(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 March 2009 (12.03.2009)

PCT

(10) International Publication Number
WO 2009/033140 A1

(51) **International Patent Classification:**
C12Q 1/68 (2006.01)   *A61P 35/00* (2006.01)
A61K 31/7088 (2006.01)

(21) **International Application Number:**
PCT/US2008/075565

(22) **International Filing Date:**
8 September 2008 (08.09.2008)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
60/967,663   6 September 2007 (06.09.2007)   US

(71) **Applicant** *(for all designated States except US)*: **THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION** [US/US]; 1960 Kenny Rd, Columbus, OH 43210-1063 (US).

(72) **Inventor; and**
(75) **Inventor/Applicant** *(for US only)*: **CROCE, Carlo, M.** [US/US]; 2140 Cambridge Boulevard, Columbus, OH 43221 (US).

(74) **Agent: MARTINEAU, Catherine, B.**; Macmillan, Sobanski & Todd, LLC, One Maritime Plaza, 5th Floor, 720 Water Street, Toledo, OH 43604 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(54) **Title:** MICRORNA SIGNATURES IN HUMAN OVARIAN CANCER

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

microRNA SIGNATURES IN HUMAN OVARIAN CANCER

Cross-Reference to Related Applications

This application claims the benefit of PCT application No. PCT/US08/075565 filed 8 September 2008 which claims priority to United States Provisional Application No. 60/967,663, filed 6 September 2007, the disclosures of which are expressly incorporated herein by reference.

Field of Invention

The present invention relates generally to the field of molecular biology. More particularly, it concerns methods and compositions involving microRNA (miRNAs or miRs) molecules. Methods and compositions for isolating, labeling, preparing miRNAs for analysis or as a tool for analysis are described, such as miRNA arrays. In addition, there are applications for miRNAs in diagnostics, therapeutics, and prognostics.

Background of the Invention

Epithelial ovarian cancer is the most common gynecological malignancy and the sixth most common cancer in women worldwide, with highly aggressive natural history causing almost 125,000 deaths yearly (1). Despite advances in detection and cytotoxic therapies, only 30% of patients with advanced-stage ovarian cancer survive 5 years after initial diagnosis (2). The high mortality of this disease is mainly due to late stage diagnosis for more than 70% of ovarian cancers. In fact, when ovarian cancer is diagnosed in its early stage, that is still organ-confined, the five year survival rate exceeds 90%. Unfortunately, only 19% of all ovarian cancers are diagnosed at this early stage. Indeed, this rather poor prognosis is due to (i) the insidious asymptomatic nature of this disease in its early onset, (ii) the lack of robust and minimally invasive methods for early detection, and (iii) tumor resistance to chemotherapy. The vast majority of human ovarian carcinomas are represented by ovarian epithelial cancers (OECs), deriving from the ovarian surface epithelium (OSE) (3).

Ovarian adenocarcinomas occur as four major histological subtypes,

serous, mucinous, endometrioid and clear cell, with serous being the most common.
Current data indicate that each of these histological types is associated with distinct
morphologic and molecular genetic alterations (4), but further investigations of the
molecular mechanisms promoting ovarian cancer are necessary to determine how each of
the subtypes emerges.

[0006]   Over the last five years expression profiling technologies greatly improved,
thus expanding the knowledge on cancer etiology and biomarkers with clinical
applications (5, 6). However, although these technologies have provided most of the new
biomarkers with potential use for diagnosis, drug development, and tailored therapy, they
have so far shed little insight into the detailed mechanisms at the origin of this neoplasia,
thus suggesting that ovarian tumorigenesis may occur through novel or poorly
characterized pathways.

[0007]   A new class of small non-coding RNAs, named microRNAs, was recently
discovered and shown to regulate gene expression at post-transcriptional level, for the
most part by binding through partial sequence homology to the 3' untranslated region (3'
UTR) of target mRNAs, and causing block of translation and/or mRNA degradation (7).
MicroRNAs are 19-25 nt long molecules cleaved from 70-100 nt hairpin pre-miRNA
precursors. The precursor is cleaved by cytoplasmic RNase III Dicer into ~22-nt miRNA
duplex: one strand (miRNA*) of the short-lived duplex is degraded, while the other strand,
that serves as mature miRNA, is incorporated into the RNA-induced silencing complex
(RISC) and drives the selection of target mRNAs containing antisense sequences.

[0008]   Several studies have demonstrated that miRNAs play important roles in
essential processes, such as differentiation, cell growth and cell death (8, 9).

[0009]   Moreover, it has been shown that miRNAs are aberrantly expressed or
mutated in cancers, suggesting that they may play a role as a novel class of oncogenes or
tumor suppressor genes, depending on the targets they regulate: let-7, downregulated in
lung cancer, suppresses RAS (10) and HMGA2 (11, 12) mir-15 and mir-16, deleted or
down-regulated in leukemia, suppress BCL2 (13); mir-17-5p and mir-20a control the
balance of cell death and proliferation driven by the proto-oncogene c-Myc (14).

[0010]   Clear evidences indicate that miRNA polycistron mir-17-92 acts as an
oncogene in lymphoma and lung cancer (15); mir-372 and mir-373 are novel oncogenes in
testicular germ cell tumors by numbing p53 pathway (16), miR-155, overexpressed in B
cell lymphomas and solid tumors, leads to the development of B cell malignancies in an in

vivo model of transgenic mice (17).

The use of microRNA microarray technologies has been used as a powerful tool to recognize microRNAs differentially expressed between normal and tumor samples (18-20), and also to identify miRNA expression signatures associated with well-defined clinico-pathological features and disease outcome (21, 22). Several studies have also investigated the molecular mechanisms leading to an aberrant microRNAs expression, identifying the presence of genomic abnormalities in microRNA genes (21, 23, 24). More recently, few evidences have shown that microRNAs genes may be regulated also by epigenetic mechanisms, as changes in genomic DNA methylation pattern: miR-127 (25) and miR-124a (26) are transcriptionally inactivated by CpG island hypermethylation, while in lung cancer the overexpression of let-7a-3 seems to be due to DNA hypomethylation (27).

In spite of considerable research into therapies for ovarian cancer, ovarian cancer remains difficult to diagnose and treat effectively, and the mortality observed in patients indicates that improvements are needed in the diagnosis, treatment and prevention of the disease.

Summary of the Invention

According to a first embodiment of the invention, there is provided a method of diagnosing whether a subject has, or is at risk for developing, ovarian cancer, comprising measuring the level of a miR signature in a test sample from the subject, wherein the miR signature comprises miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, and wherein an alteration in the level of the miR signature in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject either having, or being at risk for developing, ovarian cancer.

The present invention is based, in part, on the identification of an ovarian cancer-specific signature of miRNAs that are differentially-expressed in ovarian cancer cells, relative to normal control cells.

Accordingly, the invention encompasses methods of diagnosing whether a subject has, or is at risk for developing, ovarian cancer, comprising measuring the level of at least one miR in a test sample from the subject, wherein an alteration in the level of the miR in the test sample, relative to the level of a corresponding miR in a control sample, is

indicative of the subject either having, or being at risk for developing, ovarian cancer.

In a particular aspect, there is provided herein a method of diagnosing whether a subject has, or is at risk for developing, ovarian cancer, comprising measuring the level of at least one miR in a test sample from the subject. An alteration in the level of the miR in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject either having, or being at risk for developing, ovarian cancer.

In another particular aspect, there is provided herein a method that includes

wherein the at least one isolated miR in step (1) is selected from the group consisting of miR-199a, miR-140, miR-145 and miR-125b1, and combinations thereof; and/or

wherein the at least one isolated miR in step (2) is selected from the group consisting of miR-200a, miR-200b, miR-200c and miR-141, and combinations thereof.

According to a seventh embodiment of the invention, there is provided a method of treating ovarian cancer in a subject, comprising:

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

(2)   altering the amount of the at least one miR expressed in the ovarian cancer cells by:

i)   administering to the subject an effective amount of at least one isolated miR, if the amount of the miR expressed in the cancer cells is less than the amount of the miR 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, if the amount of the miR expressed in the cancer cells is greater than the amount of the miR expressed in control cells, such that proliferation of cancer cells in the subject is inhibited;

wherein the at least one isolated miR in step (i) and/or (ii) is selected from the group consisting of miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, and combinations thereof.

According to an eighth embodiment of the invention, there is provided a pharmaceutical composition for treating ovarian cancer, comprising at least one isolated miR and a pharmaceutically-acceptable carrier, wherein the isolated miR is selected from the group consisting of miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, and combinations thereof.

According to a ninth embodiment of the invention, there is provided a method of identifying an anti-ovarian cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR associated with decreased expression levels in ovarian cancer cells, wherein an increase in the level of the miR in the cell, relative to a suitable control cell, is indicative of the test agent being an anti-ovarian cancer agent, wherein the miR is selected from the group consisting of miR-199a, miR-140, miR-145 and miR-125b1, and combinations thereof.

2734840-1:ecc

According to a tenth embodiment of the invention, there is provided a method of identifying an anti-ovarian cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR associated with increased expression levels in ovarian cancer cells, wherein an decrease in the level of the miR in the cell, relative to a suitable control cell, is indicative of the test agent being an anti-ovarian cancer agent, wherein the miR is selected from the group consisting of miR-200a, miR-200b, miR-200c, miR-141, and combinations thereof.

According to an eleventh embodiment of the invention, there is provided a kit for detecting ovarian cancer in an individual comprising

one or more reagents for detecting one or more miRs selected from the group consisting of: miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, and combinations thereof,

one or more target genes of one or more miRs selected from the group consisting of miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, or a combination thereof.

The present invention is based, in part, on the identification of an ovarian cancer-specific signature of miRNAs that are differentially-expressed in ovarian cancer cells, relative to normal control cells.

Accordingly, the invention encompasses methods of diagnosing whether a subject has, or is at risk for developing, ovarian cancer, comprising measuring the level of at least one miR in a test sample from the subject, wherein an alteration in the level of the miR in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject either having, or being at risk for developing, ovarian cancer.

In a particular aspect, there is provided herein a method of diagnosing whether a subject has, or is at risk for developing, ovarian cancer, comprising measuring the level of at least one miR in a test sample from the subject. An alteration in the level of the miR in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject either having, or being at risk for developing, ovarian cancer.

In another particular aspect, there is provided herein a method that includes

identifying a correlation between miR expression and ovarian cancer or a predisposition for ovarian cancer, comprising: (a) labeling the miR isolated from a sample from a subject having or suspected of having a disease or condition; (b) hybridizing the miR to an miR array; (c) determining miR hybridization to the array; and (d) identifying miR differentially expressed in a sample representative of the disease or condition compared to a reference.

[0017]   In a particular aspect, there is provided herein a method where identifying miR differentially expressed comprises generating an miR profile for the sample and evaluating the miR profile to determine whether miR in the sample are differentially expressed compared to a normal sample. In certain embodiments, the miR profile is selected from one or more of the miRs shown in Table 1. Also, in certain embodiments, the miR profile is selected from one or more of the miRs shown in Figure 3A or Figure 3B.

[0018]   In a particular aspect, the ovarian cancer is one or more of clear cell, serous or endometrioid ovarian cancer. In a particular aspect, the miR profile is selected from one or more of the miRs shown in Table 3, whereby ovarian cancer cells are distinguished from normal cells. Also, in certain embodiments, the miR profile is selected from one or more of the miRs shown in Table 4, whereby ovarian cancer cells are distinguished by histotype among: serous, non-serous endometrioid, non-endometrioid, clear cell, non-clear cell, poorly differentiated and non-poorly differentiated.

[0019]   In a particular embodiment, the miR profile involves at least one miR selected from the group consisting of miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, wherein a difference in expression of one or more of the miRNA compared to a normal sample is indicative of ovarian cancer. Also, in certain embodiments, the miR profile involves at least miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, wherein a difference in expression of one or more of the miR compared to a normal sample is indicative of ovarian cancer.

[0020]   In a particular aspect, there is provided herein a method wherein an increase in expression of miR-200a, miR-200b, miR-200c or miR-141, and/or a decrease in expression of miR-199a, miR-140, miR-145 or miR-125b1, as compared to a normal sample, is indicative of ovarian cancer.

[0021]   In a particular aspect, there is provided herein a method where the miR

profile involves at least one miRNA selected from the group consisting of miR-200a, miR-200b, miR-200c and miR-141, wherein a difference in expression of one or more of the miRNA compared to a normal sample is indicative of serous ovarian cancer.

[0022]   In a particular aspect, there is provided herein a method where the miR profile involves at least one miRNA selected from the group consisting of miR-205, miR-21, miR-182, miR-200b and miR-141, wherein a difference in expression of one or more of the miRNA compared to a normal sample is indicative of endometrioid ovarian cancer.

[0023]   In a particular aspect, there is provided herein a method of distinguishing among ovarian cancer histotypes of serous, endometriod, clear cell and/or poorly differentiated ovarian cancer. In certain embodiments, the miR profile is selected from one or more of the miRs shown in Figure 3A or Figure 3B, and is indicative of serous ovarian cancer. In certain other embodiments, the miR profile is selected from one or more of the miRs shown in Figure 3A or Figure 3B, and is indicative of endometriod ovarian cancer. In certain other embodiments, the miR profile is selected from one or more of the miRs shown in Figure 3A or Figure 3B, and is indicative of clear cell ovarian cancer.

[0024]   In a particular aspect, there is provided herein a method of inhibiting proliferation of an ovarian cancer cell comprising: i) introducing into the cell one or more agents which inhibit expression or activity of one or more miRs selected from the group shown in Table 3; ii) introducing into the cell one or more agents which enhances expression of one or more target genes of the miRs, or introducing into the cell a combination of the one or more agents of i) and ii), and maintaining the cells under conditions in which the one or more agents inhibits expression or activity of the miR, enhances expression or activity of one or more target genes of the miR, or results in a combination thereof, thereby inhibiting proliferation of the ovarian cancer cell. In a particular embodiment, the cell is a human cell.

[0025]   In a particular aspect, there is provided herein a method where the expression of miR-200a, miR-200b, miR-200c and miR-141 are up-regulated, and have as common putative target the oncosuppressor BAP1, BRCA1-associated protein, that is down-modulated in ovarian cancer.

[0026]   In a particular aspect, there is provided herein a method for modulating levels of one or more of miR-21, miR-203, miR-146, miR-205, miR-30-5p and miR-30c in an ovarian caner cell compared with normal tissues, comprising administering an effective

amount of a demethylation agent. In a particular embodiment, the levels are increased after 5-aza-2'-deoxycytidine demethylating treatment.

[0027]   In a particular aspect, there is provided herein a method for altering expression of one or more of miR-21, miR-203, miR-146, miR-205, miR-30-5p and miR-30c, comprising controlling the DNA hypomethylation mechanism responsible for their overexpression.

[0028]   The level of the at least one miR can be measured using a variety of techniques that are well known to those of skill in the art. In one embodiment, the level of the at least one miR is measured using Northern blot analysis. In another embodiment, the level of the at least one miR in the test sample is less than the level of the corresponding miR in the control sample. Also, in another embodiment, the level of the at least one miR in the test sample can be greater than the level of the corresponding miR in the control sample.

[0029]   The invention also provides methods of diagnosing a cancer associated with one or more prognostic markers in a subject, comprising measuring the level of at least one miR in a cancer sample from the subject, wherein an alteration in the level of the at least one miR in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject having a cancer associated with the one or more prognostic markers. In one embodiment, the level of the at least one miR 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 comprising miR-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 miR is indicative of the subject either having, or being at risk for developing, such cancer.

[0030]   The invention also encompasses methods of treating cancer in a subject, wherein the signal of at least one miR, relative to the signal generated from the control sample, is de-regulated (e.g., down-regulated, up-regulated).

[0031]   The invention also encompasses methods of diagnosing whether a subject has, or is at risk for developing, a cancer associated with one or more adverse prognostic markers in a subject, 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 comprising miR-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 is indicative of the subject either having, or being at risk for developing, the cancer.

[0032]   The invention also encompasses methods of treating cancer in a subject who has a cancer in which at least one miR is down-regulated or up-regulated in the cancer cells of the subject relative to control cells. When the at least one miR is down-regulated in the cancer cells, the method comprises administering to the subject an effective amount of at least one isolated miR, such that proliferation of cancer cells in the subject is inhibited. When the at least one miR 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, such that proliferation of cancer cells in the subject is inhibited.

[0033]   In related embodiments, the invention provides methods of treating cancer in a subject, comprising: determining the amount of at least one miR in cancer cells, relative to control cells; and altering the amount of miR expressed in the cancer cells by: administering to the subject an effective amount of at least one isolated miR, if the amount of the miR expressed in the cancer cells is less than the amount of the miR expressed in control cells; or administering to the subject an effective amount of at least one compound for inhibiting expression of the at least one miR, if the amount of the miR expressed in the cancer cells is greater than the amount of the miR expressed in control cells, such that proliferation of cancer cells in the subject is inhibited.

[0034]   The invention further provides pharmaceutical compositions for treating cancer, comprising at least one isolated miR and a pharmaceutically-acceptable carrier. In a particular embodiment, the pharmaceutical compositions the at least one isolated miR corresponds to a miR that is down-regulated in cancer cells relative to suitable control cells.

[0035]   In another particular embodiment, the pharmaceutical composition comprises at least one miR expression inhibitor compound and a pharmaceutically-acceptable carrier. Also, in a particular embodiment, the pharmaceutical composition comprises at least one miR expression inhibitor compound is specific for a miR that is down regulated and/or up-regulated in cancer cells relative to suitable control cells.

[0036]   In other embodiments, the present invention provides methods of

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

[0037]   The present invention also provides methods of identifying an anti-cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR associated with increased expression levels in cancer cells, wherein an decrease in the level of the miR in the cell, relative to a suitable control cell, is indicative of the test agent being an anti-cancer.

[0038]   In a specific aspect, as disclosed herein, at least one miR is selected the group shown in Table 3. In a particular embodiment the miR is selected from the group consisting of miR-200a, miR-141, miR-200c, and miR-200b, miR-199a, miR-140, miR-145, and miR-125b1.

[0039]   In a specific aspect, there is also provided herein the identification of miRNAs whose expression is correlated with specific ovarian cancer biopathologic features, such as histotype, lymphovascular and organ invasion, and involvement of ovarian surface.

[0040]   In another specific aspect, it is disclosed herein that the levels of miR-21, miR-203, and miR-205, up-modulated in ovarian carcinomas compared with normal tissues, were significantly increased after 5-aza-2'-deoxycytidine demethylating treatment of OVCAR3 cells.

[0041]   In another particular aspect, there is also disclosed herein a method for altering the expression of these miRs by controlling the DNA hypomethylation mechanism responsible for their overexpression.

[0042]   Various objects and advantages of this invention will become apparent to those skilled in the art from the following detailed description of the preferred embodiment, when read in light of the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043]   The patent or application file may contain one or more drawings executed in color and/or one or more photographs. Copies of this patent or patent application publication with color drawing(s) and/or photograph(s) will be provided by the United States Patent and Trademark Office upon request and payment of the necessary fee.

[0044]   **Figures 1A-1C**: Cluster analysis of ovarian carcinomas and normal ovarian tissues:

[0045]   **Figure 1A**: Tree generated by the hierarchical cluster analysis showing the separation of normal tissues from ovarian cancers on the basis of all human miRNAs spotted on the chip.

[0046]   **Figure 1B**: Some of the microRNAs most significantly down-modulated in tumors versus normal ovary.

[0047]   **Figure 1C**: The 4 microRNAs most significantly up-modulated in tumors versus normal ovary.

[0048]   **Figure 2A**: Northern blot analysis of human ovarian carcinomas with probes of miR-200a, miR-141, miR-199a, miF-125b1, miR-145. Evaluation of miR-199a, miR-125b1 and miR-145 on human ovarian cell lines. The 5S probe was used for normalization of expression levels in the different lanes.

[0049]   **Figure 2B**: Real Time PCR to verify the miR-140 down-modulation in tumors compared to normal samples.

[0050]   **Figures 3A** and **3B**: Venn diagram showing the microRNA signatures characterizing different ovarian carcinoma histotypes (serous, endometrioid and clear cell) compared to the normal tissue (**Fig. 3A**, miRs up-modulated; **Fig. 3B**, down-modulated).

[0051]   **Figure 4A**: T-test graphic representation of miR-222 microarray data expression in serous and endometrioid tumors.

[0052]   **Figure 4B**: Verification by Northern Blot on a smallest set of samples.

[0053]   **Figures 5A-5D**: Expression pattern of microRNAs in OVCAR3 cell line before and after treatment with the demethylating agent 5'-AZA.

[0054]   **Figure 5A**: Table reporting the most significant miRs differentially expressed resulting from the Microarray profiling.

[0055]   **Figure 5B**: Hierarchical cluster tree representation.

[0056]   **Figure 5C**: Real-Time PCR to verify the up-modulation of the 5 most significantly induced miRs, reported as graphical representation of miRs expression levels (each bar is an independent experiment resulting from the average of 3 technical replicates).

[0057]   **Figure 5D**: Northern Blot showing the up-modulation of miR-21 after treatment, normalized with EtBr gel staining.

[0058]   **Figure 6A** and **6B**: The PAM analysis displaying the graphical

representation of the probabilities (0.0 to 1.0) of each sample for being a cancer or a normal tissue according to the miR signature reported in **Fig. 8 - Table 1**, which describes a smaller set of 29 miRs, 4 up-modulated (miR-200a, miR -200b, miR -200c and miR -141) and 25 down-modulated (being miR-199a,miR-140, miR-145 and miR-125b1 among the most significant) differentiating normal versus tumor with a classification rate of 89%.

[0059]   **Figures 7A** and **7B**: Northern Blotting (**Fig. 7A**) on a panel of human ovarian carcinomas and two normal tissues; miR-21 and miR-203 are associated with CpG islands, being the miR-203 embedded in a CpG island 875 bp long, and the miR-21 characterized by a CpG island -2 kb upstream the mature sequence (**Fig. 7B**), while miR-205 does not show any CpG island in a region spanning 2 Kb upstream its mature form.

[0060]   **Figure 8: Table 1**. PAM analysis of microRNAs differentially expressed between tumors and normals. Out of the 39 miRs found by SAM analysis, 29 miRs, 4 up-modulated and 25 down-modulated, were able to classify normal and tumor samples with a classification rate of 89%. The four miRs up-modulated were found amplified in the genomic study performed by Zhang et al., 2005; among the miRs down-modulated, 10 out of 25 were found deleted, 4 are discordant and 11 do not show any copy loss or gain in Zhang study.

[0061]   **Figure 9 - Table 2**: miRs differentially expressed in tumors samples versus normal ovarian tissues. SAM analysis of microRNAs differentially expressed between tumors and normal tissues shows 10 microRNAs up-modulated and 29 down-modulated (q-value<1% and fold change >3). Out of 10 miRs up-modulated, 6 were found amplified in the genomic study performed by Zhang et al., 2005, and 4 did not show any copy loss or gain; among the miRs down-modulated, 12 out of 29 were found deleted, 6 are discordant and 11 do not show any copy loss or gain in Zhang study.

[0062]   **Figure 10 - Table 3**: SAM analyses of different histological subtypes compared to the normal tissues.

[0063]   **Figure 11 - Table 4**: SAM analyses of miRNA expression of different histotypes of tumors compared in pairs.

[0064]   **Figure 12 - Table 5**: SAM analyses identifying microRNAs associated with EOC clinico-pathological features.

[0065]   **Figure 13 - Table 6**: Table summarizing validated and the important predicted targets of the most significant microRNAs resulting from our analyses.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0066]   The present invention is directed to compositions and methods relating to preparation and characterization of miRNAs, as well as use of miRNAs for therapeutic, prognostic, and diagnostic applications.

[0067]   As used herein interchangeably, a "miR ," "microRNA," "miR," or "miRNA" refers to the unprocessed or processed RNA transcript from an miR gene. As the miRs are not translated into protein, the term "miRs" does not include proteins. The unprocessed miR gene transcript is also called an "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, or 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. It is to be understood that the term "miR" as used herein can include one or more of miR-oligonucleotides, including mature miRs, pre-miRs, pri-miRs, or a miR seed sequence. In certain embodiments, a mixture of various miR nucleic acids can also be used. Also, in certain embodiments, the miRs may be modified to enhance delivery.

[0068]   The miRNA (miR) information is available from the Sanger Institute, which maintains a registry of miRNA at *http:/microrna.sanger.ac.uk/sequences/*. The miRBase Sequence database includes the nucleotide sequences and annotations of published miRNA from a variety of sources. The miRBase Registry provides unique names for novel miRNA genes that comply with conventional naming nomenclature for new miRNA prior to publication. Also, the miRBase Targets is a resource for predicated miRNA targets in animals.

[0069]   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 RNAase III). It is understood that the active 19-25 nucleotide RNA molecule can also be produced directly by biological or chemical synthesis, without having been processed from the miR precursor.

[0070]   The present invention encompasses methods of diagnosing whether a subject has, or is at risk for developing, cancer, comprising measuring the level of at least one miR in a test sample from the subject and comparing the level of the miR in the test

sample to the level of a corresponding miR in a control sample. As used herein, a "subject" can be any mammal that has, or is suspected of having, breast cancer. In a particular embodiment, the subject is a human who has, or is suspected of having, cancer.

[0071]   The level of at least one miR 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 ovarian cancer associated with by conventional biopsy techniques. In another example, 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 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 produced from a given miR gene in cells from the subject's sample can be compared to the corresponding miR levels from cells of the control sample.

[0072]   An alteration (*i.e.*, an increase or decrease) in the level of a miR in the sample obtained from the subject, relative to the level of a corresponding miR in a control sample, is indicative of the presence of cancer in the subject. In one embodiment, the level of the at least one miR in the test sample is greater than the level of the corresponding miR in the control sample (i.e., expression of the miR is "up-regulated"). As used herein, expression of a miR is "up-regulated" when the amount of miR in a cell or tissue sample from a subject is greater than the amount the same in a control cell or tissue sample. In another embodiment, the level of the at least one miR in the test sample is less than the level of the corresponding miR in the control sample (i.e., expression of the miR is "down-regulated"). As used herein, expression of an miR gene is "down-regulated" when the amount of miR 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, or the average level of miR gene expression previously obtained for a population of normal human controls.

[0073]   The level of a miR in a sample can be measured using any technique that is suitable for detecting RNA expression levels in a biological sample. Suitable techniques for determining RNA expression levels in cells from a biological sample (e.g., Northern blot analysis, RT-PCR, *in situ* hybridization) are well known to those of skill in the art. In a particular embodiment, the level of at least one miR 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.

[0074]   Suitable probes for Northern blot hybridization of a given miR can be produced from the nucleic acid sequences of the given miR. 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.

[0075]   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; or 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.

[0076]   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 the Molecular Dynamics 400-B 2D Phosphorimager available from Amersham Biosciences, Piscataway, NJ.

[0077]   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.

[0078]   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. Pat. No. 5,427,916, the entire disclosure of which is incorporated herein by reference. Suitable probes for *in situ* hybridization of a given miR can be produced from the nucleic acid sequences.

[0079]   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). The methods for quantitative RT-PCR and variations thereof are within the skill in the art.

[0080]   In some instances, it may be desirable to simultaneously determine the expression level of a plurality of different miRs in a sample. In other instances, it may be

14

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 is time consuming and requires a large amount of total RNA (at least 20 µg for each Northern blot) and autoradiographic techniques that require radioactive isotopes.

[0081]   To overcome these limitations, an oligolibrary, in microchip format (i.e., a microarray), may be constructed containing a set of probe oligodeoxynucleotides 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 oligodeoxynucleotides 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 cancer.

[0082]   As used herein, "probe oligonucleotide" or "probe oligodeoxynucleotide" refers to an oligonucleotide that is capable of hybridizing to a target oligonucleotide.

[0083]   "Target oligonucleotide" or "target oligodeoxynucleotide" refers to a molecule to be detected (e.g., via hybridization).

[0084]   By "miR-specific probe oligonucleotide" or "probe oligonucleotide specific for an miR" is meant a probe oligonucleotide that has a sequence selected to hybridize to a specific miR, or to a reverse transcript of the specific miR.

[0085]   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 cells may be distinguished from cancer cells, and within cancer cells, different prognosis states (good or poor long term survival prospects, for example) may be determined. By comparing expression profiles of cancer cells in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained.

[0086]   The identification of sequences that are differentially expressed in cancer cells or normal cells, 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 act to improve the long-term prognosis in a particular patient).

Similarly, diagnosis may be done or confirmed by comparing patient samples with the
known expression profiles. Furthermore, these gene expression profiles (or individual
genes) allow screening of drug candidates that suppress the cancer expression profile or
convert a poor prognosis profile to a better prognosis profile.

[0087]   Accordingly, the invention provides methods of diagnosing whether a
subject has, or is at risk for developing, cancer, comprising reverse transcribing RNA from
a test sample obtained from the subject to provide a set of target oligo-deoxynucleotides,
hybridizing the target oligo-deoxynucleotides 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, cancer.

[0088]   In one embodiment, the microarray comprises miRNA-specific probe
oligonucleotides for a substantial portion of the human miRNome.

[0089]   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
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.

[0090]   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.

[0091]   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.

[0092]   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.

[0093]   According to the expression profiling methods described herein, total RNA from a sample from a subject suspected of having 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, i.e., noncancerous, control sample. An alteration in the signal is indicative of the presence of the cancer in the subject.

[0094]   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.

[0095]   The invention also provides methods of diagnosing a cancer associated with one or more prognostic markers, comprising measuring the level of at least one miR in a cancer test sample from a subject and comparing the level of the at least one miR in the cancer test sample to the level of a corresponding miR in a control sample. An alteration (e.g., an increase, a decrease) 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, cancer associated with the one or more prognostic markers.

[0096]   The cancer can be associated with one or more prognostic markers or features, including, a marker associated with an adverse (i.e., negative) prognosis, or a marker associated with a good (i.e., positive) prognosis. In certain embodiments, the cancer that is diagnosed using the methods described herein is associated with one or more adverse prognostic features.

[0097]   Particular microRNAs whose expression is altered in cancer cells associated with each of these prognostic markers are described herein. In one embodiment, the level of the at least one miR 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.

[0098]   Without wishing to be bound by any one theory, it is believed that alterations in the level of one or more miRs in cells can result in the deregulation of one or

more intended targets for these miRs, which can lead to the formation of cancer.

[0099]   Therefore, altering the level of the miR (e.g., by decreasing the level of a miR that is up-regulated in CLL cells, by increasing the level of a miR that is down-regulated in cancer cells) may successfully treat the cancer. Examples of putative gene targets for miRNAs that are deregulated in cancer cells are described herein.

[00100]   Accordingly, the present invention encompasses methods of treating cancer in a subject, wherein at least one miR is de-regulated (e.g., down-regulated, up-regulated) in the cancer cells of the subject. When the at least one isolated miR is down-regulated in the cancer cells, the method comprises administering an effective amount of the at least one isolated miR such that proliferation of cancer cells in the subject is inhibited. When the at least one isolated miR 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, referred to herein as miR gene expression inhibition compounds, such that proliferation of cancer cells is inhibited.

[00101]   The terms "treat", "treating" and "treatment", as used herein, refer to ameliorating symptoms associated with a disease or condition, for example, 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.

[00102]   As used herein, an "effective amount" of an isolated miR is an amount sufficient to inhibit proliferation of a cancer cell in a subject suffering from cancer. One skilled in the art can readily determine an effective amount of an miR 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.

[00103]   For example, an effective amount of an isolated miR can 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 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.

[00104]   One skilled in the art can also readily determine an appropriate dosage regimen for the administration of an isolated miR to a given subject. For example, an miR can be administered to the subject once (*e.g.*, as a single injection or deposition). Alternatively, an miR 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, an miR 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 administered to the subject can comprise the total amount of miR administered over the entire dosage regimen.

[00105]   As used herein, an "isolated" miR is one which is synthesized, or altered or removed from the natural state through human intervention. For example, a synthetic miR, or an miR partially or completely separated from the coexisting materials of its natural state, is considered to be "isolated." An isolated miR can exist in substantially-purified form, or can exist in a cell into which the miR has been delivered. Thus, an miR which is deliberately delivered to, or expressed in, a cell is considered an "isolated" miR. An miR produced inside a cell from an miR precursor molecule is also considered to be "isolated" molecule.

[00106]   Isolated miRs can be obtained using a number of standard techniques. For example, the miRs can be chemically synthesized or recombinantly produced using methods known in the art. In one embodiment, miRs 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).

[00107]   Alternatively, the miRs 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 miRs in cancer cells.

[00108]   The miRs that are expressed from recombinant plasmids can be isolated

from cultured cell expression systems by standard techniques. The miRs which 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 miRs to cancer cells is discussed in more detail below.

[00109]   The miRs can be expressed from a separate recombinant plasmid, or they can be expressed from the same recombinant plasmid. In one embodiment, the miRs are expressed as RNA precursor molecules from a single plasmid, and the precursor molecules are processed into the functional miR 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 are 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 are incorporated herein by reference).

[00110]   Selection of plasmids suitable for expressing the miRs, methods for inserting nucleic acid sequences into the plasmid to express the s, 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.

[00111]   In one embodiment, a plasmid expressing the miRs 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 are located 3' of the promoter, so that the promoter can initiate transcription of the miR coding sequences.

[00112]   The miRs can also be expressed from recombinant viral vectors. It is contemplated that the miRs 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 miRs to cancer cells is discussed in more detail below.

[00113]   The recombinant viral vectors of the invention comprise sequences encoding the miRs and any suitable promoter for expressing the RNA sequences. Suitable promoters include, for example, 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 miRs in a cancer cell.

[00114]   Any viral vector capable of accepting the coding sequences for the miRs 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.

[00115]   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.

[00116]   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.

[00117]   Particularly suitable viral vectors are those derived from AV and AAV. A suitable AV vector for expressing the miRs, 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 miRs, 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. Pat. No. 5,252,479; U.S. Pat. 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 miRs are expressed from a single recombinant AAV vector comprising the CMV intermediate early promoter.

[00118] In a certain embodiment, a recombinant AAV viral vector of the invention comprises a nucleic acid sequence encoding an 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.

[00119] In other embodiments of the treatment methods of the invention, an effective amount of at least one compound which inhibits miR expression can also be administered to the subject. As used herein, "inhibiting miR expression" means that the production of the active, mature form of miR 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 above for the diagnostic method. Inhibition can occur at the level of gene expression (i.e., by inhibiting transcription of a miR gene encoding the miR) or at the level of processing (e.g., by inhibiting processing of a miR precursor into a mature, active miR).

[00120] 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 associated with a cancer-associated chromosomal feature. One skilled in the art can readily determine an effective amount of an 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.

[00121]   For example, an effective amount of the expression-inhibiting compound can be based on the approximate or estimated body weight of a subject to be treated. Such effective amounts are administered parenterally or enterally, among others, as described herein. For example, an effective amount of the expression-inhibiting compound 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 it can be greater than about 1000 micrograms/kg of body weight.

[00122]   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. For example, an expression-inhibiting compound can be administered to the subject once (*e.g.*, as a single injection or deposition). Alternatively, an expression-inhibiting compound can be administered once or twice daily to a subject for a period of from about three to about twenty-eight days, more preferably from about seven to about ten days. In a particular dosage regimen, an expression-inhibiting compound is administered once a day for seven days. Where a dosage regimen comprises multiple administrations, it is understood that the effective amount of the expression-inhibiting compound administered to the subject can comprise the total amount of compound administered over the entire dosage regimen.

[00123]   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 and destroy or induce the destruction of the target miR.

[00124]   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 . In a particular embodiment, the dsRNA molecule is a "short or small interfering RNA" or "siRNA."

[00125]   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 which is substantially identical to a nucleic acid sequence contained within the

target miR.

[00126]   As used herein, a nucleic acid sequence in an siRNA which 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.

[00127]   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.

[00128]   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").

[00129]   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 miRs. 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 which are incorporated herein by reference.

[00130]   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 or 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, PNA) that generally comprise a nucleic acid sequence complementary to a contiguous nucleic acid sequence in an miR. 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 an miR. Nucleic acid sequences for the miRs are provided herein. 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 /antisense nucleic acid duplex.

[00131]   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.

[00132]   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 miRs. 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. Pat. No. 5,849,902 to Woolf *et al.*, the entire disclosures of which are incorporated herein by reference.

[00133]   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 an miR, and which is able to specifically cleave the miR. 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 an miR. 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.

[00134]   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 miRs. 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. Pat. No. 4,987,071 to Cech *et al*, the entire disclosures of which are incorporated herein by reference.

[00135]   Administration of at least one miR, or at least one compound for inhibiting miR expression, will inhibit the proliferation of cancer cells in a subject who has a cancer associated with a cancer-associated chromosomal feature. 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 miRs 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.

[00136]   The number of cancer cells in a subject's body 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.

[00137]   The miRs 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 miRs 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 or miR gene expression inhibiting compound.

[00138]   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.

[00139]   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.

[00140]   An miR 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 intravenous administration into the patient.

[00141]   In the present methods, an miR or miR 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 or expression inhibiting compound. Suitable delivery reagents include, e.g., the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (*e.g.*, polylysine), and liposomes.

[00142]   Recombinant plasmids and viral vectors comprising sequences that express the miRs or miR gene expression inhibiting compounds, and techniques for delivering such plasmids and vectors to cancer cells, are discussed herein.

[00143]   In a particular embodiment, liposomes are used to deliver an miR 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 s 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. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, the entire disclosures of which are incorporated herein by reference.

[00144]   The liposomes for use in the present methods can comprise a ligand

molecule that targets the liposome to cancer cells. Ligands which bind to receptors prevalent in cancer cells, such as monoclonal antibodies that bind to tumor cell antigens, are preferred.

[00145]   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 opsonization-inhibition moieties and a ligand.

[00146]   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. Pat. No. 4,920,016, the entire disclosure of which is incorporated herein by reference.

[00147]   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) derivatives; 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 derivatives thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called "PEGylated liposomes."

[00148] 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.

[00149] 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, 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 miRs or miR gene expression inhibition compounds (or nucleic acids comprising sequences encoding them) to tumor cells.

[00150] The miRs 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 cancer. In one embodiment, the pharmaceutical compositions comprise at least one isolated miR and a pharmaceutically-acceptable carrier. In a particular embodiment, the at least one miR corresponds to a miR that has a decreased level of expression in cancer cells relative to suitable control cells.

[00151] 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 cancer cells than control cells.

[00152]   Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. As used herein, "pharmaceutical formulations" 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.

[00153]   The present pharmaceutical formulations comprise at least one miR or miR gene expression inhibition compound (or at least one nucleic acid comprising sequences encoding them) (e.g., 0.1 to 90% by weight), or a physiologically acceptable salt thereof, mixed with a pharmaceutically-acceptable carrier. The pharmaceutical formulations of the invention can also comprise at least one miR or miR gene expression inhibition compound (or at least one nucleic acid comprising sequences encoding them) which are encapsulated by liposomes and a pharmaceutically-acceptable carrier.

[00154]   Especially suitable pharmaceutically-acceptable carriers are water, buffered water, normal saline, 0.4% saline, 0.3% glycine, hyaluronic acid and the like.

[00155]   In a particular embodiment, the pharmaceutical compositions of the invention comprise at least one miR or miR gene expression inhibition compound (or at least one nucleic acid comprising sequences encoding them) which is resistant to degradation by nucleases. One skilled in the art can readily synthesize nucleic acids which are nuclease resistant, for example by incorporating one or more ribonucleotides that are modified at the 2'-position into the miRs. Suitable 2'-modified ribonucleotides include those modified at the 2'-position with fluoro, amino, alkyl, alkoxy, and O-allyl.

[00156]   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.

[00157]   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.

[00158]   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 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 or miR gene expression inhibition compound (or at least one nucleic acid comprising sequences encoding them) encapsulated in a liposome as described above, and a propellant. A carrier can also be included as desired; e.g., lecithin for intranasal delivery.

[00159]   The invention also encompasses methods of identifying an anti- cancer agent, comprising providing a test agent to a cell and measuring the level of at least one miR 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 associated with decreased expression levels in cancer cells. An increase in the level of the miR in the cell, relative to a suitable control cell, is indicative of the test agent being an anti- cancer agent.

[00160]   In other embodiments the method comprises providing a test agent to a cell and measuring the level of at least one miR associated with increased expression levels in cancer cells. A decrease in the level of the miR in the cell, relative to a suitable control cell, is indicative of the test agent being an anti- cancer agent.

[00161]   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 (e.g., Northern blotting, *in situ* hybridization, RT-PCR, expression profiling) are also well known in the art.

[00162]   The invention will now be illustrated by the following non-limiting examples. e following examples are intended to illustrate preferred embodiments of the invention and should not be interpreted to limit the scope of the invention as defined in the claims, unless so specified.

[00163]   **EXAMPLES**

[00164]   Presented herein are the results of a genome-wide miRNA expression profiling in a large set of normal and tumor ovarian tissues. It is now demonstrated here the existence of an ovarian cancer specific miRNA signature. Also, the altered methylation of microRNA genes is identified as a possible epigenetic mechanism responsible for their aberrant expression.

[00165]   Materials and Methods

[00166]   Ovarian cancer samples and cell lines.

[00167]   A total of 84 snap-frozen normal and malignant ovarian tissues were collected at the GOG Tissues Bank, Columbus Children's Hospital, Columbus (OH, USA). The tissue collection used for microarray analysis included 15 normal ovarian tissue sections, and 69 malignant tissues, all ovarian epithelial carcinomas, including 31 serous (29 out of them showed a papillary pattern), 8 endometrioid, 4 clear cell, 9 poorly differentiated and 1 mucinous carcinomas. The ovarian cancer cell line IGROV1 was originally derived by Dr. Bernard (Institute Gustave Roussy, Villejuf, France), from a moderately differentiated ovarian carcinoma of an untreated patient, OAW-42 from Dr. Ulrich U. (Department of Obstetrics and Gynecology, University of Ulm, Germany), while OVCAR3, OVCAR8 and SK-OV3 were purchased from the American Type Culture Collection. All the cell lines were maintained in RPMI medium (Life Technologies, Rockville, MD), supplemented with 10% (v/v) fetal bovine serum (FCS), 3mM L-Glutamine and 100 U/ml penicillin/streptomycin.

[00168]   miRNA microarray Hybridization and Quantification

[00169]   Total RNA isolation was performed with Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA labeling and hybridization on microRNA microarray chips were performed as previously described (28) using 5 µg of total RNA from each sample. Hybridization was carried out on our microRNA microarray (Ohio State Comprehensive Cancer Center, version 2.0), which contains probes for 460 mature microRNAs spotted in quadruplicate (235 homo sapiens, 222 mus musculus, and three Arabidopsis thaliana) with annotated active sites. Often, more than one probe set exists for a given mature microRNA. Additionally, there are quadruplicate probes corresponding to most precursor microRNAs. Hybridization signals were detected with Streptavidin-Alexa647 conjugate and scanned images (Axon 4000B) were quantified using the Genepix 6.0 software (Axon Instruments, Sunnyvale, CA).

[00170]   Computational Analysis of microRNA Microarray Data.

[00171]   Microarray images were analyzed by using GENEPIX PRO. Average

values of the replicate spots of each miRNA were background subtracted, normalized, and

subjected to further analysis. We performed a global median normalization of Ovary

microarray data by using BRB ArrayTools developed by Richard Simon & Amy Peng

Lam (29). Absent calls were thresholded to 4.5 before subsequent statistical analysis.

This level is the average minimum intensity level detected in the experiments. miRNA

nomenclature was according to the Genome Browser (genome.ucsc.edu) and the miRNA

database at Sanger Center (microrna.sanger.ac.uk/); in case of discrepancies the miRNA

database was followed. Differentially expressed miRNAs were identified by using the t

test procedure within significance analysis of microarrays (SAM), a method developed at

Stanford University Labs based on recent paper of Tusher, Tibshirani and Chu (30).

[00172]   To identify miRNA signatures we also applied PAM, which performs

sample classification from gene expression data, via the "nearest shrunken centroid

method" of Tibshirani, Hastie, Narasimhan and Chu (31).

[00173]   Northern Blotting.

[00174]   Northern blot analysis was performed as previously described. RNA

samples (10 μg each) were run on 15% Polyacrylamide, 7M Urea Criterion pre-casted gels

(Bio-Rad, Hercules, CA) and transferred onto Hybond-N+ membranes (Amersham,

Piscataway, NJ). The hybridization was performed at 370C in ULTRAhyb-Oligo

hybrization buffer (Ambion, Austin, TX) for 16 hours. Membranes were washed at 370C,

twice with 2X SSPE and 0.5% SDS.

[00175]   The oligonucleotides used as probes were antisense to the sequence of the

mature microRNAs (miR Registry at sanger.ac.uk/Software/Rfam/mirna/ ,which is fully

incorporated herein by reference):

miR-200a: 5'- ACA TCG TTA CCA GAC AGT GTT A -3'**[SEQ ID NO:92]**;

miR-141:   5'- CCA TCT TTA CCA GAC AGT GTT A - 3'**[SEQ ID NO:93]**;

miR-199a: 5'- GAA CAG GTA GTC TGA ACA CTG GG -3'**[SEQ ID NO:94]**;

miR-125b1:   5'TCA CAA GTT AGG GTC TCA GGG A -3'**[SEQ ID NO:95]**;

miR-145:   5'- AAG GGA TTC CTG GGA AAA CTG GAC -3'**[SEQ ID NO:96]**;

miR-222:   5'- GAG ACC CAG TAG CCA GAT GTA GCT -3'**[SEQ ID NO:97]**;

miR-21:   5'- TCA ACA TCA GTC TGA TAA GCT A -3'**[SEQ ID NO:98]**.

[00176]   5S RNA or EtBr gel staining were used to normalize. 200 ng of each probe

was end labeled with 100 μCi [gamma-32P]-ATP using the polynucleotide kinase

(Roche). Blots were stripped in boiling 0.1% SDS for 10 minutes before re-hybridization.

[00177]   Real-Time PCR

[00178]   The single tube TaqMan MicroRNA Assays were used to detect and

quantify mature microRNAs on Applied Biosystems Real-Time PCR instruments in

accordance with manufacturer's instructions (Applied Biosystems, Foster City, CA).

Normalization was performed with 18S rRNA. All RT reactions, including no-template

controls and RT minus controls, were run in a GeneAmp PCR 9700 Thermocycler

(Applied Biosystems). Gene expression levels were quantified using the ABI Prism

7900HT Sequence detection system (Applied Biosystems). Comparative real-time PCR

was performed in triplicate, including no-template controls. Relative expression was

calculated using the comparative Ct method.

[00179]   Demethylating Experiment

[00180]   OVCAR3 cells were seeded at low density 48h before treatment with

10μM 5`aza-2`deoxycytidine (5`-AZA, Sigma). After 24h of treatment, cells were

collected and total RNA was isolated using Trizol reagent (Invitrogen). 3 replicates for

both untreated cells and AZA-treated cells were used to evaluate the miR expression by

Microarray profiling. Differentially expressed microRNAs were identified by using

univariate two-classes T-test with random variance model.

[00181]   Results

[00182]   A microRNA expression signature discriminates Ovarian Cancer Tissues

from Normal Ovary.

[00183]   A custom microarray platform already validated by numerous studies (19)

was used to evaluate microRNA expression profiles on a heterogeneous set of ovarian

tissues from different patients. This set included 15 normal ovarian samples, 69 ovarian

malignant tumors, and 5 ovarian cancer cell lines, for a total of 89 biologically

independent samples. Each tumor sample derived from a single specimen (data not

shown).

[00184]   The unsupervised hierarchical clustering, based on all the human

microRNAs spotted on the chip, generated a tree with a clear distinction of samples in two

main groups, represented by normal tissues and malignant tissues (**Fig. 1**).

[00185]   To identify microRNAs differentiating normal versus cancer tissue, we

used SAM and PAM tools, and the results obtained from the two types of class prediction

analysis were largely overlapping. The SAM comparison between normal and cancer tissues identified 39 miRNAs (with q-values <1% and fold changes >3) differentially expressed, 10 up-modulated in tumors and the remaining down-modulated (the list is reported in **Fig. 9 - Table 2**).

[00186]   The PAM analysis in **Figs. 6A and 6B** displays the graphical representation of the probabilities (0.0 to 1.0) of each sample for being a cancer or a normal tissue according to the miR signature reported in **Fig. 8 - Table 1**, which describes a smaller set of 29 miRs, 4 up-modulated (miR-200a, -200b, -200c and -141) and 25 down-modulated (being miR-199a,miR-140, miR-145 and miR-125b1 among the most significant) differentiating normal versus tumor with a classification rate of 89%.

[00187]   To confirm the results obtained by microarray analysis, we carried out Northern blots (**Fig. 2A**) or Real-Time PCR (**Fig. 2B**) on some of the differentially expressed microRNAs. We analyzed the expression of miR-200a and miR-141, the most significantly up-modulated in ovarian carcinoma, and the microRNAs most significantly down-modulated: miR-199a, miR-140, miR-145 and miR-125b1. All the experiments confirmed the results obtained by microarray analysis.

[00188]   Bio-pathological features and microRNA expression.

[00189]   Considering that ovarian epithelial carcinomas occur as different histological subtypes characterized by distinct morphologic and molecular genetic alterations, we decided to compare the microRNA profile of each of them to the normal tissue to evaluate if microRNA expression profiles are different in distinct histotypes of ovarian carcinomas. Complete lists resulting from SAM analyses are reported in **Fig. 10 - Table 3**, while a summary is shown in the Venn diagrams in **Figs. 3A** and **3B**:

[00190]   Two (2) out of 4 microRNAs most significantly up-modulated (**Fig.3A**) in tumors versus normal tissue, miR-200a and miR-200c, are up-modulated in all the three histotypes considered (serous, endometrioid and clear cell), while miR-200b and miR-141 up-modulation is shared by endometrioid and serous histotypes.

[00191]   Moreover, the endometrioid histotype shows the up-modulation of 3 additional microRNAs, miR-21, miR-203 and miR-205. 19 miRs, including miR-125b1, miR-199a and miR-140, are down-modulated (**Fig. 3B**) in all the three histotypes examined in comparison with normal tissue, while 4 are shared in each paired analysis of the different signatures: miR-145, for example, is down-modulated in both serous and clear cell carcinomas; miR-222 in both endometrioid and clear cell carcinomas.

[00192]   Considering the tumors classified as "mixed" and "poorly differentiated",
we found that the first group revealed a signature with characteristics of different
histotypes, sharing for example the overexpression of miR-200c and miR-181 with the
endometrioid carcinomas, and the down-modulation of miR-214 with the serous, while the
"poorly differentiated" tumors have a quite different pattern of microRNAs expression
(**Fig. 10 - Table 3**).

[00193]   We then compared miRNA expression of different groups of tumors paired
as reported in **Fig. 11 - Table 4,** and in particular we compared the 2 most numerous
histotypes, serous and endometriod. When considering the microRNAs differentially
expressed in endometrioid carcinomas compared to serous, we found miR-212 up-
modulated, and miR-302b* and miR-222 (T-test analysis of microarray data in **Fig. 4A,**
$p<0.05$), among the microRNAs most significantly down-modulated.

[00194]   In **Fig. 4B** a Northern Blot on a small set of samples verifies miR-222
overexpression in serous tumors compared to endometrioid. We then focused our
attention on other clinico-pathological features associated with tumor specimens: while no
miRs were found significantly differentially expressed in relation to the age of patients,
other tumor characteristics seemed to affect miRs expression, such as lympho-vascular
invasion, ovarian surface, tubal, uterus and pelvic peritoneum involvement (**Fig. 12 -
Table 5**).

[00195]   To investigate if there were miRs associated with different Grade or Stage
of the disease, we performed comparative analyses considering all the tumors or only the
serous histotype, which was the most numerous, but we did not obtain any significant
microRNA differentially expressed.

[00196]   <u>Confirmed and potential targets for miRNAs members of various
signatures.</u>

[00197]   Using the DianaTarbase at "diana.pcbi.upenn.edu/tarbase", we looked for
confirmed targets of some of the most significant miRNAs resulting from our analyses,
finding some interesting data: ERBB2 and ERBB3 receptors, for example, are targeted by
miR-125 (32); miR-101, down-modulated in ovarian carcinoma, has been demonstrated
targeting the oncogene MYCN (33). We then analyzed their potential targets using the
"diana.pcbi.upenn.edu/miRGen" database, and evaluated for some of these molecules the
expression levels in ovarian carcinoma. All the four most significantly up-modulated
microRNAs, miR-200a, miR-200b, miR-200c and miR-141, for example, have as common

putative target the oncosuppressor BAP1, BRCA1-associated protein, down-modulated in ovarian cancer. The information obtained is summarized in **Fig. 13 - Table 6.**

[00198]   Epigenetic regulation of miRs expression

[00199]   To evaluate if an aberrant DNA methylation pattern could also contribute to the altered microRNA expression characterizing the human ovarian carcinoma, we analyzed the miR profiling of the ovarian cell line OVCAR3, before and after treatment with the demethylating agent 5-Aza-2'-deoxycitidine. The analysis of Microarray data showed 11 human microRNAs differentially expressed, 9 up-modulated and 2 down-modulated (significance threshold of each univariate test: $p<0.001$), being miR-21, miR-203, miR-146b, miR-205, miR-30-5p and miR-30c the most significant induced upon treatment (the miRs differentially expressed are listed in **Fig. 5A**, while the resulting hierarchical cluster tree is reported in **Fig. 5B**).

[00200]   Real-Time PCRs to verify the up-modulation of the 5 most significantly induced miRs are described in **Figs. 5C** and **5D** as graphical representation of miR expression levels (**Fig. 5C**), and miR-21 was also validated by Northern Blot (**Fig. 5D**).

[00201]   Interestingly, miR-21, miR-203 and miR-205 are overexpressed in ovarian carcinomas compared to normal tissues (see SAM analysis in **Fig. 9 - Table 2** and Venn Diagram in **Figs. 3A and 3B**): the reactivation of these miR genes after demethylating treatment suggests that the hypomethylation could be the mechanism responsible for their overexpression *in vivo*. We confirmed the overexpression of miR-21, the most significant miR induced upon treatment, performing a Northern Blotting (**Fig. 7A**) on a panel of human ovarian carcinomas and two normal tissues. Moreover, using the CpG Island Searcher Program (34), we verified that miR-21 and miR-203 are associated with CpG islands, being the miR-203 embedded in a CpG island 875 bp long, and the miR-21 characterized by a CpG island -2 kb upstream the mature sequence (**Fig. 7B**), while miR-205 does not show any CpG island in a region spanning 2 Kb upstream its mature form.

[00202]   Discussion

[00203]   In the Examples herein, it is now shown that microRNAs are aberrantly expressed in human ovarian cancer. The overall microRNA expression can clearly separate normal versus cancer tissues, identifying a number of microRNAs altered in human ovarian cancer and probably involved in the development of this neoplasia.

[00204]   The expression of all the four microRNAs we found most significantly up-modulated, miR-200a and miR-141, belonging to the same family; miR-200b (localized in

the same region of miR-200a, at chr.1p36.33); and miR-200c, (localized in the same region of miR-141, at chr.12p13.31), is concordant with the results obtained at genomic level by Zhang et al. (24), suggesting that the mechanism driving their up-modulation could be the amplification of the microRNA genes.

[00205]   Interestingly, all these miRs have a common putative target: the oncosuppressor BAP1, BRCA1-associated protein (24). The altered expression of GATA factors, found and proposed as the underlying mechanism for dedifferentiation in ovarian carcinogenesis (35), may also be driven by microRNAs deregulation. In particular GATA6, lost or excluded from the nucleus in 85% of ovarian tumors, may be regulated by miR-200a, and GATA4, absent in the majority of ovarian cancer cell lines, may be targeted by miR-200b (**Fig. 12 - Table 5**).

[00206]   Among the down-modulated genes, notably we found miR-125b1, altered also in breast cancer, as well as miR-145 (18); mir-199a, recently shown down-modulated in other tumors, as hepatocellular carcinoma (36); miR-140, deleted in ovarian carcinoma (24).

[00207]   Interestingly, miR-140 is indeed located at chr.6q22, a fragile region often deleted in ovarian tumor, and it is predicted to target important molecules as c-SRK, MMP13 and FGF2.

[00208]   Even if the normal control available in these examples is represented by whole normal ovary, our data can identify a number of microRNAs altered in human ovarian carcinoma and probably involved in the biology of this malignancy. In fact, the miRNA signatures obtained comparing different histotypes of ovarian carcinomas (serous, endometrioid, clear cell and mixed) to the normal tissue are overlapping in most cases, but they also reveal a number of microRNAs that seem to be "histotype-specific": the endometrioid tumors, for example, share with the others the 4 most significantly up-modulated miRs (miR-200a, miR200b, miR-200c and miR-141), but also present overexpression of miR-21, known to be mis-regulated in numerous solid tumors (18, 37, 38) and to exert an anti-apoptotic role in different cellular systems (39, 40), miR-205 and miR-182.

[00209]   Endometrioid tumors also present down-modulation of several microRNAs in comparison with the other classes of tumors, for example miR-222, already demonstrated targeting c-Kit (41), being involved in cancer (42-44) and down-modulated under folate-deficient conditions (45).

[00210]   These differences enforce the fact that different histotypes represent biologically and pathogenetically distinct entities of EOCs, even though they are currently treated with identical therapeutic strategies. Microarray analysis has recently confirmed that different histotypes (serous, mucinous, endometrioid and clear cell) show the alteration of different pathways, probably reflecting the gene expression pattern of the organ of origin (respectively fallopian tubes, colonic mucosa and endometrium) (46).

[00211]   Notably, many of the microRNAs differentially expressed are predicted to target molecules involved in pathways differentially activated depending on the histotype. miR-212, for example, down-modulated in serous carcinoma, has as putative target WT1, overexpressed in this subgroup of ovarian carcinomas (47). Another putative target of miR-212 is BRCA1: mutated in hereditary ovarian cancer, this molecule has been recently found involved also in the pathoetiology of sporadic ovarian epithelial cancer (OEC), where a loss of gene function due to epigenetic alterations has been observed more commonly (48). he decreased BRCA1 expression could be determined by overexpression of one or more microRNAs.

[00212]   miR-299-5p and miR-135b, up-modulated in serous histotype compared to endometrioid, are supposed to target, respectively, DLK1 (Delta-like 1) and MSX2 (msh homeobox 2), overexpressed in endometrioid carcinomas (47). Compared to the other tumors, clear cell carcinomas show expression levels of miR-30-5p and of miR-20a opposite (46) to two putative targets, RBP4 (retinol binding protein 4) and SLC40A1 (solute carrier 40-iron-regulated transporter, member 1), respectively. Compared to the normal tissue, clear cell carcinoma also show lower expression of miR-18a, miR-19a and miR-19b, suggesting a possible down-modulation of the cluster 17-92 (already validated as deleted by Zhang et al.). This cluster, involved in the intricate regulation mediated by E2F1 and c-Myc, seems to have a duplex nature of putative oncogene, as recently suggested in B-cell Lymphoma (15), or tumor-suppressor: in hepatocellular carcinoma, for example, LOH at the locus coding the miR-17-92 cluster (13q31) has been reported (49). In Ovarian Carcinoma, at least in clear cell histotype, it could also exert a role of oncosuppressor. The data shown herein now suggest indeed that microRNAs may have a regulatory role in the process of differentiation leading to the development of a specific subtype of EOC.

[00213]   Interestingly, poorly differentiated carcinomas have a quite different pattern of microRNAs expression, showing up-modulation of several microRNAs in comparison

to normal ovary. More intriguingly, one of them, miR-373, has been recently described as putative oncogene in testicular germ cell tumors (16).

[00214]   The absence of microRNAs significantly differentially expressed in relation to tumor Stage or Grade might be explained by the fact that our set of samples is mostly represented by advanced stage tumors, as expected considering the late diagnosis of this kind of neoplasia; however, the difference in size among the different groups of samples could have represented a limit for the statistical analysis. Alternatively, microRNAs might be important for the development of human ovarian carcinoma but not for the progression of the disease.

[00215]   Resulting from our analyses a number of miRs overexpressed but not reported as amplified in Zhang study, as well as down-modulated but not deleted, the involvement of an epigenetic regulatory mechanism could actually exert a role on microRNA expression in human EOC.

[00216]   Indeed, among the most significant microRNAs induced after demethylating treatment of an ovarian cell line, we found miR-21, miR-203 and miR-205, up-modulated in ovarian cancer. Moreover, miR-203 and miR-21 are associated with a CpG island (miR-203 is embedded in a CpG island, while miR-21 has a CpG island -2 kb upstream its mature sequence), supporting the idea that the demethylation leads to the reactivation of these microRNA genes. Notably, miR-21 has already been described up-modulated in several human tumors and having an anti-apoptotic role in different cellular models. These data now show that the DNA hypomethylation could be an epigenetic mechanism responsible for the in vivo overexpression of potentially oncogenic miRs.

[00217]   To the best of the inventor's knowledge, this is the first report describing a complete miRs expression profiling in human EOCs, focused on the identification of miRs differentially expressed in carcinomas versus normal ovary, and in different subgroups of tumors. The data now show the important role that microRNAs can exert on the pathogenesis and on the development of different histotypes of ovarian carcinoma, and identify altered DNA methylation as a possible epigenetic mechanism responsible for the aberrant expression of microRNAs not affected by genomic changes.

[00218]   In accordance with the provisions of the patent statutes, the principle and mode of operation of this invention have been explained and illustrated in its preferred embodiment. However, it must be understood that this invention may be practiced otherwise than as specifically explained and illustrated without departing from its spirit or

scope.

[00219]   THE miR GENE DATABASE

[00220]   The miRNAs of interest are listed in public databases. In certain preferred embodiments, the public database can be a central repository provided by the Sanger Institute http://microrna.sanger.ac.uk/sequences/ to which miRNA sequences are submitted for naming and nomenclature assignment, as well as placement of the sequences in a database for archiving and for online retrieval via the world wide web. Generally, the data collected on the sequences of miRNAs by the Sanger Institute include species, source, corresponding genomic sequences and genomic location (chromosomal coordinates), as well as full length transcription products and sequences for the mature fully processed miRNA (miRNA with a 5' terminal phosphate group). Another database can be the GenBank database accessed through the National Center for Biotechnology Information (NCBI) website, maintained by the National Institutes of Health and the National Library of Medicine. These databases are fully incorporated herein by reference.

ACCESSION NUMBER	ID	SEQUENCE	SEQ ID NO
MIMAT0000682	hsa-miR-200a	UAACACUGUCUGGUAACGAUGU	1
MIMAT0000318	hsa-miR-200b	UAAUACUGCCUGGUAAUGAUGA	2
MIMAT0000617	hsa-miR-200c	UAAUACUGCCGGGUAAUGAUGGA	3
MIMAT0000432	hsa-miR-141	UAACACUGUCUGGUAAAGAUGG	4
MIMAT0000714	hsa-miR-302b*	ACUUUAACAUGGAAGUGCUUUC	5
MIMAT0000259	hsa-mir-182	UUUGGCAAUGGUAGAACUCACACU	6
MIMAT0000771	hsa-miR-325	CCUAGUAGGUGUCCAGUAAGUGU	7
MIMAT0000726	hsa-miR-373	GAAGUGCUUCGAUUUUGGGGUGU	8
MIMAT0000264	hsa-miR-203	GUGAAAUGUUUAGGACCACUAG	9
MIMAT0000266	hsa-miR-205	UCCUUCAUUCCACCGGAGUCUG	10
MIMAT0000231	hsa-miR-199a	CCCAGUGUUCAGACUACCUGUUC	11
MIMAT0000263	hsa-miR-199b	CCCAGUGUUUAGACUAUCUGUUC	12
MIMAT0000435	hsa-miR-143	UGAGAUGAAGCACUGUAGCUC	13
MIMAT0004604	hsa-miR-127	CUGAAGCUCAGAGGGCUCUGAU	14
MIMAT0000431	hsa-miR-140	CAGUGGUUUUACCCUAUGGUAG	15
MIMAT0000441	hsa-miR-9	UCUUUGGUUAUCUAGCUGUAUGA	16
MIMAT0000427	hsa-miR-133a	UUUGGUCCCCUUCAACCAGCUG	17
MIMAT0000102	hsa-miR-105	UCAAAUGCUCAGACUCCUGUGGU	18
MIMAT0000099	hsa-miR-101	UACAGUACUGUGAUAACUGAA	19
MIMAT0000281	hsa-miR-224	CAAGUCACUAGUGGUUCCGUU	20
MIMAT0000445	hsa-miR-126	UCGUACCGUGAGUAAUAAUGCG	21
MIMAT0000098	hsa-miR-100	AACCCGUAGAUCCGAACUUGUG	22
MIMAT0000251	hsa-miR-147	GUGUGUGGAAAUGCUUCUGC	23
MIMAT0000265	hsa-miR-204	UUCCCUUUGUCAUCCUAUGCCU	24

MIMAT0000271	hsa-miR-214	ACAGCAGGCACAGACAGGCAGU	25
MIMAT0000097	hsa-miR-99a	AACCCGUAGAUCCGAUCUUGUG	26
MIMAT0000268	hsa-miR-211	UUCCCUUUGUCAUCCUUCGCCU	27
MIMAT0000437	hsa-miR-145	GUCCAGUUUUCCCAGGAAUCCCU	28
MIMAT0000065	hsa-let-7d	AGAGGUAGUAGGUUGCAUAGUU	29
MIMAT0000422	hsa-miR-124	UAAGGCACGCGGUGAAUGCC	30
MIMAT0000443	hsa-miR-125a	UCCCUGAGACCCUUUAACCUGUGA	31
MIMAT0000064	hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU	32
MIMAT0000062	hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	33
MIMAT0000681	hsa-miR-29c	UAGCACCAUUUGAAAUCGGUUA	34
MIMAT0000461	hsa-miR-195	UAGCAGCACAGAAAUAUUGGC	35
MIMAT0000423	hsa-miR-125b	UCCCUGAGACCCUAACUUGUGA	36
MIMAT0000727	hsa-miR-374	UUAUAAUACAACCUGAUAAGUG	37
MIMAT0000715	hsa-miR-302b	UAAGUGCUUCCAUGUUUUAGUAG	38
MIMAT0000086	hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA	39
MIMAT0000076	hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA	40
MIMAT0000259	hsa-miR-182	UUUGGCAAUGGUAGAACUCACACU	41
MIMAT0000270	hsa-miR-181a*	ACCAUCGACCGUUGAUUGUACC	42
MIMAT0000273	hsa-miR-216	UAAUCUCAGCUGGCAACUGUGA	43
MIMAT0000717	hsa-miR-302c	UAAGUGCUUCCAUGUUUCAGUGG	44
MIMAT0000688	hsa-miR-301a	CAGUGCAAUAGUAUUGUCAAAGC	45
MIMAT0000096	hsa-miR-98	UGAGGUAGUAAGUUGUAUUGUU	46
MIMAT0000074	hsa-miR-19b	UGUGCAAAUCCAUGCAAAACUGA	47
MIMAT0000100	hsa-miR-29b	UAGCACCAUUUGAAAUCAGUGUU	48
MIMAT0000072	hsa-miR-18a	UAAGGUGCAUCUAGUGCAGAUAG	49
MIMAT0000452	hsa-miR-154	UAGGUUAUCCGUGUUGCCUUCG	50
MIMAT0000073	hsa-miR-19a	UGUGCAAAUCUAUGCAAAACUGA	51
MIMAT0000439	hsa-miR-153	UUGCAUAGUCACAAAAGUGAUC	52
MIMAT0000436	hsa-miR-144	UACAGUAUAGAUGAUGUACU	53
MIMAT0000279	hsa-miR-222	AGCUACAUCUGGCUACUGGGU	54
MIMAT0000416	hsa-miR-1	UGGAAUGUAAAGAAGUAUGUAU	55
MIMAT0000684	hsa-miR-302a	UAAGUGCUUCCAUGUUUUGGUGA	56
MIMAT0000686	hsa-miR-34c-5p	AGGCAGUGUAGUUAGCUGAUUGC	57
MIMAT0000272	hsa-miR-215	AUGACCUAUGAAUUGACAGAC	58
MIMAT0000085	hsa-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	59
MIMAT0000770	hsa-miR-133b	UUUGGUCCCCUUCAACCAGCUA	60
MIMAT0002890	hsa-miR-299-5p	UGGUUUACCGUCCCACAUACAU	61
MIMAT0000252	hsa-miR-7	UGGAAGACUAGUGAUUUUGUUGU	62
MIMAT0000250	hsa-miR-139-5p	UCUACAGUGCACGUGUCUCCAG	63
MIMAT0000722	hsa-miR-370	GCCUGCUGGGGUGGAACCUGGU	64
MIMAT0000429	hsa-miR-137	UUAUUGCUUAAGAAUACGCGUAG	65
MIMAT0000442	hsa-miR-9*	AUAAAGCUAGAUAACCGAAAGU	66
MIMAT0002809	hsa-miR-146b-5p	UGAGAACUGAAUUCCAUAGGCU	67
MIMAT0000087	hsa-miR-30	UGUAAACAUCCUCGACUGGAAG	68
MIMAT0000095	hsa-miR-96	UUUGGCACUAGCACAUUUUUGCU	69

MIMAT0000646	hsa-miR-155	UUAAUGCUAAUCGUGAUAGGGGU	70
MIMAT0000738	hsa-miR-383	AGAUCAGAAGGUGAUUGUGGCU	71
MIMAT0000244	hsa-miR-30c	UGUAAACAUCCUACACUCUCAGC	72
MIMAT0002819	hsa-miR-193b	AACUGGCCCUCAAAGUCCCGCU	73
MIMAT0002811	hsa-miR-202	AGAGGUAUAGGGCAUGGGAA	74
MIMAT0000447	hsa-miR-134	UGUGACUGGUUGACCAGAGGGG	75
MIMAT0004696	hsa-miR-323-5p	AGGUGGUCCGUGGCGCGUUCGC	76
MIMAT0004695	hsa-miR-337-5p	GAACGGCUUCAUACAGGAGUU	77
MIMAT0000254	hsa-miR-10b	UACCCUGUAGAACCGAAUUUGUG	78
MIMAT0000077	hsa-miR-22	AAGCUGCCAGUUGAAGAACUGU	79
MIMAT0001080	hsa-miR-196b	UAGGUAGUUUCCUGUUGUUGGG	80
MIMAT0000460	hsa-miR-194	UGUAACAGCAACUCCAUGUGGA	81
MIMAT0000761	hsa-miR-324-5p	CGCAUCCCCUAGGGCAUUGGUGU	82
MIMAT0000758	hsa-miR-135b	UAUGGCUUUUCAUUCCUAUGUGA	83
MIMAT0000269	hsa-miR-212	UAACAGUCUCCAGUCACGGCC	84
MIMAT0000451	hsa-miR-150	UCUCCCAACCCUUGUACCAGUG	85
MIMAT0000759	hsa-miR-148b	UCAGUGCAUCACAGAACUUUGU	86
MIMAT0000692	hsa-miR-30e	UGUAAACAUCCUUGACUGGAAG	87
MIMAT0000075	hsa-miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	88
MIMAT0000256	hsa-miR-181a	AACAUUCAACGCUGUCGGUGAGU	89
MIMAT0000449	hsa-miR-146a	UGAGAACUGAAUUCCAUGGGUU	90
MIMAT0004614	hsa-miR-193a-5p	UGGGUCUUUGCGGGCGAGAUGA	91

[00221]　　　REFERENCES

[00222]　　　The references discussed above and the following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

1.　　　Cannistra SA. Cancer of the ovary. N Engl J Med 2004;351:2519–29.

2.　　　Greenlee RT, Hill-Harmon MB, Murray T and Thun M. Cancer statistics, 2001. CA Cancer J. Clin. 2001;51:15-36.

3.　　　Feeley KM and Wells M. Precursor lesions of ovarian epithelial malignancy. Histopathology 2001;38:87–95.

4.　　　Bell DA. Origins and molecular pathology of ovarian cancer. Mod Pathol 2005;18 Suppl 2:S19-32.

5.　　　Schwartz DR, Kardia SL, Shedden KA et al. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. Cancer Res 2002;62:4722-9.

6.   De Cecco L, Marchionni L, Gariboldi M et al. Gene expression profiling of advanced ovarian cancer: characterization of a molecular signature involving Fibroblast Growth Factor 2. Oncogene 2004;23:8171-83.

7.   He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature Rev Genet 2004;5:522-31.

8.   Miska EA. How microRNAs control cell division, differentiation and death. Curr Opin Genet Dev 2005;5:563-8.

9.   Zamore PD, Haley B. Ribo-gnome: the big world of small RNAs. Science 2005;309:1519-24.

10.   Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635-47.

11.   Mayr C, Hemann MT, Bartel D. Disrupting the pairing between let-7 and HMGA2 enhances oncogenic transformation. Science 2007;315:1576-9.

12.   Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev. 2007;21:1025-30.

13.   Cimmino A, Calin GA, Fabbri M et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 2005;102:13944-9.

14.   O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839-43.

15.   He L, Thomson JM, Hemann MT et al. A microRNA polycistron as a potential human oncogene. Nature 2005;435:828-33.

16.   Voorhoeve PM, le Sage C, Schrier M et al. A genetic screen implicates miRNA-372 and miRNA-373 as Oncogenes in Testicular Germ Cell Tumors. Cell 2006;124:1169-81.

17.   Costinean S, Zanesi N, Pekarsky Y et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci U S A. 2006;103:7024-9.

18.   Iorio MV, Ferracin M, Liu CG et al. Cancer Res. 2005;65:7065-70.

19.   Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857-66.

20.   Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-69.

21.   Calin GA, Ferracin M, Cimmino A et al. MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005;353:1793-801.

22.   Yanaihara N, Caplen N, Bowman E et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006;9:189-98.

23.   Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. Oncogene 2006;25:6202-10.

24.   Zhang L, Huang J, Yang N et al. MicroRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A. 2006;103:9136-41.

25.   Saito Y, Liang G, Egger G et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 2006;9:435-43.

26.   Lujambio A, Ropero S, Ballestar E et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 2007;67:1424-9.

27.   Brueckner B, Stresemann C, Kuner R et al. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res 2007;67:1419-23.

28.   Liu CG, Calin GA, Meloon B, et al. An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. Proc Natl Acad Sci U S A 2004;101:9740-4.

29.   Wright GW, Simon RM. A random variance model for detection of differential gene expression in small microarray experiments. Bioinformatics. 2003;19:2448-55.

30.   Tusher VG, Tibshirani R and Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001;98:5116-21.

31.   Tibshirani R, Hastie T, Narasimhan B, Chu G. Diagnosis of multiple cancer types by shrunken centroids of gene expression. Proc Natl Acad Sci U S A 2002;99:6567-6572.

32.   Scott GK, Goga A, Bhaumik D et al. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. J Biol Chem 2007;282:1479-86.

33.   Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003;115:787-98.

34.   Takai D, Jones PA. The CpG island searcher: a new WWW resource. In Silico Biol 2003;3:325-40.

35.   Capo-chichi CD, Roland IH, Vanderveer L et al. Anomalous expression of epithelial differentiation-determining GATA factors in ovarian tumorigenesis. Cancer Res 2003;63:4967-77.

36.   Murakami Y, Yasuda T, Saigo K et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene. 2006;25:2537-45.

37.   Roldo C, Missaglia E, Hagan JP et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol 2006;24:4677-84.

38.   Volinia S, Calin GA, Liu CG et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257-61.

39.   Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029-33.

40.   Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). J Biol Chem 2007;282:14328-36.

41.   Felli N, Fontana L, Pelosi E et al. MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci U S A. 2005;102:18081-6.

42.   He H, Jazdzewski K, Li W et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2005;102:19075-80.

43.   Pallante P, Visone R, Ferracin M et al. MicroRNA deregulation in human thyroid papillary carcinomas. Endocr Relat Cancer 2006;13:497-508.

44.   Lee EJ, Gusev Y, Jiang J et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer 2007;120:1046-54.

45.   Marsit CJ, Eddy K, Kelsey KT. MicroRNA responses to cellular stress. Cancer Res 2006;66:10843-8.

46.   Marquez RT, Baggerly KA, Patterson AP et al. Patterns of gene expression in different histotypes of epithelial ovarian cancer correlate with those in normal fallopian tube, endometrium and colon. Clin Cancer Res 2005;11:6116-26.

47.   Shedden KA, Kshirsagar MP, Schwartz DR et al. Histologic type, organ of origin, and Wnt pathway status: effect on gene expression in ovarian and uterine carcinomas. Clin Cancer Res 2005;11:2123-31.

48.   Thrall M, Gallion HH, Kryshio R et al. BRCA1 expression in a large series of sporadic ovarian carcinomas: a Gynecologic Oncology Group study. Int J Gynecol Cancer 2006;16 Suppl 1:166-71.

49.   Lin YW, Sheu JC, Liu LY et al. Loss of heterozygosity at chromosome 13q in hepatocellular carcinoma: identification of three independent regions. Eur J Cancer 1999;35:1730-4.

CLAIMS

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

measuring the level of a miR signature in a test sample from the subject, wherein the miR signature comprises miR-200a, miR-200b, miR-200c, miR-141, miR-199a, miR-140, miR-145 and miR-125b1, and

wherein an alteration in the level of the miR signature in the test sample, relative to the level of a corresponding miR in a control sample, is indicative of the subject either having, or being at risk for developing, ovarian cancer.

2.   The method of claim 1, including identifying a correlation between miR expression and ovarian cancer or a predisposition for ovarian cancer, comprising:

(a)   labeling the miRs isolated from a sample from a subject having or suspected of having a disease or condition;

(b)   hybridizing the miRs to an miR array;

(c)   determining miR hybridization to the array; and

(d)   identifying miRs differentially expressed in a sample representative of the disease or condition compared to a reference.

3.   The method of claim 1, wherein the ovarian cancer is one or more of clear cell, serous or endometrioid ovarian cancer.

4.   The method of claim 1, wherein the test sample comprises ovarian cancer cells, whereby ovarian cancer cells are distinguished by histotype among: serous, non-serous endometrioid, non-endometrioid, clear cell, non-clear cell, poorly differentiated and non-poorly differentiated.

5.   The method of claim 1, wherein an increase in expression of at least one of miR-200a, miR-200b, miR-200c or miR-141, and/or a decrease in expression of at least one of

miR-199a, miR-140, miR-145 or miR-125b1, as compared to a normal sample, is indicative of ovarian cancer.

6.   The method of claim 1, wherein the miR signature further includes one or more of the miRs shown in Figure 3A or Figure 3B, wherein the miR signature is indicative of serous ovarian cancer.

7.   The method of claim 1, wherein the miR signature further includes one or more of the miRs shown in Figure 3A or Figure 3B, wherein the miR signature is indicative of endometrioid ovarian cancer.

8.   The method of claim 1, wherein the miR signature further includes one or more of the miRs shown in Figure 3A or Figure 3B, wherein the miR signature is indicative of clear cell ovarian cancer.

9.   The method of claim 1, wherein the subject is a human.

10.   The method of claim 1, comprising:

(1)   reverse transcribing RNA from the 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 miR is indicative of the subject either having, or being at risk for developing, ovarian cancer.

11.   The method of claim 1, further combining the expression levels of two or more of the miRs.

12.   The method of claim 10, further combining the expression levels of two or more of the miRs.

The Ohio State University Research Foundation

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

Figure 1A
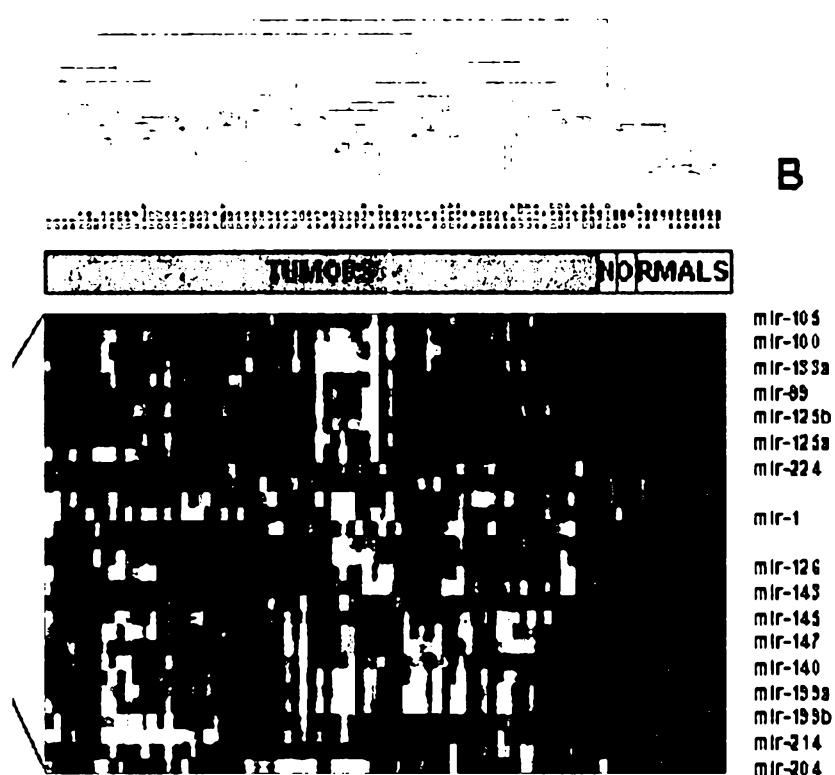
Figure 1B

Figure 1C
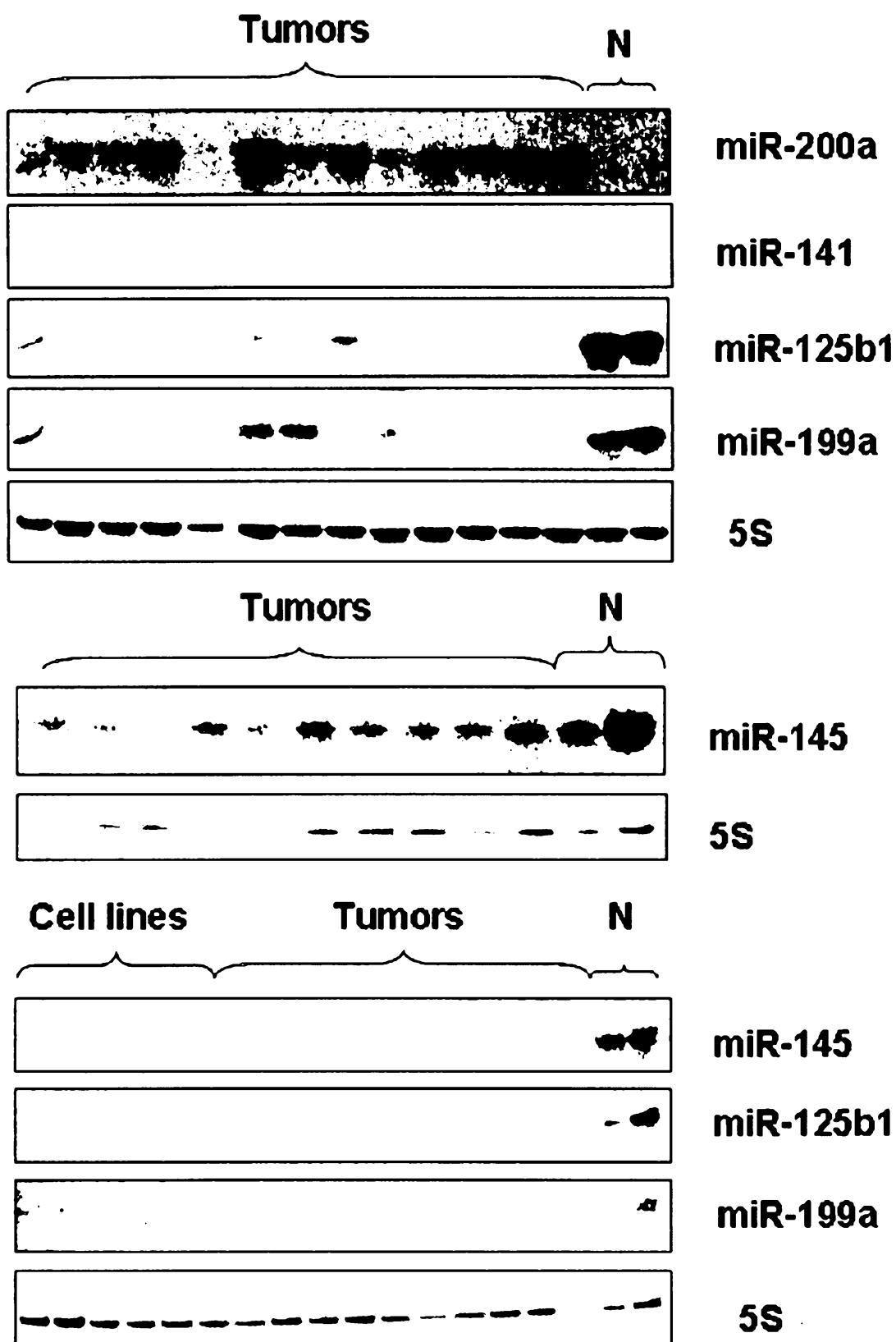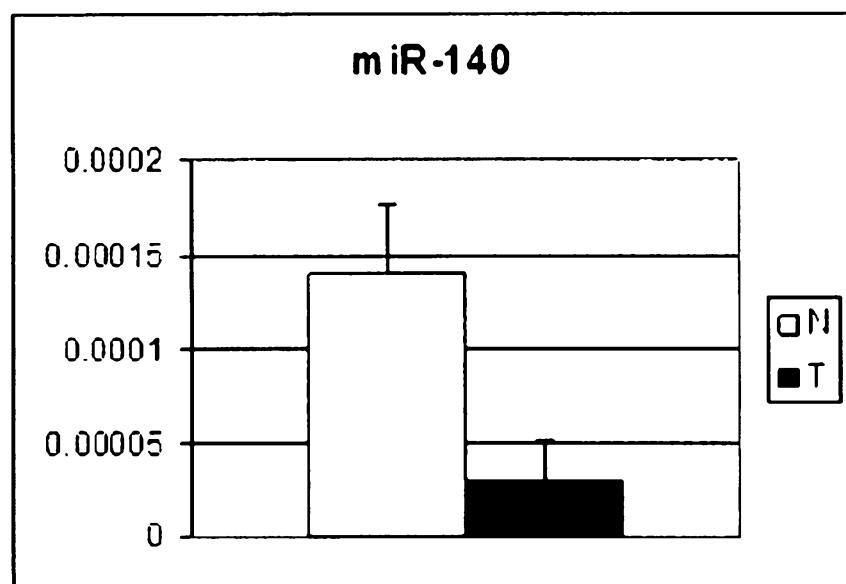
Figure 2A

Figure 2B
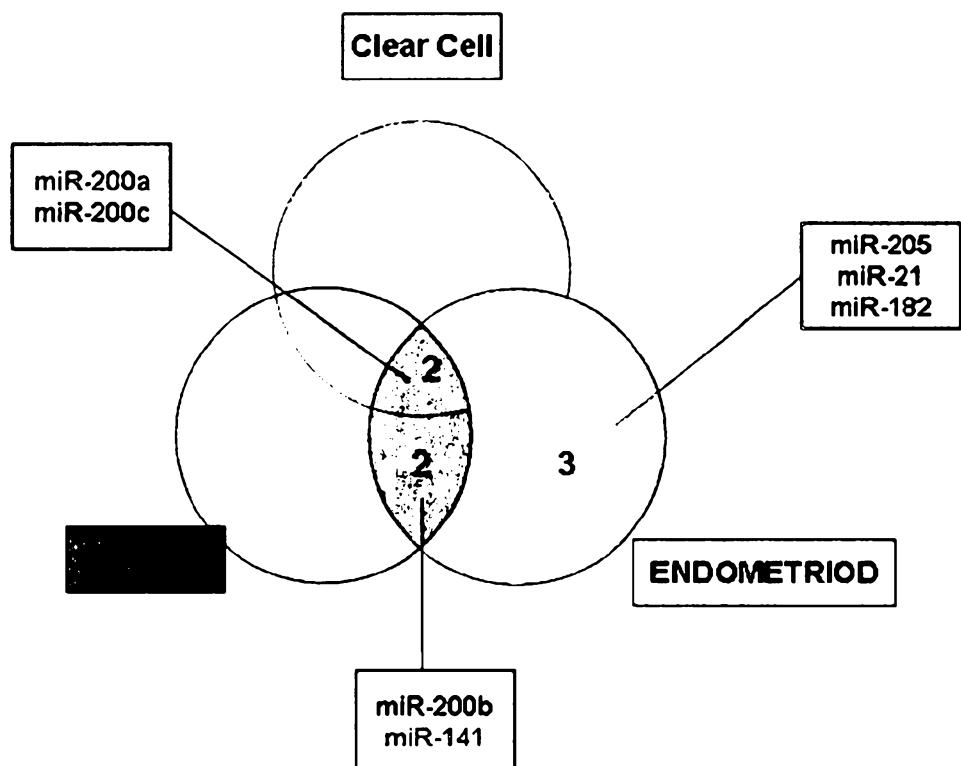
UP-MODULATED IN TUMORS VS NORMALS

Figure 3A

2008296022   31 May 2010

DOWN-MODULATED IN TUMORS VS NORMALS

mir-301
mir-98
mir-19b
mir-29b
mir-18a
mir-154
mir-19a
mir-153

let-7c
mir-145
mir-29c
mir-181a*

Clear Cell

mir-124a
mir-211
mir-216
mir-302c

mir-144
mir-222
mir-1
mir-302a

let-7d
mir-100
mir-101
mir-105
mir-125a
mir-125b-1
mir-126
mir-133a
mir-137
mir-140
mir-143
mir-147
mir-193a
mir-199b
mir-224
mir-302b
mir-9
mir-9*
mir-99a

mir-204
mir-101-1
mir-214
mir-34c
mir-374
mir-195
mir-215
mir-28
mir-29a
mir-133b

4   4

19

4   8

10

ENDOMETRIOD

mir-299-5p
mir-7-2
let-7f
mir-139
mir-193
mir-323
mir-134
mir-370

Figure 3B

Figure 4A

Figure 4B

miR name	FDR	Ratio of geom means AZA/NT
hsa-mir-21	0.002013	4.277
hsa-mir-203	0.0067368	3.826
hsa-mir-146b	0.0095114	2.968
hsa-mir-205	0.0121302	2.877
hsa-miR-30a-5p	0.0160979	2.047
hsa-miR-96	0.0161969	0.476
hsa-mir-155	0.018638	1.784
hsa-mir-383	0.018638	0.474
hsa-miR-30c	0.018638	2.286
hsa-mir-193b	0.018638	2.09
hsa-mir-202	0.018638	1.782

Figure 5A

hsa-miR-30a-5p
hsa-mir-30c
hsa-mir-146b
hsa-mir-193b
hsa-mir-21
hsa-mir-203
hsa-mir-205
hsa-mir-155
hsa-mir-202
hsa-mir-96
hsa-mir-383
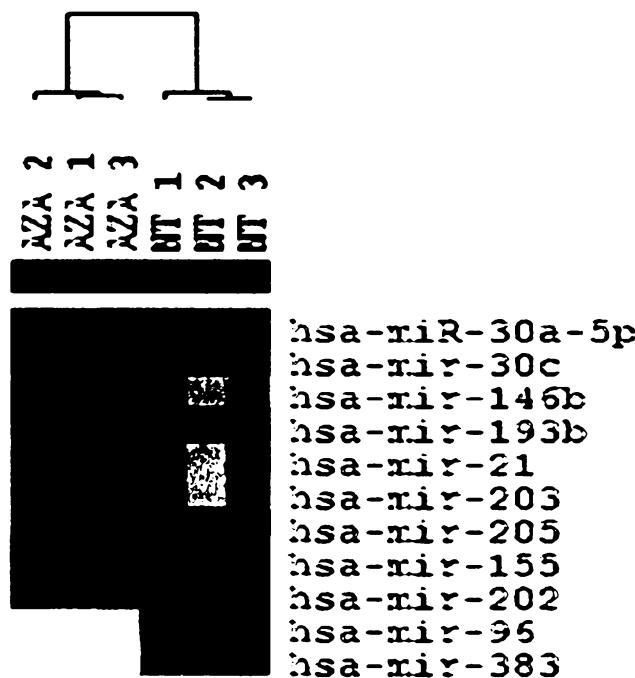
Figure 5B

Figure 5C

9/21
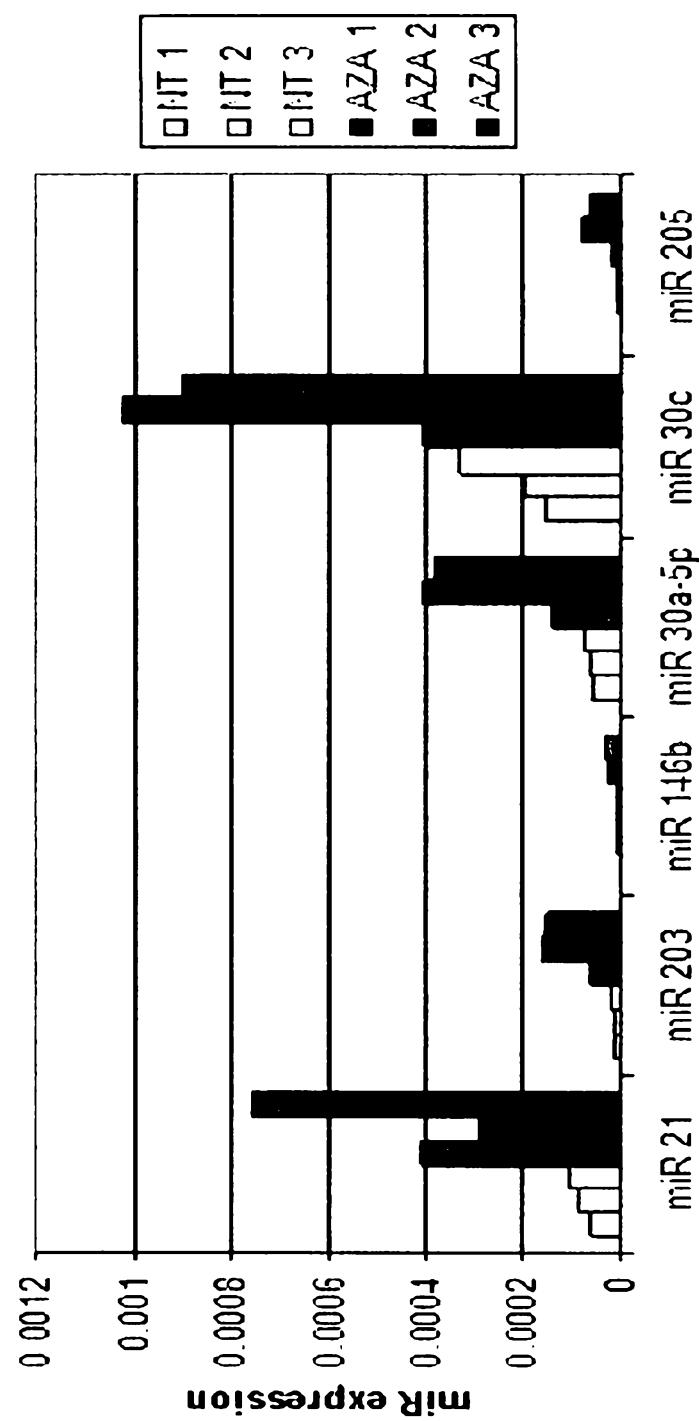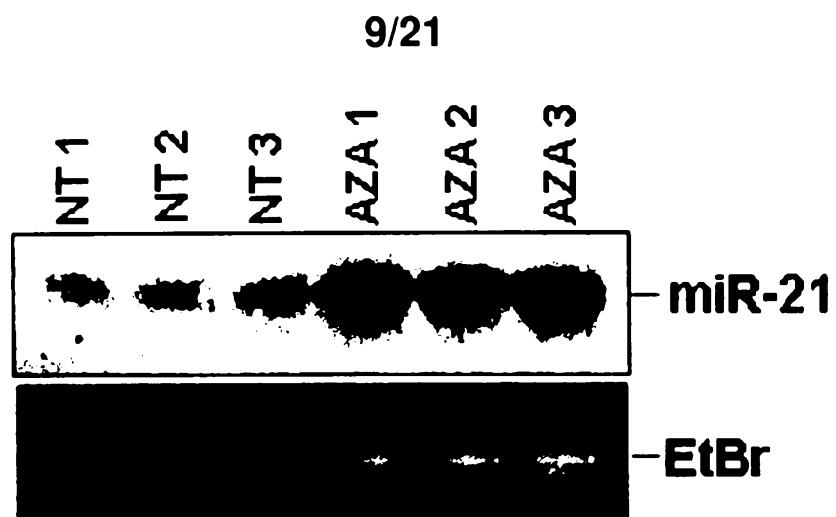
Figure 5D

Individual CV Plots

Figure 6A

Cross-validated Probabilities (Threshold=3.2)
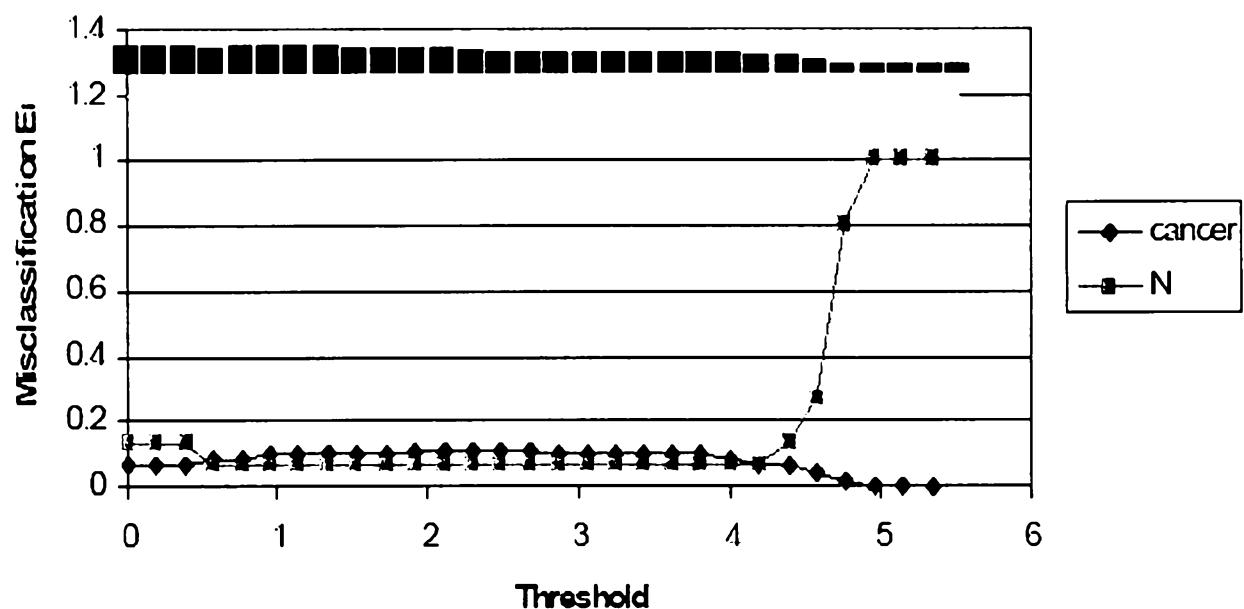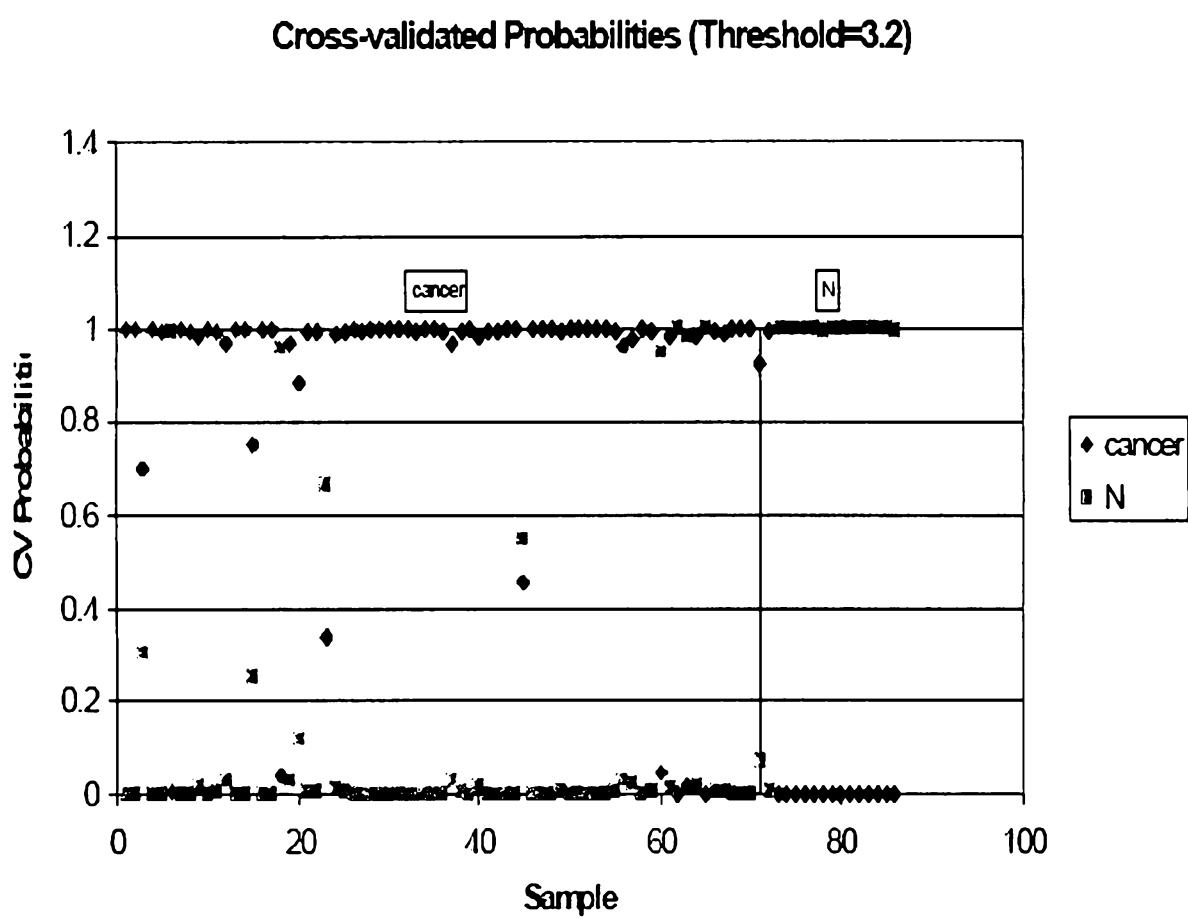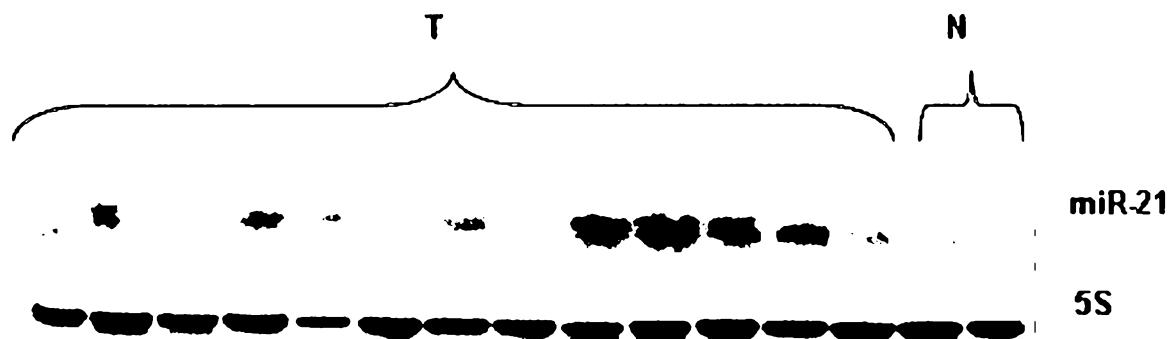
Figure 6B

Figure 7A

2008296022   31 May 2010

800

1600

2400

mature miR-21

↑

1

CpG Island 1

801

1601
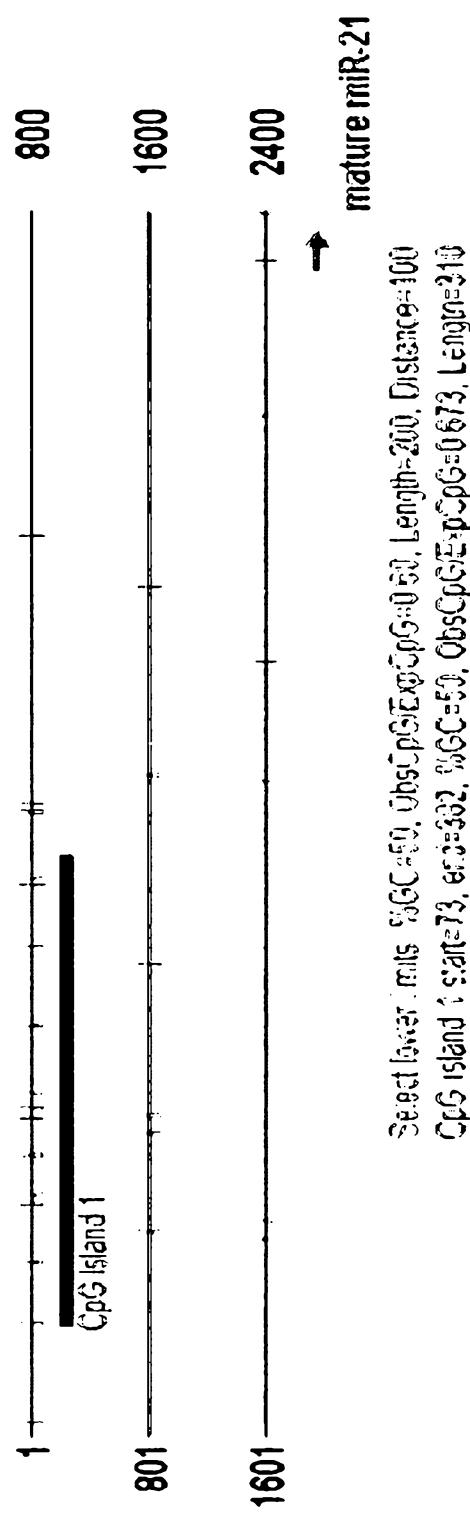
Select lower limits %GC=50, ObsCpGExpCpG=0.60, Length=200, Distance=100
CpG Island 1 start=73, end=352, %GC=50, ObsCpGExpCpG=0.673, Length=210

Figure 7B

Figure 7B cont.

31 May 2010

2008296022
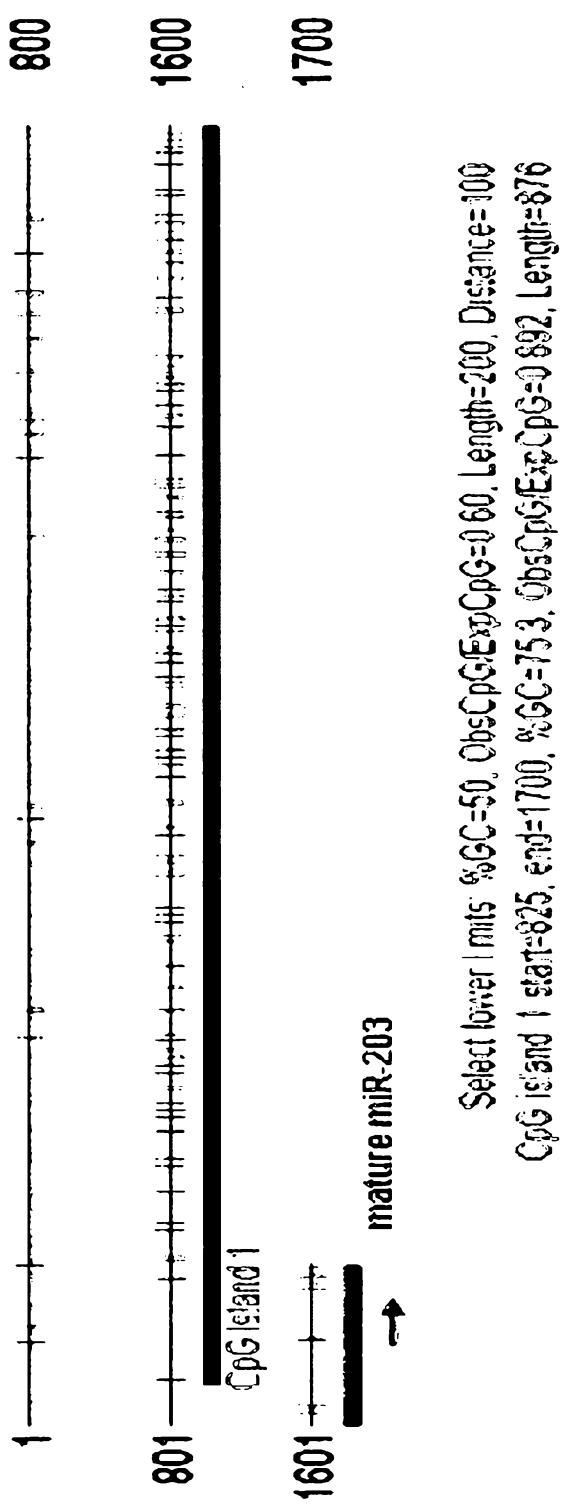
CV Confusion Matrix
(Threshold=3.23866)

True\Predicted	cancer	N	Class Error rate
cancer	63	8	0.112676056
N	1	14	0.066666667

MISCLASSIFICATION ERROR = 0.11

microRNAs	cancer score	N score	
Hsa-mir-200c	0.1152	-0.5454	
Hsa-mir-200a	0.1059	-0.5012	concordant
Hsa-mir-199a	-0.098	0.4637	discordant
			unchanged in Zhang
Hsa-mir-143	-0.0946	0.4479	study
Hsa-mir-199b	-0.0887	0.4197	
Hsa-mir-141	0.0874	-0.4138	
Hsa-mir-145	-0.0734	0.3473	
Hsa-mir-147	-0.0679	0.3212	
Hsa-mir-133a	-0.0671	0.3176	
Hsa-mir-101	-0.0616	0.2917	
Hsa-mir-214	-0.0607	0.2873	
Hsa-mir-100	-0.0535	0.2533	
Hsa-mir-140	-0.0523	0.2474	
Hsa-mir-126	-0.0501	0.2371	
Hsa-mir-224	-0.0485	0.2294	
Hsa-mir-9	-0.0481	0.2277	
Hsa-mir-105	-0.0461	0.2184	
Hsa-mir-99a	-0.037	0.1753	
Hsa-mir-125a	-0.0315	0.1489	
Hsa-mir-211	-0.0248	0.1174	
Hsa-mir-127	-0.0232	0.11	
Hsa-mir-200b	0.0179	-0.0847	
Hsa-mir-125b-1	-0.0177	0.0837	
Hsa-let-7c	-0.0152	0.0719	
Hsa-let-7d	-0.0138	0.0654	
Hsa-mir-124a	-0.0121	0.0574	
Hsa-mir-374	-0.0119	0.0563	
Hsa-let-7a	-0.0113	0.0533	
Hsa-mir-134	-0.0014	0.0068	

Figure 8 – Table 1

microRNAs up-modulated

	Score(d)	Fold Change	
hsa-miR-200a	6.029559054	5.909228959	concordant
miR-141	5.282215009	6.676969496	discordant
hsa-miR-200c	5.072214667	8.522477876	unchanged in Zhang study
hsa-mir-200b	3.833075725	3.305192114	NA
hsa-miR-302b*	2.735468464	7.576624548	
hsa-miR-182	2.474388072	4.629588641	
has-mir-325	2.427712097	3.582141857	
has-mir-373	2.366832731	3.10378255	
hsa-mir-203	2.106735098	3.924966016	
hsa-mir-205	1.912402407	3.913918092	

microRNAs down-modulated

	Score(d)	1/Fold Change
hsa-mir-199a	-6.660234251	0.149170383
hsa-mir-199b	-6.411673443	0.158503458
hsa-mir-143	-6.25941986	0.208966195
hsa-mir-127	-6.239314997	0.36263205
hsa-mir-140	-6.141381353	0.284933473
hsa-mir-9	-6.109935294	0.213902307
hsa-mir-133a	-5.997775401	0.201323582
hsa-mir-105	-5.971698987	0.256386384
hsa-mir-101	-5.951436701	0.140368004
hsa-mir-224	-5.92099781	0.221057654
hsa-mir-126	-5.818430779	0.194310151
hsa-mir-100	-5.768583066	0.217155847
hsa-mir-147	-5.743104574	0.225260363
hsa-mir-204	-5.642915363	0.135983578
hsa-mir-214	-5.63781891	0.226868132
hsa-mir-99a	-5.61022158	0.29031027
hsa-mir-211	-5.52630143	0.151341246
hsa-mir-145	-5.522715864	0.268284181
hsa-let-7d	-5.353956412	0.292557531
hsa-mir-124a	-5.308572049	0.30918176
hsa-mir-125a	-5.275376888	0.285112062
hsa-let-7c	-5.155238667	0.313348909
hsa-let-7a	-5.043893498	0.346201662
hsa-mir-29c	-4.992819698	0.284842858
hsa-mir-195	-4.880474865	0.333200438
hsa-mir-125b	-4.706869872	0.319219655
hsa-mir-374	-4.625938602	0.260209699
hsa-miR-302b	-4.540380253	0.26329528
hsa-miR-29a	-4.538932739	0.330079052

Figure 9 – Table 2

SEROUS VS NORMALS
q value=0, fold change>3

Positive genes

Gene Name	Score(d)	Fold Change
hsa-mir-200c	4.874822	9.316226469
hsa-mir-141	4.105882	6.297820292
hsa-mir-200a	5.22409	6.21082388
hsa-mir-200b	3.028617	3.354261291

Negative genes

Gene Name	Score(d)	Fold Change
hsa-mir-137	-4.79547	0.148869513
hsa-mir-101	-4.20173	0.156683391
hsa-mir-199a	-4.95379	0.157135174
hsa-mir-211	-3.6933	0.159546591
hsa-mir-204	-4.35187	0.160197536
hsa-mir-199b	-4.77294	0.169477272
hsa-mir-101-1	-4.61299	0.205344813
hsa-mir-126	-4.31991	0.205709452
hsa-mir-224	-4.51103	0.210351178
hsa-mir-214	-4.4747	0.211015924
hsa-mir-143	-4.66151	0.225560787
hsa-mir-133a	-4.27006	0.22613586
hsa-mir-147	-4.19961	0.244193755
hsa-mir-34c	-3.49226	0.244581699
hsa-mir-100	-4.031	0.244948271
hsa-mir-302c	-2.7653	0.263419845
hsa-mir-374	-3.44084	0.264221816
hsa-mir-9	-4.22971	0.271871858
hsa-mir-101	-4.10703	0.281023282
hsa-mir-125a	-3.96362	0.284404726
hsa-mir-195	-4.22039	0.288096703
hsa-mir-29c	-3.81131	0.289651915
hsa-mir-105	-4.22771	0.290067186
hsa-mir-145	-4.16437	0.291445115
hsa-let-7d	-3.95134	0.300703735
hsa-mir-302b	-3.13456	0.30356362
hsa-mir-9*	-3.40033	0.307833212
hsa-mir-216	-2.98653	0.309688574
hsa-mir-99a	-4.06488	0.311194043
hsa-mir-140	-5.02723	0.313106918
hsa-mir-125b-1	-3.54736	0.314166789
hsa-mir-215	-3.62675	0.315298054
hsa-let-7c	-3.8424	0.31529976
hsa-mir-181a*	-3.69974	0.319272615
hsa-mir-124a	-3.80122	0.320240298
hsa-mir-28	-2.56159	0.33142251
hsa-let-7d	-3.80275	0.332028327
hsa-mir-29a	-3.46281	0.333446637
hsa-mir-133b	-2.68467	0.333883896

Figure 10 - Table 3

ENDOMETRIOID VS NORMALS
q value=0, fold change>3
Positive genes

Gene Name	Score(d)	Fold Change	Score(d)	Fold Change
hsa-mir-200c	5.602789	11.35184732	4.285621	5.169317934
hsa-mir-141	6.66868	8.134845605	3.175124	4.632327322
hsa-mir-200a	5.873119	5.577590602	2.847984	5.089229918
hsa-mir-205	4.091189	4.268810393	2.74905	3.473502434
hsa-mir-21	3.308304	3.933420772	2.685224	2.25107383
hsa-mir-182	3.336352	3.755931307	2.318534	2.000271165
hsa-mir-200b	3.822882	3.58677048	2.264066	2.501721471
			2.25371	2.455112892
			2.231062	2.385770368
			2.200151	3.904843321
			2.167726	2.263162617
			1.998298	2.69462407
			1.898917	2.611066113
			1.893946	2.183311521

Negative genes

Gene Name	Score(d)	Fold Change
hsa-mir-302c	-4.5238	0.057739797
hsa-mir-140	-4.32451	0.122075074
hsa-mir-101	-4.28657	0.132249108
hsa-mir-9	-4.06022	0.165300935
hsa-mir-147	-5.31115	0.170117845
hsa-mir-302b	-3.40316	0.177459541
hsa-mir-9*	-3.96329	0.177591359
hsa-mir-133a	-4.81939	0.1833621
hsa-mir-137	-2.7233	0.18888881
hsa-mir-299-5p	-4.39191	0.192261887
hsa-mir-105	-4.82847	0.202341368
hsa-mir-199b	-4.4869	0.209194024
hsa-mir-199a	-4.52646	0.210975698
hsa-mir-124a	-4.58559	0.214502778
hsa-mir-100	-4.17774	0.21482284
hsa-mir-211	-2.41054	0.240456539
hsa-mir-224	-3.8354	0.242984707
hsa-mir-143	-4.79084	0.249925609
hsa-mir-126	-3.76437	0.250497146
hsa-mir-1	-3.88978	0.271896204
hsa-mir-7-2	-2.91243	0.280749401
hsa-mir-302a	-2.203	0.289793304
hsa-mir-99a	-3.7768	0.294318696
hsa-mir-216	-1.97425	0.311440033
hsa-mir-125b-1	-3.72135	0.313838284
hsa-let-7d	-3.92129	0.314438715
hsa-let-7f	-4.38303	0.317245972
hsa-mir-139	-2.99323	0.32021126
hsa-mir-125a	-3.54416	0.321469706
hsa-mir-193	-3.35024	0.3275396
hsa-mir-144	-2.56969	0.32905162
hsa-mir-222	-2.70438	0.330732291
hsa-mir-323	-5.14298	0.335905205
hsa-mir-134	-4.3392	0.336555522
hsa-mir-370	-3.40042	0.339804603

Figure 10 cont - Table 3 cont

31 May 2010   2008296022

CLEAR CELL VS NORMALS
q value=0, fold change>3
Positive genes

Gene Name	Score(d)	Fold Change
hsa-mir-200c	3.727262	8.735679382
hsa-mir-200a	4.251752	3.777659435

Negative genes

Gene Name	Score(d)	Fold Change
hsa-mir-133a	-2.93133	0.088082129
hsa-mir-140	-2.97299	0.121810358
hsa-mir-301	-2.39538	0.123963114
hsa-mir-137	-2.27518	0.14653595
hsa-mir-1	-2.33148	0.147859091
hsa-mir-9	-2.96241	0.148568071
hsa-mir-101	-3.2276	0.166648407
hsa-mir-100	-3.83532	0.168061777
hsa-mir-9*	-2.83792	0.174089614
hsa-mir-199b	-3.67083	0.178632358
hsa-mir-105	-3.64152	0.196628255
hsa-mir-98	-3.50536	0.205661462
hsa-mir-302a	-2.1459	0.211204706
hsa-mir-199a	-3.56433	0.212611204
hsa-mir-126	-3.07167	0.22443264
hsa-mir-125a	-3.94158	0.22469509
hsa-mir-143	-3.8477	0.229220021
hsa-mir-19b	-2.84997	0.231739108
hsa-mir-125b-1	-3.97578	0.232452007
hsa-mir-29a	-2.55383	0.236887361
hsa-mir-147	-3.22581	0.237154001
hsa-mir-29c	-2.28388	0.243218229
hsa-mir-222	-2.5152	0.244824885
hsa-mir-99a	-3.2997	0.25237176
hsa-mir-224	-3.01436	0.252667402
hsa-mir-29b	-2.32129	0.25626224
hsa-mir-18a	-1.87174	0.261229849
hsa-mir-144	-2.11054	0.263378089
hsa-mir-100	-3.22544	0.275300179
hsa-mir-154	-2.4006	0.278357698
hsa-mir-302b	-1.87776	0.282435823
hsa-mir-29b	-2.36214	0.284442159
hsa-mir-125b	-3.37335	0.285579607
hsa-let-7d	-3.39786	0.285816741
hsa-let-7c	-3.1888	0.295568469
hsa-mir-145	-3.61294	0.297295886
hsa-mir-181a*	-1.97299	0.29770489
hsa-mir-19b	-2.24934	0.300862177
hsa-mir-153	-2.10042	0.300931686
hsa-let-7d	-2.98829	0.332705239

MIXED VS NORMALS
q value=0, fold change>3
Positive genes

Gene Name	Score(d)	Fold Change
hsa-mir-200c	4.94136514	13.03477954
mir-182	4.75314464	8.234584867
mir-141	6.52792749	8.114549554
hsa-mir-200a	8.59775165	6.749192792
hsa-mir-205	3.15296179	6.612809032
mir-203	3.95187923	6.023471984
hsa-mir-200b	4.21752758	4.559403288
hsa-mir-302b*	2.97964866	3.793211487
hsa-mir-337	2.7911355	3.476209352

Negative genes

Gene Name	Score(d)	Fold Change
hsa-mir-1	-2.9664742	0.104268828
hsa-mir-140	-3.2747728	0.106318579
hsa-mir-199a	-4.3900871	0.130723644
hsa-mir-137	-2.1620126	0.160523671
hsa-mir-214	-5.0278438	0.160582932
hsa-mir-145	-5.3314714	0.174664643
hsa-mir-143	-4.5203659	0.179176097
hsa-mir-199b	-3.7977335	0.180079351
hsa-mir-133a	-2.2671953	0.193349456
hsa-mir-9	-2.5636558	0.195399985
hsa-mir-101	-2.0553425	0.223886714
hsa-mir-9*	-3.1686816	0.243634991
hsa-mir-100	-2.8720969	0.258813665
hsa-mir-147	-3.2823363	0.264405641
hsa-mir-126	-2.5427505	0.273636302
hsa-mir-29c	-1.996995	0.287851362
hsa-mir-134	-4.3148185	0.293654878
hsa-mir-34c	-2.1549977	0.29946464
hsa-mir-10b	-2.9384249	0.303075071
hsa-mir-125a	-3.2480671	0.305281943
hsa-mir-224	-2.5926058	0.305719697
hsa-mir-105	-2.6330954	0.316376728
hsa-mir-181a*	-1.862744	0.321375183

Figure 10 cont – Table 3 cont

31 May 2010   2008296022

**POORLY DIFFERENTIATED VS
NORMALS**
q value=0, fold change>2
Positive genes

Gene Name	Score(d)	Fold Change
hsa-mir-325	4.285621	5.169317934
hsa-mir-22	3.175124	4.632327322
hsa-miR-302c	2.847984	5.089229918
hsa-miR-299-5p	2.74905	3.473502434
hsa-miR-373*	2.685224	2.25107383
hsa-miR-196b	2.318534	2.000271165
hsa-miR-194	2.264066	2.501721471
hsa-mir-373	2.25371	2.455112892
hsa-miR-324-3p	2.231062	2.385770368
hsa-miR-302b*	2.200151	3.904843321
hsa-mir-222	2.167726	2.263162617
hsa-miR-105	1.998298	2.69462407
hsa-miR-124a	1.898917	2.611066113
hsa-miR-1	1.893946	2.183311521

Figure 10 cont - Table 3 cont

2008296022   31 May 2010

SEROUS VS ENDOMETRIOID

Positive genes

Gene Name	Score(d)	Fold Change
hsa-miR-302b*	2.1162067	4.562238026
hsa-miR-325	1.957964	2.804324646
hsa-miR-299-5p	1.9831227	2.733850997
hsa-miR-222	2.0673155	2.519055674
hsa-miR-324-3p	2.012373	2.40273827
hsa-miR-196b	2.222754	1.951149725
hsa-miR-135b	2.087203	1.817543895

Negative genes

Gene Name	Score(d)	Fold Change
hsa-miR-212	-2.533951	0.455394471
hsa-miR-150	-2.4778111	0.531181718

SEROUS VS NON-SEROUS

Positive genes

Gene Name	Score(d)	Fold Change
hsa-miR-302b*	1.8856931	2.387166664
hsa-miR-22	1.8760835	2.289141433
hsa-mir-373	1.9671527	2.026991587

Negative genes

Gene Name	Score(d)	Fold Change
hsa-miR-148b	-1.9350385	0.473389
hsa-miR-211	1.5246626	0.380840675

POORLY DIFFERENTIATED (PD) VS NON-PD

Negative genes

Gene Name	Score(d)	Fold Change
hsa-mir-9	-1.667661896	0.30496814
hsa-mir-18	-1.573399409	0.275779483

ENDOMETRIOID VS NON-ENDOMETRIOID

Positive genes

Gene Name	Score(d)	Fold Change
hsa-miR-212	2.432924647	2.073840527

Negative genes

Gene Name	Score(d)	Fold Change
hsa-miR-22	-1.656798515	0.204982703
hsa-miR-302b*	-2.041611412	0.248819032
hsa-miR-299-5p	-2.034194437	0.341389128
hsa-mir-325	-2.065466938	0.357016955
hsa-miR-194	-1.727883478	0.378903888
hsa-miR-101	-1.785001084	0.379589721
hsa-mir-373	-1.696286422	0.387321213
hsa-miR-324-3p	-1.977704757	0.424134141
hsa-mir-222	-1.783218284	0.42620566

CLEAR CELL VS NON-CLEAR CELL

Positive genes

Gene Name	Score(d)	Fold Change
hsa-miR-30a-5p	2.567858911	3.180690498
hsa-miR-29b	2.548556062	2.674746178
hsa-miR-30e-5p	2.090920369	2.632760423

Negative genes

Gene Name	Score(d)	Fold Change
hsa-miR-20a	-2.08649871	0.408227411

Figure 11 - Table 4

LYMPHOVASCULAR INVASION

Negative genes

Gene Name	Score(d)	Fold Change	q-value(%)
hsa-mir-144	-1.96	0.215959317	0
hsa-mir-216	-1.33255	0.41718005	0

OVARIAN SURFACE INVOLVEMENT

Positive genes

Gene Name	Score(d)	Fold Change	q-value(%)
hsa-mir-101	2.018272	2.243033058	0
hsa-miR-182*	1.561247	2.065541993	0
hsa-mir-22	1.401532	2.457950079	0
hsa-miR-133a	1.381055	3.450487913	0

TUBAL INVOLVEMENT

Negative genes

Gene Name	Score(d)	Fold Change	q-value(%)
hsa-mir-137	-1.9090693	0.37256783	0
hsa-mir-101	-1.7469971	0.390419388	0
hsa-mir-215	-1.4178719	0.324648273	0
hsa-mir-211	-1.1978493	0.407288227	0

PELVIC PERITONEUM

Positive genes

Gene Name	Score(d)	Fold Change	q-value(%)
hsa-miR-302c	1.150474	2.293373976	0

UTERUS INVOLVEMENT

Positive genes

Gene Name	Score(d)	Fold Change	q-value(%)
hsa-mir-133a-2	1.27588177	3.266174858	0
hsa-mir-143	1.91132897	2.362716504	0
hsa-mir-145	1.82832273	2.282302908	0
hsa-mir-1	1.57556619	2.278201035	0
hsa-mir-147	0.99659977	2.115494772	0
hsa-miR-126	1.36023908	2.08659655	0

Figure 12 - Table 5

31 May 2010

2008296022

miRs UP-modulated in ovarian carcinoma	CONFIRMED TARGET	EXPRESSION IN OVARIAN CARCINOMA	PUTATIVE TARGETS	EXPRESSION IN OVARIAN CARCINOMA
miR-200a			BAP1	DOWN
			PDCD4	down in other tumors
			GATA6	DOWN
			VCAM-1	DOWN
miR-200b			BAP1	DOWN
			GATA4	DOWN
			FN1 (fibronectin 1)	DOWN
miR-200c			BAP1	DOWN
			FN1 (fibronectin 1)	DOWN
miR-141	CLOCK (Kiriakidou M. Et al, 2004)		BAP1	DOWN
miRs DOWN-modulated in ovarian carcinoma				
miR-140	HDAC4 (Tuddenham L. et al, 2006)	UP	c-SRK	UP
			MMP13	UP
			FGF2	UP
miR-199a			CCNL1	UP (other tumor types)
miR-199b	LAMC2 (Kiriakidou M. Et al, 2004)	UP (other cancers)		
miR-145	FLJ21308 (Kiriakidou M. Et al, 2004)			
miR-143	ERK5 (Esau et al, 2004)	UP (other cancers)	COX-2 (strongly correlates with resistance to chemotherapy and poor outcome in ovarian cancer)	UP
miR-125a	ERBB2, ERBB3 (Scott et al, 2007)	UP		
miR-125b	ERBB2, ERBB3 (Scott et al, 2007)	UP		
miR-101	Enx-1 (Lewis et al, 2003)		COX-2	UP
	MYCN (Lewis et al, 2003)	UP		
miR-212			WT1, BRCA1	
miR-222	c-KIT (Felli et al, 2005)	higher in high-grade compared with low-grade OSC		

Figure 13 - Table 6

<220>

<223> Description of Artificial Sequence: Synthetic
    probe

<400> 98
tcaacatcag tctgataagc ta   22

<110> THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION

<120> MICRORNA SIGNATURES IN HUMAN OVARIAN CANCER

<130> 53-29381

<140> PCT/US2008/075565
<141> 2008-09-08

<150> 60/967,663
<151> 2007-09-06

<160> 98

<170> PatentIn version 3.5

<210> 1
<211> 22
<212> RNA
<213> Homo sapiens

<400> 1
uaacacuguc ugguaacgau gu   22

<210> 2
<211> 22
<212> RNA
<213> Homo sapiens

<400> 2
uaauacugcc ugguaaugau ga   22

<210> 3
<211> 23
<212> RNA
<213> Homo sapiens

<400> 3
uaauacugcc ggguaaugau gga   23

<210> 4
<211> 22
<212> RNA
<213> Homo sapiens

<400> 4
uaacacguc ugguaaagau gg   22

<210> 5
<211> 22
<212> RNA
<213> Homo sapiens

<400> 5
acuuuaacau ggaagugcuu uc   22

<210> 6
<211> 24
<212> RNA
<213> Homo sapiens

<400> 6
uuuggcaaug guagaacuca cacu   24

2008296022   31 May 2010

<210> 7
<211> 23
<212> RNA
<213> Homo sapiens

<400> 7
ccuaguaggu guccaguaag ugu   23

<210> 8
<211> 23
<212> RNA
<213> Homo sapiens

<400> 8
gaagugcuuc gauuuugggg ugu   23

<210> 9
<211> 22
<212> RNA
<213> Homo sapiens

<400> 9
gugaaauguu uaggaccacu ag   22

<210> 10
<211> 22
<212> RNA
<213> Homo sapiens

<400> 10
uccuucauuc caccggaguc ug   22

<210> 11
<211> 23
<212> RNA
<213> Homo sapiens

<400> 11
cccaguguuc agacuaccug uuc   23

<210> 12
<211> 23
<212> RNA
<213> Homo sapiens

<400> 12
cccaguguuu agacuaucug uuc   23

<210> 13
<211> 21
<212> RNA
<213> Homo sapiens

<400> 13
ugagaugaag cacuguagcu c   21

<210> 14
<211> 22
<212> RNA
<213> Homo sapiens

<400> 14
cugaagcuca gagggcucug au   22

<210> 15
<211> 22
<212> RNA
<213> Homo sapiens

<400> 15
cagugguuuu acccuauggu ag   22

<210> 16
<211> 23
<212> RNA
<213> Homo sapiens

<400> 16
ucuuugguua ucuagcugua uga   23

<210> 17
<211> 22
<212> RNA
<213> Homo sapiens

<400> 17
uuuggucccc uucaaccagc ug   22

<210> 18
<211> 23
<212> RNA
<213> Homo sapiens

<400> 18
ucaaaugcuc agacuccugu ggu   23

<210> 19
<211> 21
<212> RNA
<213> Homo sapiens

<400> 19
uacaguacug ugauaacuga a   21

<210> 20
<211> 21
<212> RNA
<213> Homo sapiens

<400> 20
caagucacua gugguuccgu u   21

<210> 21
<211> 22
<212> RNA
<213> Homo sapiens

<400> 21
ucguaccgug aguaauaaug cg   22

<210> 22
<211> 22
<212> RNA
<213> Homo sapiens

<400> 22
aacccguaga uccgaacuug ug   22

<210> 23
<211> 20
<212> RNA
<213> Homo sapiens

<400> 23
gugaguggaa augcuucugc   20

<210> 24
<211> 22
<212> RNA
<213> Homo sapiens

<400> 24
uucccuuugu cauccuaugc cu   22

<210> 25
<211> 22
<212> RNA
<213> Homo sapiens

<400> 25
acagcaggca cagacaggca gu   22

<210> 26
<211> 22
<212> RNA
<213> Homo sapiens

<400> 26
aacccguaga uccgaucuug ug   22

<210> 27
<211> 22
<212> RNA
<213> Homo sapiens

<400> 27
uucccuuugu cauccuucgc cu   22

<210> 28
<211> 23
<212> RNA
<213> Homo sapiens

<400> 28
guccaguuuu cccaggaauc ccu   23

<210> 29
<211> 22
<212> RNA
<213> Homo sapiens

<400> 29
agagguagua gguugcauag uu   22

<210> 30
<211> 20
<212> RNA
<213> Homo sapiens

<400> 30
uaaggcacgc ggugaaugcc   20

<210> 31
<211> 24
<212> RNA
<213> Homo sapiens

<400> 31
ucccugagac ccuuuaaccu guga   24

<210> 32
<211> 22
<212> RNA
<213> Homo sapiens

<400> 32
ugagguagua gguuguaugg uu   22

<210> 33
<211> 22
<212> RNA
<213> Homo sapiens

<400> 33
ugagguagua gguuguauag uu   22

<210> 34
<211> 22
<212> RNA
<213> Homo sapiens

<400> 34
uagcaccauu ugaaaucggu ua   22

<210> 35
<211> 21
<212> RNA
<213> Homo sapiens

<400> 35
uagcagcaca gaaauauugg c   21

<210> 36
<211> 22
<212> RNA
<213> Homo sapiens

<400> 36
ucccugagac ccuaacuugu ga   22

<210> 37
<211> 22
<212> RNA
<213> Homo sapiens

<400> 37
uuauaauaca accugauaag ug   22

<210> 38
<211> 23
<212> RNA
<213> Homo sapiens

<400> 38
uaagugcuuc cauguuuuag uag   23

<210> 39
<211> 22
<212> RNA
<213> Homo sapiens

<400> 39
uagcaccauc ugaaaucggu ua   22

<210> 40
<211> 22
<212> RNA
<213> Homo sapiens

<400> 40
uagcuuauca gacugauguu ga   22

<210> 41
<211> 24
<212> RNA
<213> Homo sapiens

<400> 41
uuuggcaaug guagaacuca cacu   24

<210> 42
<211> 22
<212> RNA
<213> Homo sapiens

<400> 42
accaucgacc guugauugua cc   22

<210> 43
<211> 22
<212> RNA
<213> Homo sapiens

<400> 43
uaaucucagc uggcaacugu ga   22

<210> 44
<211> 23
<212> RNA
<213> Homo sapiens

<400> 44
uaagugcuuc cauguuucag ugg   23

<210> 45
<211> 23
<212> RNA
<213> Homo sapiens

<400> 45
cagugcaaua guauugucaa agc   23

<210> 46
<211> 22
<212> RNA
<213> Homo sapiens

<400> 46
ugagguagua aguuguauug uu   22

<210> 47
<211> 23
<212> RNA
<213> Homo sapiens

<400> 47
ugugcaaauc caugcaaaac uga   23

<210> 48
<211> 23
<212> RNA
<213> Homo sapiens

<400> 48
uagcaccauu ugaaaucagu guu   23

<210> 49
<211> 23
<212> RNA
<213> Homo sapiens

<400> 49
uaaggugcau cuagugcaga uag   23

<210> 50
<211> 22
<212> RNA
<213> Homo sapiens

<400> 50
uagguuaucc guguugccuu cg   22

<210> 51
<211> 23
<212> RNA
<213> Homo sapiens

<400> 51
ugugcaaauc uaugcaaaac uga   23

<210> 52
<211> 22
<212> RNA
<213> Homo sapiens

<400> 52
uugcauaguc acaaaaguga uc   22

<210> 53
<211> 20
<212> RNA
<213> Homo sapiens

<400> 53
uacaguauag augauguacu   20

<210> 54
<211> 21
<212> RNA
<213> Homo sapiens

<400> 54
agcuacaucu ggcuacuggg u   21

<210> 55
<211> 22
<212> RNA
<213> Homo sapiens

<400> 55
uggaauguaa agaaguaugu au   22

<210> 56
<211> 23
<212> RNA
<213> Homo sapiens

<400> 56
uaagugcuuc cauguuuugg uga   23

<210> 57
<211> 23
<212> RNA
<213> Homo sapiens

<400> 57
aggcagugua guuagcugau ugc   23

<210> 58
<211> 21
<212> RNA
<213> Homo sapiens

<400> 58
augaccuaug aauugacaga c   21

<210> 59
<211> 22
<212> RNA
<213> Homo sapiens

<400> 59
aaggagcuca cagucuauug ag   22

<210> 60
<211> 22
<212> RNA
<213> Homo sapiens

<400> 60
uuugguccccc uucaaccagc ua   22

<210> 61
<211> 22
<212> RNA
<213> Homo sapiens

<400> 61
ugguuuaccg ucccacauac au   22

<210> 62
<211> 23
<212> RNA
<213> Homo sapiens

<400> 62
uggaagacua gugauuuugu ugu   23

<210> 63
<211> 22
<212> RNA
<213> Homo sapiens

<400> 63
ucuacagugc acgugucucc ag   22

<210> 64
<211> 22
<212> RNA
<213> Homo sapiens

<400> 64
gccugcuggg guggaaccug gu   22

<210> 65
<211> 23
<212> RNA
<213> Homo sapiens

<400> 65
uuauugcuua agaauacgcg uag   23

<210> 66
<211> 22
<212> RNA
<213> Homo sapiens

<400> 66
auaaagcuag auaaccgaaa gu   22

<210> 67
<211> 22
<212> RNA
<213> Homo sapiens

<400> 67
ugagaacuga auuccauagg cu   22

<210> 68
<211> 22
<212> RNA
<213> Homo sapiens

<400> 68
uguaaacauc cucgacugga ag   22

<210> 69
<211> 23
<212> RNA
<213> Homo sapiens

<400> 69
uuuggcacua gcacauuuuu gcu   23

<210> 70
<211> 23
<212> RNA
<213> Homo sapiens

<400> 70
uuaaugcuaa ucgugauagg ggu   23

<210> 71
<211> 22
<212> RNA
<213> Homo sapiens

<400> 71
agaucagaag gugauugugg cu   22

<210> 72
<211> 23
<212> RNA
<213> Homo sapiens

<400> 72
uguaaacauc cuacacucuc agc   23

<210> 73
<211> 22
<212> RNA
<213> Homo sapiens

<400> 73
aacuggcccu caaagucccg cu   22

<210> 74
<211> 20
<212> RNA
<213> Homo sapiens

<400> 74
agagguauag ggcaugggaa   20

<210> 75
<211> 22
<212> RNA
<213> Homo sapiens

<400> 75
ugugacuggu ugaccagagg gg   22

<210> 76
<211> 22
<212> RNA
<213> Homo sapiens

<400> 76
aggugguccg uggcgcguuc gc   22

<210> 77
<211> 21
<212> RNA
<213> Homo sapiens

<400> 77
gaacggcuuc auacaggagu u   21

<210> 78
<211> 23
<212> RNA
<213> Homo sapiens

<400> 78
uacccguag aaccgaauuu gug   23

<210> 79
<211> 22
<212> RNA
<213> Homo sapiens

<400> 79
aagcugccag uugaagaacu gu   22

<210> 80
<211> 22
<212> RNA
<213> Homo sapiens

<400> 80
uagguaguuu ccuguuguug gg   22

<210> 81
<211> 22
<212> RNA
<213> Homo sapiens

<400> 81
uguaacagca acuccaugug ga   22

<210> 82
<211> 23
<212> RNA
<213> Homo sapiens

<400> 82
cgcauccccu agggcauugg ugu   23

<210> 83
<211> 23
<212> RNA
<213> Homo sapiens

<400> 83
uauggcuuuu cauuccuaug uga   23

<210> 84
<211> 21
<212> RNA
<213> Homo sapiens

<400> 84
uaacagucuc cagucacggc c   21

<210> 85
<211> 22
<212> RNA
<213> Homo sapiens

<400> 85
ucucccaacc cuuguaccag ug   22

<210> 86
<211> 22
<212> RNA
<213> Homo sapiens

<400> 86
ucagugcauc acagaacuuu gu   22

```
<210> 87
<211> 22
<212> RNA
<213> Homo sapiens

<400> 87
uguaaacauc cuugacugga ag                                                   22


<210> 88
<211> 23
<212> RNA
<213> Homo sapiens

<400> 88
uaaagugcuu auagugcagg uag                                                  23


<210> 89
<211> 23
<212> RNA
<213> Homo sapiens

<400> 89
aacauucaac gcugucggug agu                                                  23


<210> 90
<211> 22
<212> RNA
<213> Homo sapiens

<400> 90
ugagaacuga auuccauggg uu                                                   22


<210> 91
<211> 22
<212> RNA
<213> Homo sapiens

<400> 91
ugggucuuug cgggcgagau ga                                                   22


<210> 92
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 92
acatcgttac cagacagtgt ta                                                   22


<210> 93
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 93
ccatctttac cagacagtgt ta                                                   22


<210> 94
```

<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 94
gaacaggtag tctgaacact ggg   23

<210> 95
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 95
tcacaagtta gggtctcagg ga   22

<210> 96
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 96
aagggattcc tgggaaaact ggac   24

<210> 97
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 97
gagacccagt agccagatgt agct   24

<210> 98
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      probe

<400> 98
tcaacatcag tctgataagc ta   22