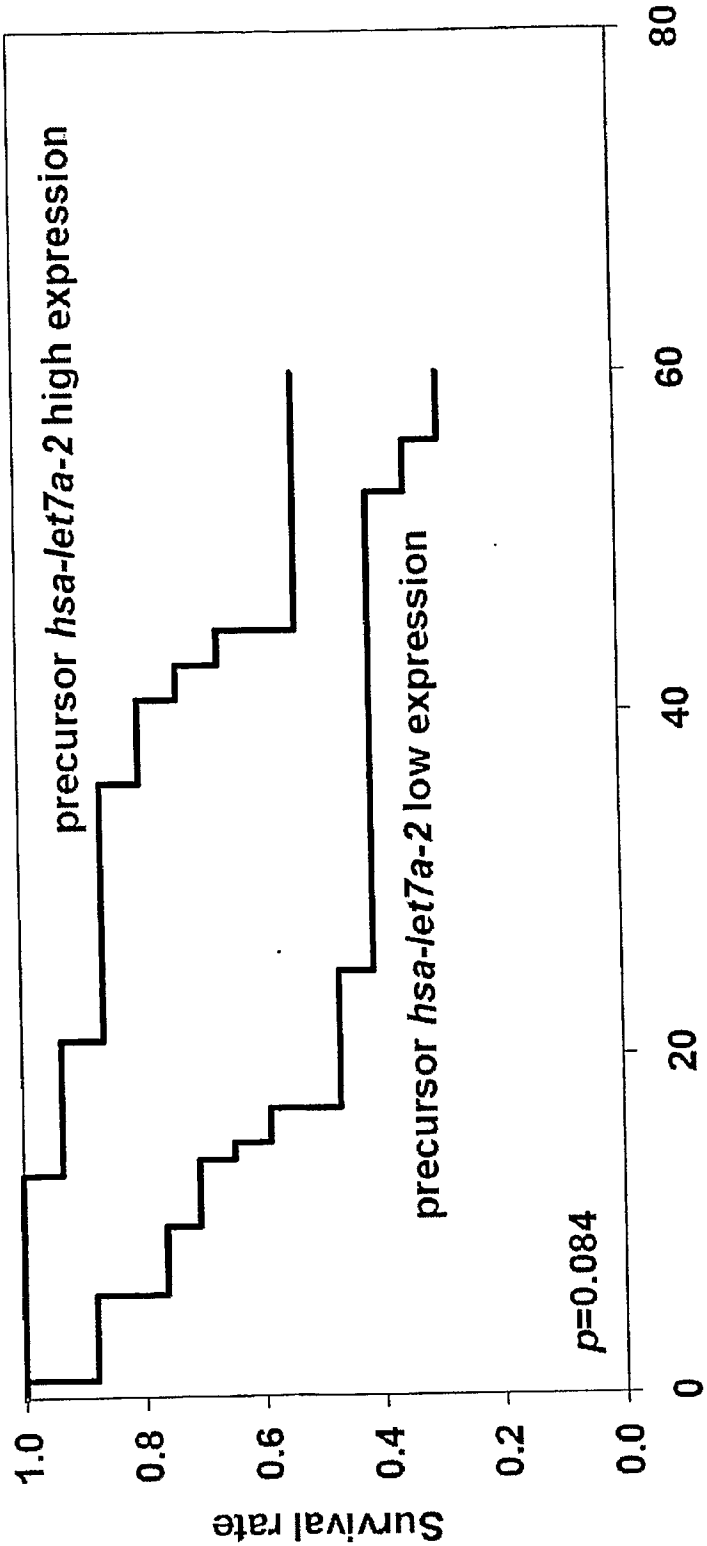
precursor hsa-let7a-2 high expression
precursor hsa-let7a-2 low expression
p=0.084
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Months

FIG. 9

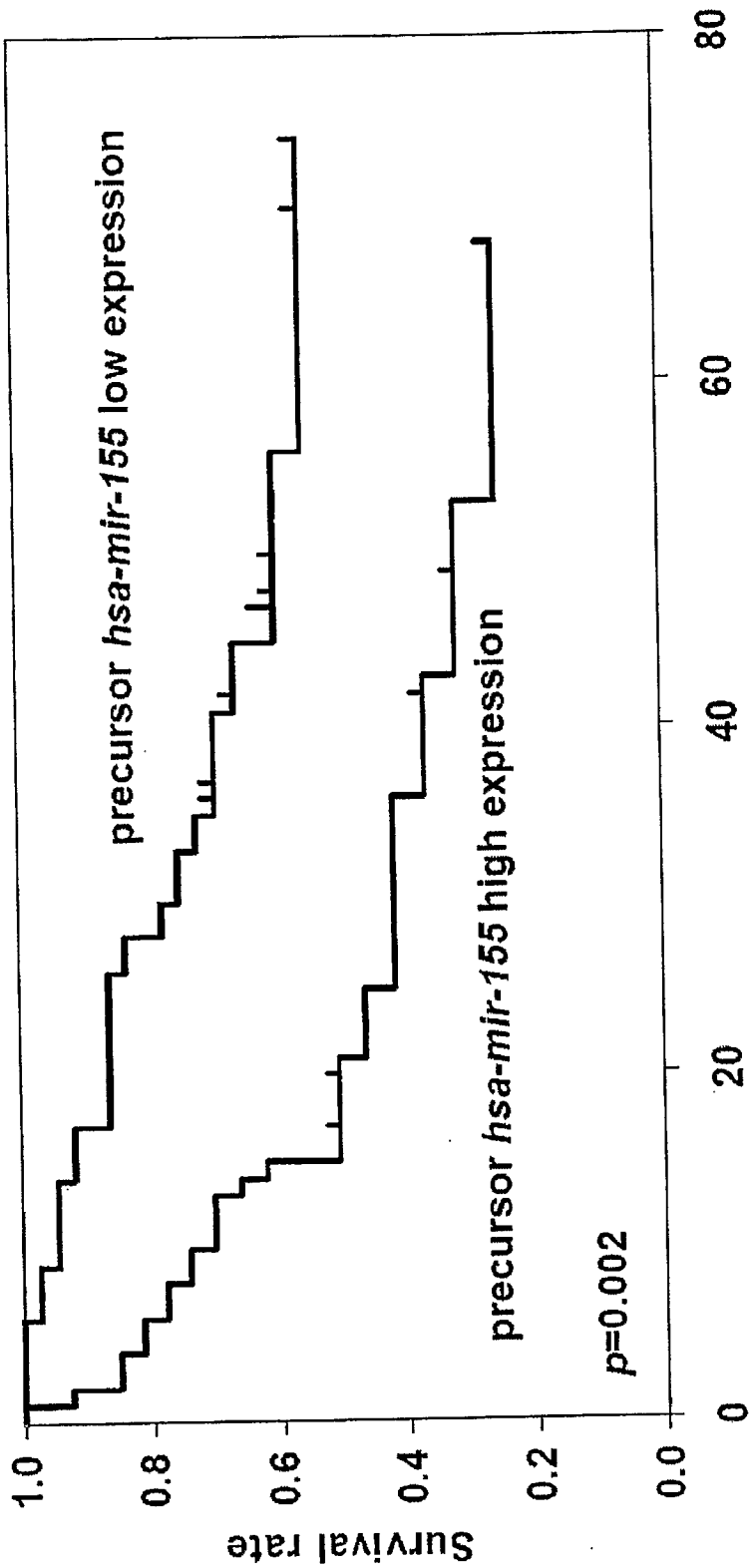
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Months
precursor hsa-mir-155 low expression
precursor hsa-mir-155 high expression
p=0.002

FIG. 10

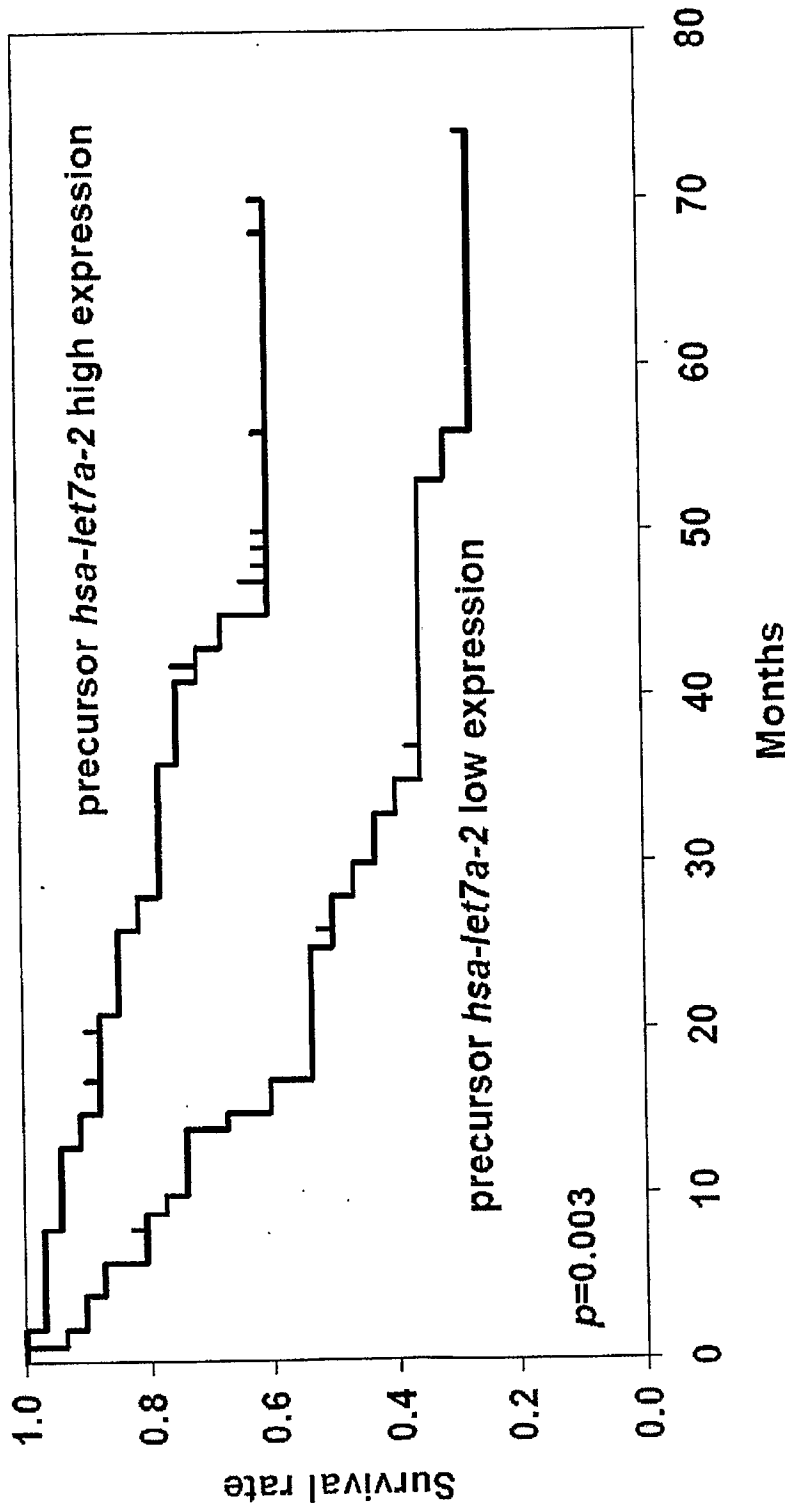
precursor hsa-let7a-2 high expression
precursor hsa-let7a-2 low expression
p=0.003
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
Months

FIG. 11


H157
A549
1 2 3 4 1 2 3 4
139 bp
330 bp
MYO18B (35 cycles)
GAPDH (25 cycles)

FIG. 12