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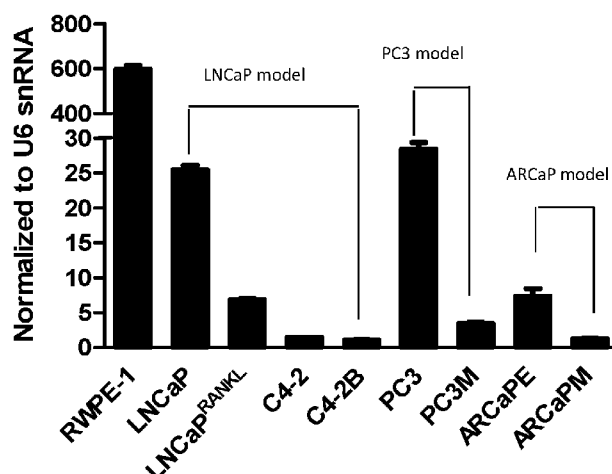
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(54) Title: METHODS OF DIAGNOSING AND TREATING CANCER WITH MICRORNAS

FIG. 1B

miR-n1



(57) Abstract: Described herein are methods of treating and diagnosing cancer, as well as selecting a cancer treatment for a subject in need thereof. In particular, prostate and metastatic cancer are treated or diagnosed. The treatment can comprise administering an miRNA or miRNA mimics described herein to a patient in need thereof. The diagnostic can comprise measuring the miRNA expression levels, as described herein.



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METHODS OF DIAGNOSING AND TREATING CANCER WITH MICRORNAS**FIELD OF INVENTION**

[0001] This invention relates to micro RNAs and cancer; particularly, to prostate cancer and metastasis.

BACKGROUND

[0001] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0002] MiRNAs are non-coding RNAs of 18-24 nucleotides that bind to sites of complementarity in the 3' untranslated regions of messenger RNAs and inhibit their translation. A single miRNA can target several mRNA and regulate cellular pathways and cell fate. Several miRNA have been dysregulated in cancer, some of these are oncogenic (oncomiR) or they function as tumor suppressors.

[0003] The lack of effective treatment for cancers, and particularly drug resistant cancers, along with the prevalence of metastasis shows a need in the art for additional therapies as well as biomarkers to diagnose cancer, to differentiate the status of malignancy of cancer and to discover and develop cancer therapeutics.

SUMMARY OF THE INVENTION

[0004] The following embodiments and aspects thereof are described and illustrated in conjunction with compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0005] Various embodiments of the present invention provide a method of treating cancer in a subject in need thereof, comprising: administering an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-

n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, to the subject in need thereof to treat the cancer.

[0006] In various embodiments the method can further comprise administering a JMJD3 inhibitor.

[0007] In various embodiments, the cancer can be prostate cancer. In various embodiments, the cancer can be metastatic prostate cancer.

[0008] Various embodiments of the present invention provide for a method of inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells comprising: administering an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to a subject in need thereof to inhibit growth, proliferation, migration, invasion and/or metastasis of cancer cells.

[0009] In various embodiments, the method can further comprise administering a JMJD3 inhibitor.

[0010] In various embodiments, the cancer can be prostate cancer. In various embodiments, the cancer can be metastatic prostate cancer.

[0011] Various embodiments of the present invention provide for a method of promoting cellular differentiation, inducing terminal differentiation of a cell, or inducing apoptosis of a cell, comprising: contacting an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to the cell to promote cellular differentiation, induce terminal differentiation of the cell, or induce apoptosis of the cell.

[0012] In various embodiments, the method can further comprise contacting a JMJD3 inhibitor to the cell.

[0013] In various embodiments, the cell can be a prostate cancer cell. In various embodiments, the cell can be a metastatic prostate cancer cell.

[0014] Various embodiments provide for a method, comprising: subjecting a biological sample from a subject to analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof; comparing the miRNA expression level to a reference value; and determining the subject has cancer or cancer with metastatic potential if the

miRNA expression level is lower than the reference value or determining that the subject does not have cancer or cancer with metastatic potential if the miRNA expression level is not lower than the reference value.

[0015] In various embodiments, the method further comprises selecting a cancer treatment for the subject if the subject is determined to have cancer.

[0016] In various embodiments, the cancer treatment selected can be an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof.

[0017] In various embodiments, the method further comprises comprising selecting a JMJD3 inhibitor.

[0018] Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0019] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0020] Figures 1A-1D depict miR-n1 validation in lineage-related human prostate cancer cell lines and in clinical samples. The northern blots were probed with U6 RNA (control) or miRNAs (Fig. 1A). qRT-PCR was used to quantify, normalized by RNU6B in cells and tissues (n=36, Fig. 1C) and by miR-1228 in sera (n=12, Fig. 1D) (Figs. 1B-1D). Northern blot detects various miRNAs, normalizes by RNU6B in lineage-related progressive prostate cancer cell line models, LNCaP, PC3 and ARCaP (Fig. 1B), and tissues (Fig. 1C) by probing with an oligonucleotide complementary to U6 RNA (an internal control for RNA content) or corresponding miRNA.

[0021] Figures 2A-2E show that miR-n1 inhibits growth, migration and invasion of an aggressive human prostate cancer LNCaP^{RANKL} cells (similar level of growth was noted in mock and normal control group, Figs. 2B-2E). qRT-PCR was used to quantify novel miRNAs, normalized by RNU6B (Fig. 2A).

[0022] Figures 3A-3E shows that miR-n1 inhibits growth, migration and invasion of castration-resistant human prostate cancer C4-2B cells (similar level of growth was noted in mock and normal control group, Figs. 3B-3E). qRT-PCR was used to quantify novel miRNAs, normalized by RNU6B (Fig. 3A).

[0023] Figures 4A-4H show that miR-n1 knockdown increases JMJD3 and promotes anchorage-independent growth. Fig. 4A: Bioinformatics predicted the positions of 5 putative miR-n1 target sites in JMJD3 3'UTR. Fig. 4B: 3'UTR binding dual luciferase assay using WT and mutant 3'UTR (JMJD3) construct and miR-n1 mimics in 293T cells. Fig. 4C: qRT-PCR assessed the endogenous JMJD3 expression after transfecting with miR-n1 mimics in 293T cells. Fig. 4D: Inverse relationship between miR-n1 and JMJD3 were noted consistent the indolent nature of a normal prostate epithelial cell line, RWPE-1, and a prostate cancer cell line, LNCaP cells. Fig. 4E: Knockdown miR-n1 in LNCaP cell promoted JMJD3 expression rather than EZH2. Fig. 4F: Knockdown efficiency of miR-n1 inhibitor in LNCaP cells is concentration-dependent. Fig. 4G: Anchorage-dependent cell proliferation on plastic dishes did not change after knocking down miR-n1 in LNCaP cell. Fig. 4H: Knocking down miR-n1 promoted anchorage-independent growth of LNCaP cell as assessed by colony formation in soft agar.

[0024] Figures 5A-5F show that JMJD3 inhibitor, GSK-J4, inhibits prostate cancer cell anchorage-independent growth. Fig. 5A: molecular structure of GSK-J4; Fig. 5B, Fig. 5C, Fig. 5D, Fig. 5E: GSK-J4 inhibits anchorage-dependent growth of LNCaP, LNCaP^{RANKL}, C4-2B and PC3 cells. Fig. 5F: GSKJ4 inhibits anchorage-independent colony-forming growth of LNCaP, LNCaP^{RANKL}, C4-2B and PC3 cells.

[0025] Figure 6 depicts dual luciferase reporter assay indicated that novel miRNA n1 targets RAF1 and CREBBP through the novel miRNA n1 binding sequences located at the 3'-UTR of the RAF1 and CREBBP genes. As is shown in the figure, when miRNA n1-mimics were cotransfected with the reporter plasmids, the relative luciferase activity of the reporter plasmid containing wild-type RAF1 or CREBBP 3'-UTR was markedly reduced, while the luciferase activity of the reporter plasmid containing mutant RAF1 or CREBBP 3'-UTR was unaltered. These results suggest that among genes regulating prostate cancer cell growth, migration, invasion and metastasis, RAF and CREBBP are the targets of miRNA n1.

DESCRIPTION OF THE INVENTION

[0026] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology* 3rd ed., Revised, J. Wiley & Sons (New York, NY 2006); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* 7th ed., J. Wiley & Sons (New York, NY 2013); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual* 4th ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application.

[0027] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0028] “Cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, lung cancer, prostate cancer, breast cancer, colon cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma, melanoma, head and neck cancer, and brain cancer.

[0029] “Contacting” as used here with reference to contacting a cell with an agent (e.g., a miRNA or miRNA mimics) refers to any method that is suitable for placing the agent on, in or adjacent to a target cell. For example, when the cells are *in vitro*, contact the cells with the agent can comprise adding the agent to culture medium containing the cells. For example, when the cells are *in vivo*, contacting the cells with the agent can comprise administering the agent to the subject.

[0030] As used herein, the term “label” with reference to probes refers to a composition capable of producing a detectable signal indicative of the presence of a target. Suitable labels include quantum dots, fluorescent molecules, radioisotopes, nucleotide chromophores, enzymes, substrates, chemiluminescent moieties, magnetic particles, bioluminescent moieties, and the like. As such, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means needed for the methods and devices described herein.

[0031] Exemplary fluorescent labeling reagents include, but are not limited to, Hydroxycoumarin, Succinimidyl ester, Aminocoumarin, Methoxycoumarin, Cascade Blue, Hydrazide, Pacific Blue, Maleimide, Pacific Orange, Lucifer yellow, NBD, NBD-X, R-Phycoerythrin (PE), a PE-Cy5 conjugate (Cychrome, R670, Tri-Color, Quantum Red), a PE-Cy7 conjugate, Red 613, PE-Texas Red, PerCP, Peridinin chlorophyll protein, TruRed (PerCP-Cy5.5 conjugate), FluorX, Fluoresceinisothiocyanate (FITC), BODIPY-FL, TRITC, X-Rhodamine (XRITC), Lissamine Rhodamine B, Texas Red, Allophycocyanin (APC), an APC-Cy7 conjugate, Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500, Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, Alexa Fluor 750, Alexa Fluor 790, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5 or Cy7.

[0032] “Mammal” as used herein refers to any member of the class Mammalia, including, without limitation, humans and nonhuman primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus adult and newborn subjects, as well as fetuses, whether male or female, are intended to be including within the scope of this term.

[0033] “miRNA mimics” as used herein are small, double-stranded RNAs that mimic endogenous miRNAs. MiRNA mimics can enable miRNA functional analysis by up-regulation of miRNA activity. “miRNA mimics” can also be referred to as “miRNA mimetics.” Stabilizing modifications can be added to miRNAs to create miRNA mimics for additional stability in nuclease-rich environments.

[0034] “Treatment” and “treating,” as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, slow down and/or lessen the disease even if the treatment is ultimately unsuccessful.

Treatment methods

[0035] Various embodiments provide for a method of treating cancer in a subject in need thereof. In various embodiments, method comprises administering an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7,

and combinations thereof to the subject in need thereof to treat the cancer. In various embodiments, the method comprises first providing the miRNA.

[0036] In various embodiments, the method comprises administering a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to the subject in need thereof to treat the cancer. In various embodiments, the method comprises first providing the miRNA mimic.

[0037] In various embodiments, the cancer is prostate cancer. In other embodiments, the cancer is metastatic prostate cancer.

[0038] In various embodiments, the method of treating cancer further comprises administering a JMJD3 inhibitor. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0039] Various embodiments provide for a method of inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells. In various embodiments, the method comprises administering an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to a subject in need thereof to inhibit growth, proliferation, migration, invasion and/or metastasis of cancer cells. In various embodiments, the method comprises first providing the miRNA.

[0040] In various embodiments, the method comprises administering a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to a subject in need thereof to inhibit growth, proliferation, migration, invasion and/or metastasis of cancer cells. In various embodiments, the method comprises first providing the miRNA mimic.

[0041] In various embodiments, the cancer cell is a prostate cancer cell. In other embodiments, the cancer cell is a metastatic prostate cancer cell.

[0042] In various embodiments, the method of inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells further comprises administering a JMJD3 inhibitor. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0043] Various embodiments provide for a method of promoting cellular differentiation. In various embodiments, the method comprises contacting an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, to a cell to promote cellular differentiation. In various embodiments, the method comprises first providing the miRNA.

[0044] In various embodiments, the method comprises contacting a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5,

miR-n6, miR-n7, and combinations thereof, to a cell to promote cellular differentiation. In various embodiments, the method comprises first providing the miRNA mimic.

[0045] In various embodiments, the cell is a prostate cancer cell. In other embodiments, the cell is a metastatic prostate cancer cell.

[0046] In various embodiments, the method of promoting cellular differentiation further comprises contacting a JMJD3 inhibitor to the cell. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0047] Various embodiments provide for a method of inducing terminal differentiation of a cell. In various embodiments, the method comprises contacting an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, to the cell to induce terminal differentiation. In various embodiments, the method comprises first providing the miRNA.

[0048] In various embodiments, the method comprises contacting a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to a cell to induce terminal differentiation. In various embodiments, the method comprises first providing the miRNA mimic.

[0049] In various embodiments, the cell is a prostate cancer cell. In other embodiments, the cancer cell is a metastatic cancer cell.

[0050] In various embodiments, the method of inducing terminal differentiation of a cell further comprises contacting a JMJD3 inhibitor to the cell. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0051] Various embodiments provide for a method of inducing apoptosis of a cell. In various embodiments, the method comprises contacting a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, to the cell to induce apoptosis. In various embodiments, the method comprises first providing the miRNA.

[0052] In various embodiments, the method comprises contacting a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, to a cell to induce apoptosis. In various embodiments, the method comprises first providing the miRNA mimic.

[0053] In various embodiments, the cell is a prostate cancer cell. In other embodiments, the cell is a metastatic prostate cancer cell.

[0054] In various embodiments, the method of inducing apoptosis of a cell further comprises contacting a JMJD3 inhibitor to the cell. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0055] Various embodiments provide for a method for treating cancer, or inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells, comprising administering an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, to a subject who has been determined to have an miRNA expression level lower than a reference value, wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof.

[0056] Various embodiments provide for a method for treating cancer, or inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells, comprising administering an cancer therapeutic to a subject who has been determined to have an miRNA expression level lower than a reference value, wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof.

[0057] In various embodiments, the cancer is prostate cancer. In other embodiments, the cancer is metastatic prostate cancer.

[0058] In various embodiments, the method of treating cancer further comprises administering a JMJD3 inhibitor to the subject. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0059] Various embodiments provide for a method for treating cancer, or inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells, comprising administering a JMJD3 inhibitor to a subject who has been determined to have an miRNA expression level lower than a reference value, wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof.

[0060] In various embodiments, the cancer is prostate cancer. In other embodiments, the cancer is metastatic prostate cancer. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

[0061] In various embodiments, the present invention provides pharmaceutical compositions including a pharmaceutically acceptable excipient along with a therapeutically effective amount of an miRNA or miRNA mimic of the present invention. “Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[0062] In various embodiments, the pharmaceutical compositions according to the invention may be formulated for delivery via any route of administration. “Route of administration” may refer to any administration pathway known in the art, including but not limited to aerosol, nasal, oral, transmucosal, transdermal or parenteral. “Transdermal” administration may be accomplished using a topical cream or ointment or by means of a transdermal patch.

[0063] “Parenteral” refers to a route of administration that is generally associated with injection, including intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the compositions may be in the form of solutions or suspensions for infusion or for injection, or as lyophilized powders.

[0064] Via the enteral route, the pharmaceutical compositions can be in the form of tablets, gel capsules, sugar-coated tablets, syrups, suspensions, solutions, powders, granules, emulsions, microspheres or nanospheres or lipid vesicles or polymer vesicles allowing controlled release.

[0065] Via the topical route, the pharmaceutical compositions based on compounds according to the invention may be formulated for treating the skin and mucous membranes and are in the form of ointments, creams, milks, salves, powders, impregnated pads, solutions, gels, sprays, lotions or suspensions. They can also be in the form of microspheres or nanospheres or lipid vesicles or polymer vesicles or polymer patches and hydrogels allowing controlled release. These topical-route compositions can be either in anhydrous form or in aqueous form depending on the clinical indication. Via the ocular route, they may be in the form of eye drops.

[0066] The pharmaceutical compositions according to the invention can also contain any pharmaceutically acceptable carrier. “Pharmaceutically acceptable carrier” as used

herein refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a combination thereof. Each component of the carrier must be “pharmaceutically acceptable” in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it may come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0067] The pharmaceutical compositions according to the invention can also be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

[0068] The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

[0069] The pharmaceutical compositions according to the invention may be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine

a therapeutically effective amount through routine experimentation, for instance, by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see *Remington: The Science and Practice of Pharmacy* (Gennaro ed. 20th edition, Williams & Wilkins PA, USA) (2000).

Diagnostics

[0070] Various embodiments provide for a method of diagnosing cancer.

[0071] In various embodiments, the method comprises subjecting a biological sample from a subject to analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof; comparing the miRNA expression level to a reference value; and determining the subject has cancer if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value. In further embodiments, the method comprises first obtaining the biological sample.

[0072] In various embodiments, the method comprises requesting an analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, of a biological sample obtained from a subject; comparing the miRNA expression level to a reference value; and determining the subject has cancer if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value. In further embodiments, the method comprises first obtaining the biological sample.

[0073] In various embodiments, the method comprises detecting the expression level of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, wherein an expression level lower than a reference level indicated that the subject has cancer and an expression level that is not lower than the reference level indicates that the subject does not have cancer.

[0074] In various embodiments, the cancer is prostate cancer. In other embodiments, the cancer is metastatic prostate cancer.

Treatment selection

[0075] Various embodiments of the present invention provide for selecting a treatment for a subject suspected of having cancer. In various embodiments, the method comprises subjecting a biological sample from a subject to analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof; comparing the miRNA expression level to a reference value; determining the subject has cancer if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value; and selecting a cancer treatment for the subject if the subject is determined to have cancer. In further embodiments, the method comprises first obtaining the biological sample.

[0076] Various embodiments of the present invention provide for selecting a treatment for a subject suspected of having cancer. In various embodiments, the method comprises requesting the results of a test regarding miRNA expression level, wherein results are obtained by: subjecting a biological sample from a subject to analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, and comparing the miRNA expression level to a reference value; determining the subject has cancer if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value; and selecting a cancer treatment for the subject if the subject is determined to have cancer.

[0077] In various embodiments, the method of selecting a treatment comprises requesting the results of an analysis of miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof; and selecting a cancer treatment for the subject if the miRNA expression level is lower than a reference value.

[0078] In various embodiments, the cancer treatment selected is an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, as the treatment for the subject.

[0079] In various embodiments, the cancer is prostate cancer. In other embodiments, the cancer is metastatic prostate cancer.

[0080] In various embodiments, the method of selecting a treatment further comprises selecting a JMJD3 inhibitor. A non-limiting example of a JMJD3 inhibitor is GSK-J4.

Expression level analysis and biological samples

[0081] In various embodiments, analyzing miRNA expression level or detecting the expression level comprises using qRT-PCR.

[0082] In various embodiments, analyzing miRNA expression level or detecting the expression level comprises using *in situ* hybridization. In other embodiments, analyzing miRNA expression level or detecting the expression level comprises using northern blot.

[0083] Probes comprising a nucleic acid having an antisense sequence of the miRNA and a label can be used to analyze, detect or measure the expression level. A nonlimiting example of the label is example is multiplexed *in situ* hybridization quantum dot (ISH-QD) labeling. Examples of other labels that can be used are as discussed above.

[0084] In various embodiments, the expression level of the miRNA is the normalized expression level. MiRNA expression can be normalized by RNU6B, for example, in cells and tissues. MiRNA expression can also be normalized by miR-1228, for example, in sera.

[0085] Biological sample used in the methods described herein can be tissues and cells (for example from the prostate or prostate tumor), body fluids, sera such as blood (including whole blood as well as its plasma and serum), CSF (spinal fluid), urine, sweat, saliva, tears, pulmonary secretions, breast aspirate, prostate fluid, seminal fluid, stool, cervical scraping, cysts, amniotic fluid, intraocular fluid, mucous, moisture in breath, animal tissue, cell lysates, tumor tissue, hair, skin, buccal scrapings, nails, bone marrow, cartilage, prions, bone powder, ear wax, etc. or even from external or archived sources such as tumor samples (i.e., fresh, frozen or paraffin-embedded).

MicroRNAs and MicroRNA Mimics

[0086] In various embodiments, miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, and miR-n7 have the sequence as provided by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, respectively. In various embodiments, the miR-n1 comprises the following sequence: GTCGGTAGAGCATCAGACTTTTAATCTGAGGGTCCAGGGTTCAAGTCCCTGTTTCG GCGGGAGTGGTGGCTTTTAGTACCTGA (SEQ ID NO:8), which is the precursor sequence.

[0087] In various embodiments, the miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, and miR-n7 have the sequence as provided by SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, respectively, wherein n is T or U. In various embodiments, the miR-n1 comprises the following sequence: G_nCGG_nAGAGCA_nCAGAC_nnnnnA_nC_nGAGGG_nCCAGGG_nCAAG_nCCC_nG_nCGGGCGGGAG_nGG_nGGC_nnnnnAG_nACC_nGA (SEQ ID NO:20), wherein n is T or U, which is the precursor sequence.

[0088] In various embodiments, the miRNA mimic comprises a nucleic acid having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and combinations thereof

[0089] Table 1. Sequence of miRNA

	Sequence	SEQ ID NO:
miR-n1	TCGGGCGGGAGTGGTGGCTTTT	1
miR-n2	CACCGACTCTGTCTCCTGCAG	2
miR-n3	TTGTGGAAACAATGGTACGGCA	3
miR-n4	TGGGAGGAACAAGTATGCATT	4
miR-n5	CGCGCCTGCAGGAAGTGGTAGA	5
miR-n6	GGAGGAACCTTGGAGCTTCGGCA	6
miR-n7	TGCCCCGGCGGTGTGCGGCCACA	7

[0090] Table 2. Sequence of miRNA

	Sequence	SEQ ID NO:
miR-n1	ncgggcgggagngnggcnnnn	13
miR-n2	caccgacncngncncngcag	14
miR-n3	nngnggaacaanggnacggca	15
miR-n4	ngggaggaacaagnangcann	16
miR-n5	cgcgccngcaggaacnggnaga	17

miR-n6	ggaggaaccnnggagcnnccggca	18
miR-n7	ngcccggcggnngcggccaca	19

Wherein “n” can be U or T.

[0091] In various embodiments, the miRNA mimic comprises a nucleic acid having a sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and combinations thereof.

Reference values

[0092] In various embodiments of the present invention, the reference value is the miRNA's expression level in non-cancerous cells.

[0093] In various embodiments of the present invention, the reference value can be one or more housekeeping genes.

EXAMPLES

[0094] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1

[0095] Paired normal human prostate and prostate cancer tissue specimens were analyzed by RNA-seq and revealed 7 novel miRNAs. miRn-1 was downregulated in lineage-related prostate cancer cell lines with increased aggressiveness, lower level of expression in human prostate cancer tissues and in sera of metastatic prostate cancer patients.

[0096] We found that an miRn-1 mimic suppresses the growth of prostate cancer cells *in vitro*.

[0097] Genetic knockdown of miRn-1 enhances prostate cancer anchorage-independent growth possibly through upregulation of JMJD3.

[0098] Consistent with the functional regulatory loop of miRn-1-JMJD3 axis, inhibitor of this axis by GSKJ4 strongly inhibited prostate cancer anchorage-independent growth *in vitro*.

[0099] We predict that miR-n1 target genes are one or more of the following JMJD3, CREBBP, ETS1, CSK, RAF1, CXCR3, IGF1R, RUNX1, DHH, PARK2, and NFIX and their respective signaling axes.

Example 2

[0100] Dual luciferase reporter assay indicated that novel miRNA n1 targets RAF1 and CREBBP through the novel miRNA n1 binding sequences located at the 3'-UTR of the RAF1 and CREBBP genes. As is shown in the figure 6, when miRNA n1 mimics were cotransfected with the reporter plasmids, the relative luciferase activity of the reporter plasmid containing wild-type RAF1 or CREBBP 3'-UTR was markedly reduced, while the luciferase activity of the reporter plasmid containing mutant RAF1 or CREBBP 3'-UTR was unaltered.

[0101] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0102] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[0103] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. It will be understood by those within the art that, in general, terms used herein are generally intended as “open” terms (*e.g.*, the term “including” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as “includes but is not limited to,” etc.).

[0104] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not. It will be understood by those within the art that, in general, terms used herein are generally intended as “open” terms (*e.g.*, the term “including” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as “includes but is not limited to,” etc.). Although the open-ended term “comprising,” as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as “consisting of” or “consisting essentially of.”

WHAT IS CLAIMED IS:

1. A method of treating cancer in a subject in need thereof, comprising:
administering a miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, to the subject in need thereof to treat the cancer.
2. The method of claim 1, further comprising administering a JMJD3 inhibitor.
3. The method of claim 2, wherein the JMJD3 inhibitor is GSK-J4.
4. The method of claim 1, wherein the cancer is prostate cancer.
5. The method of claim 1, wherein the cancer is metastatic prostate cancer.
6. A method of inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells comprising:
administering a miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to a subject in need thereof to inhibit growth, proliferation, migration, invasion and/or metastasis of cancer cells.
7. The method of claim 6, further comprising administering a JMJD3 inhibitor.
8. The method of claim 6, wherein the cancer is prostate cancer.
9. The method of claim 6, wherein the cancer is metastatic prostate cancer.
10. A method of promoting cellular differentiation, inducing terminal differentiation of a cell, or inducing apoptosis of a cell, comprising:
contacting an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of an miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof to the cell to promote cellular differentiation, induce terminal differentiation of the cell, or induce apoptosis of the cell.
11. The method of claim 10, further comprising contacting a JMJD3 inhibitor to the cell.
12. The method of claim 10, wherein the cell is a prostate cancer cell.
13. The method of claim 10, wherein the cell is a metastatic prostate cancer cell.

14. A method, comprising:
 - subjecting a biological sample from a subject to analysis for miRNA expression level wherein the miRNA is selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof;
 - comparing the miRNA expression level to a reference value; and
 - determining the subject has cancer or cancer with metastatic potential if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value.
15. The method of claim 14, further comprising selecting a cancer treatment for the subject if the subject is determined to have cancer.
16. The method of claim 15, wherein the cancer treatment selected is a miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof, or a mimic of a miRNA selected from the group consisting of miR-n1, miR-n2, miR-n3, miR-n4, miR-n5, miR-n6, miR-n7, and combinations thereof.
17. The method of claim 15, further comprising administering the selected cancer treatment.
18. The method of claim 16, further comprising selecting a JMJD3 inhibitor.
19. The method of claim 18, further comprising administering the JMJD3 inhibitor.
20. The method of claim 18, wherein the JMJD3 inhibitor is GSK-J4.

FIG. 1A

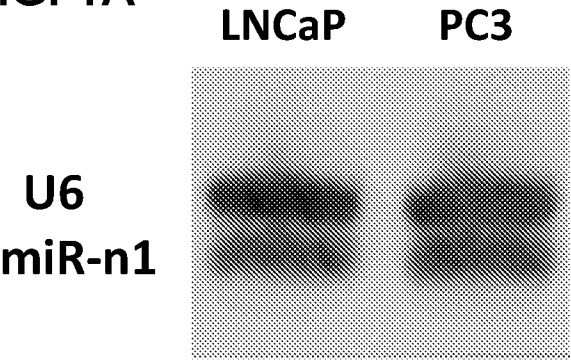
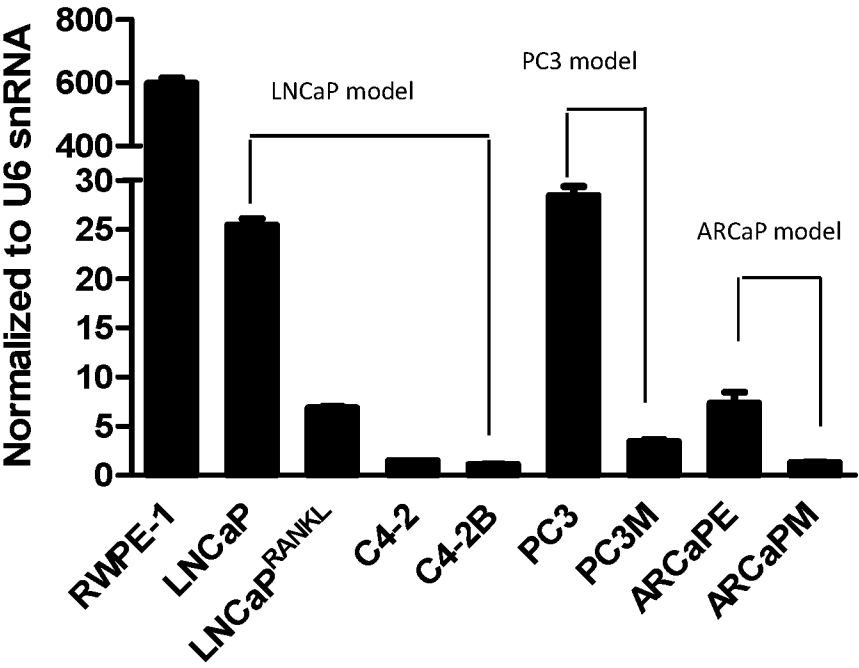


FIG. 1B

miR-n1



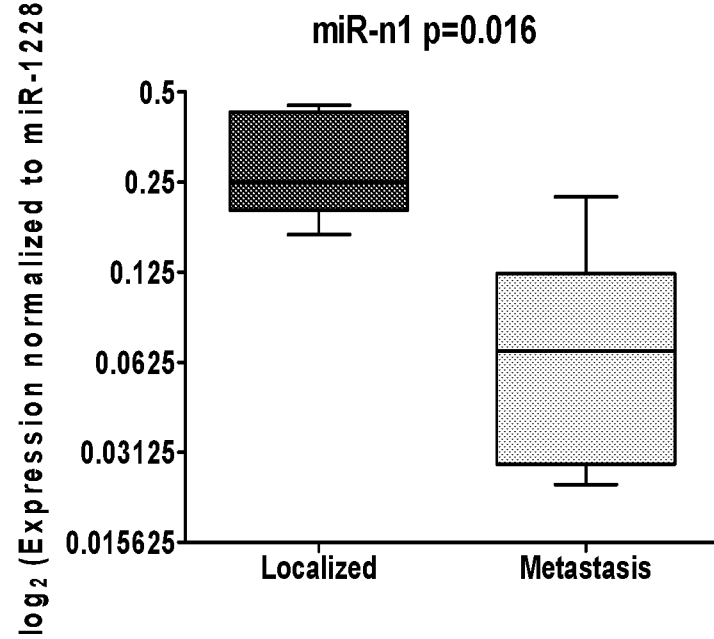


FIG. 2A

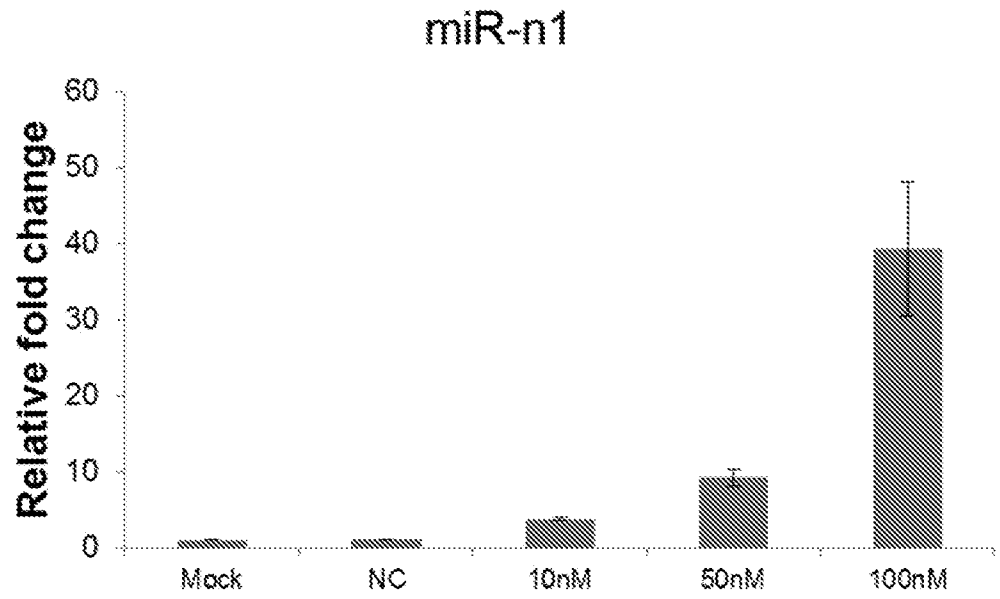


FIG. 2B

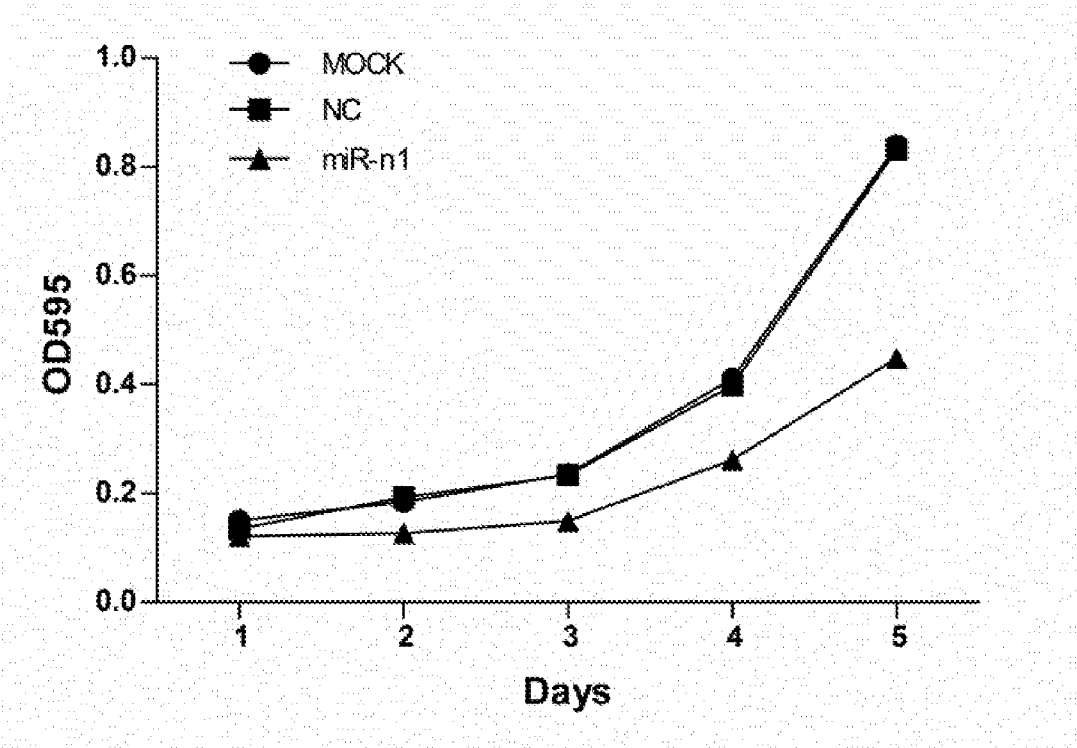


FIG. 2C

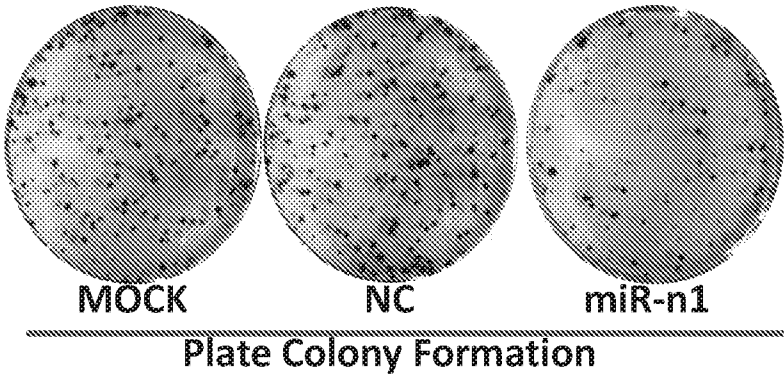


FIG. 2D

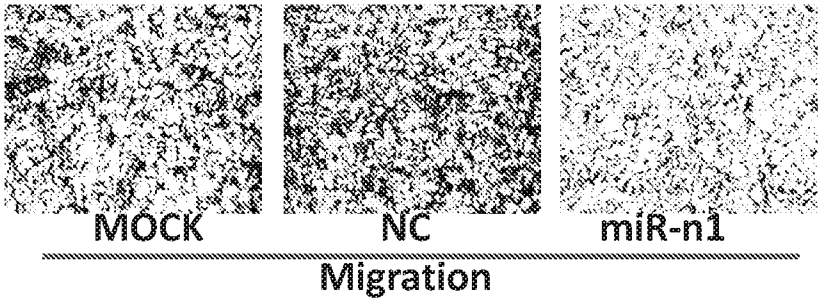


FIG. 2E

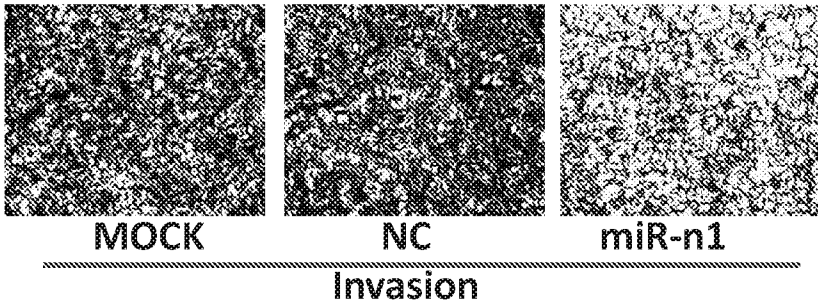


FIG. 3A

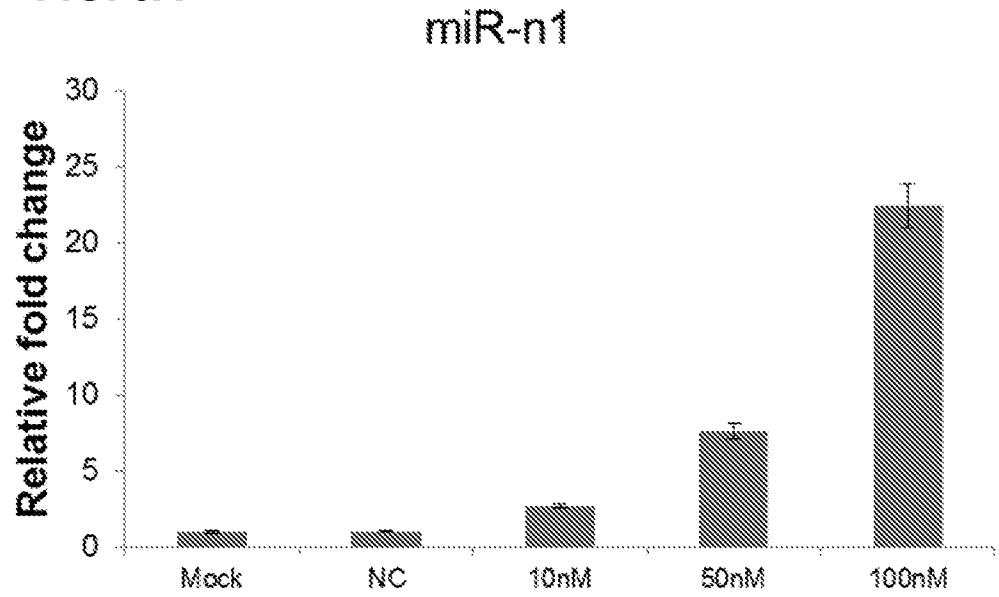


FIG. 3B

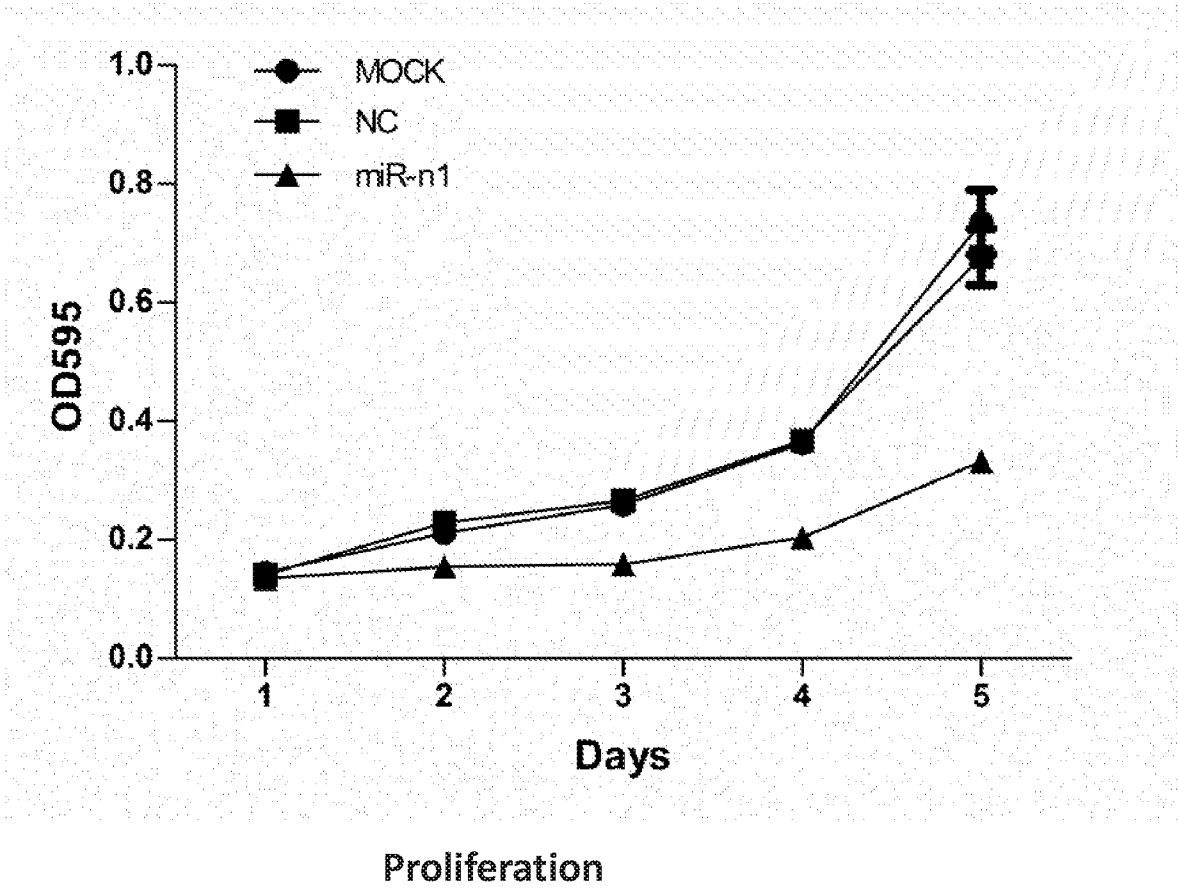


FIG. 3C

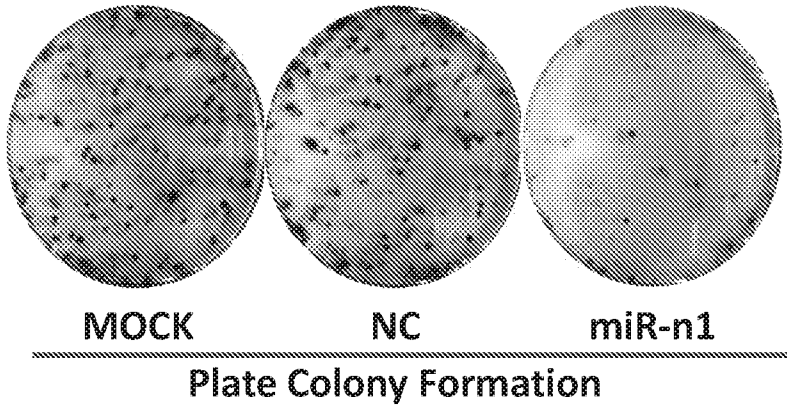


FIG. 3D

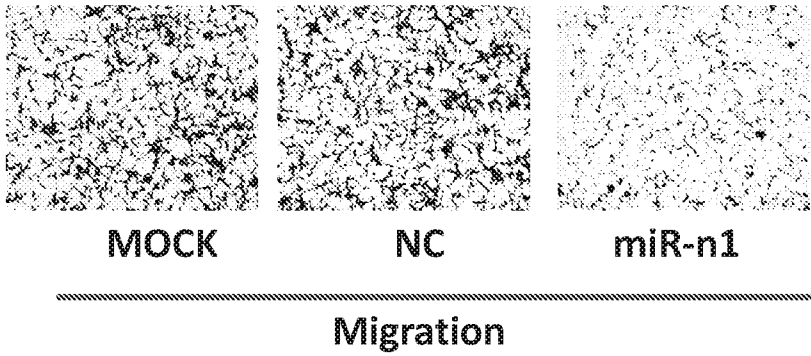


FIG. 3E

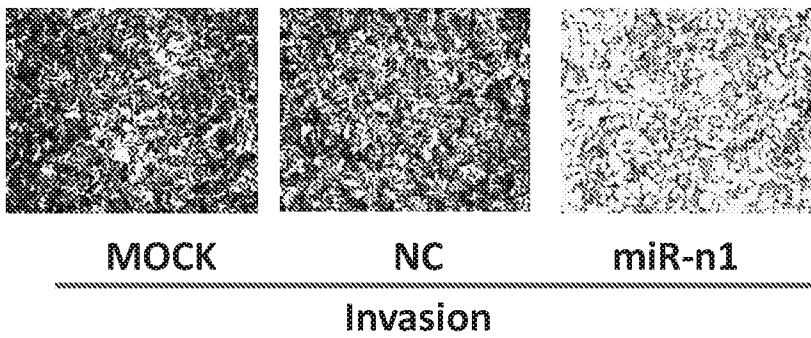


FIG. 4A

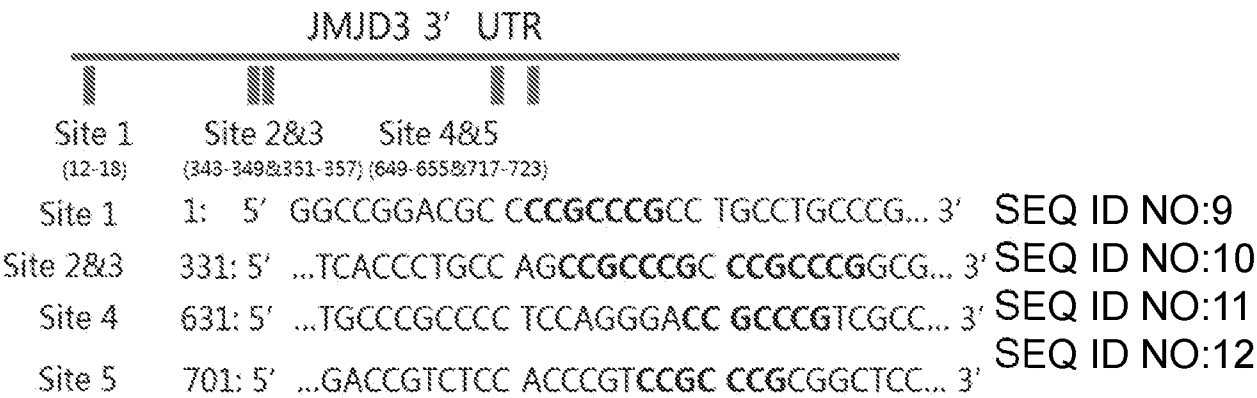


FIG. 4B

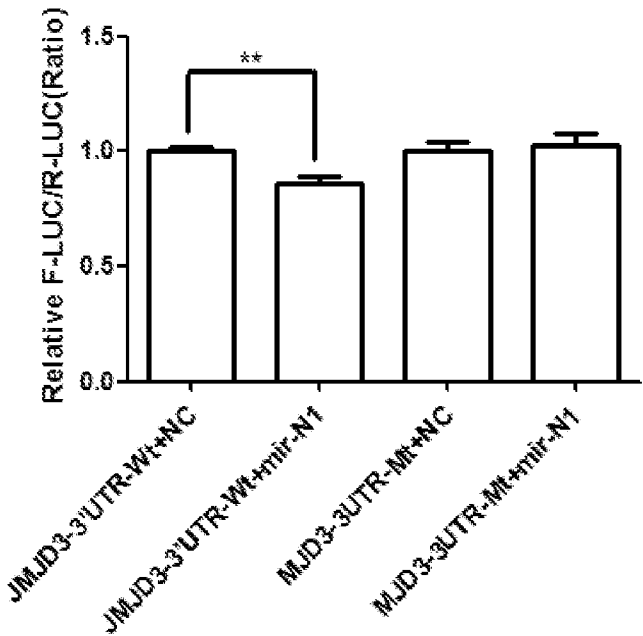


FIG. 4C

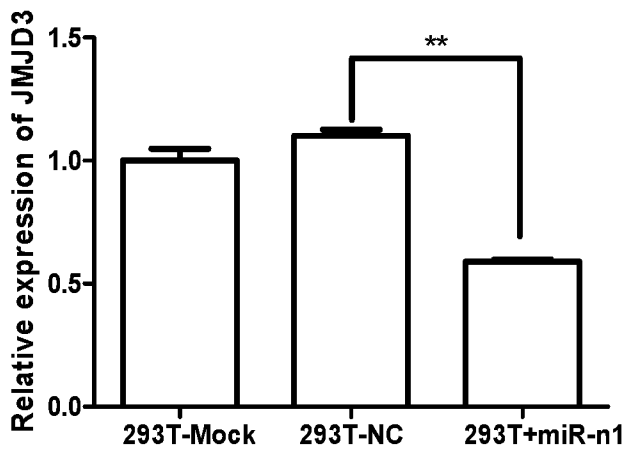


FIG. 4D

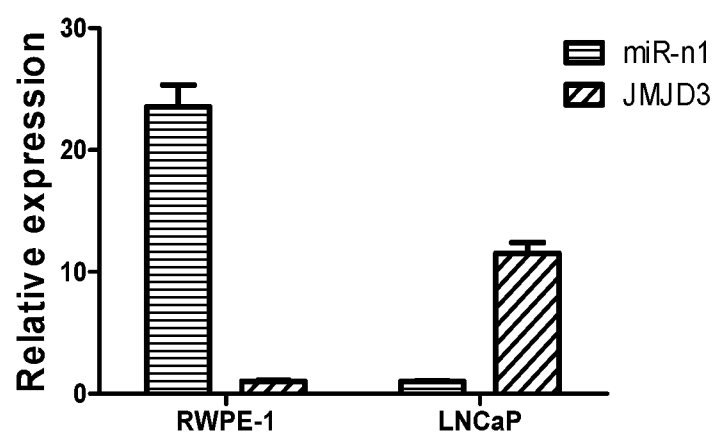


FIG. 4E

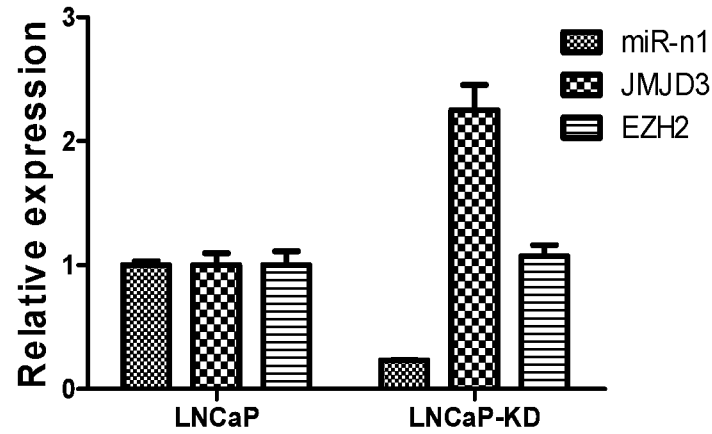


FIG. 4F

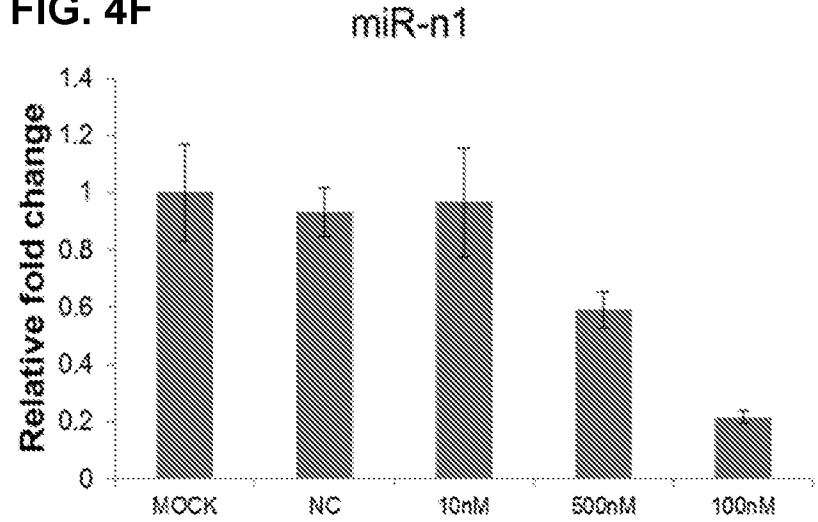


FIG. 4G

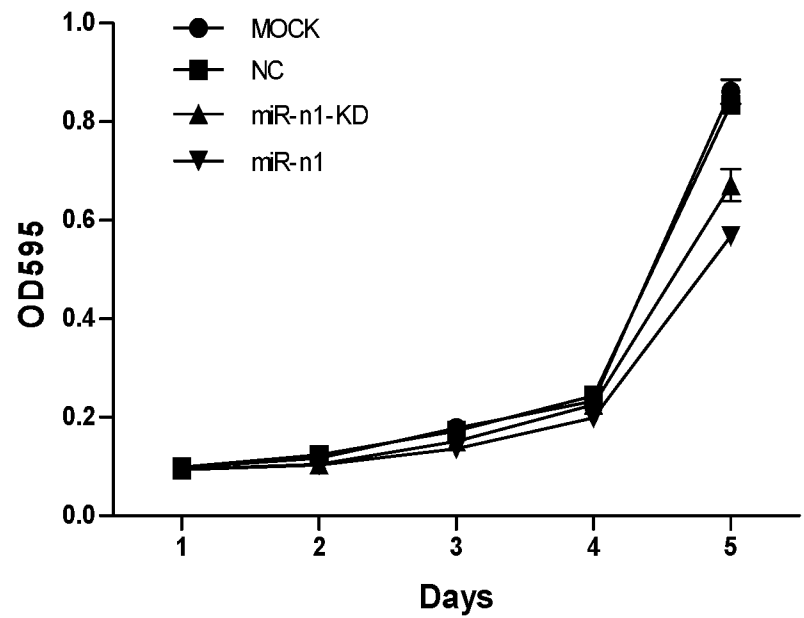
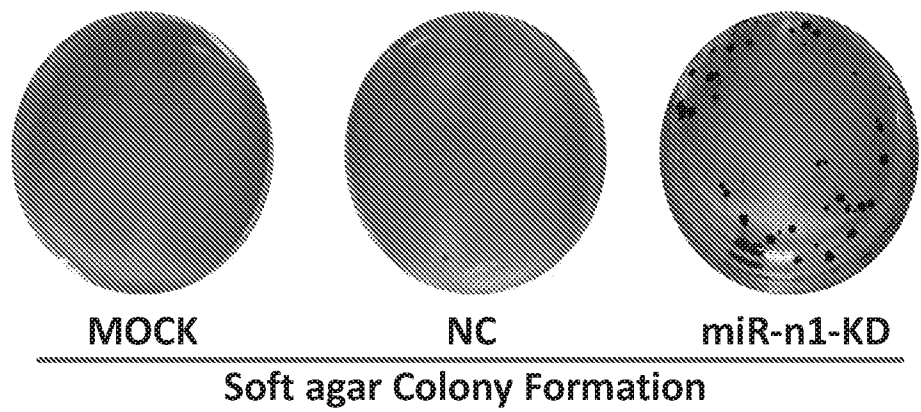
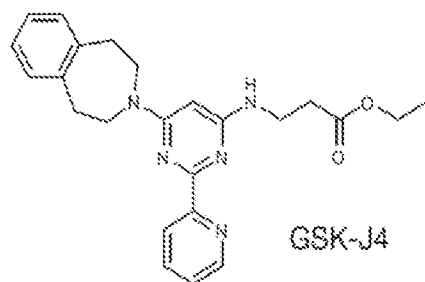


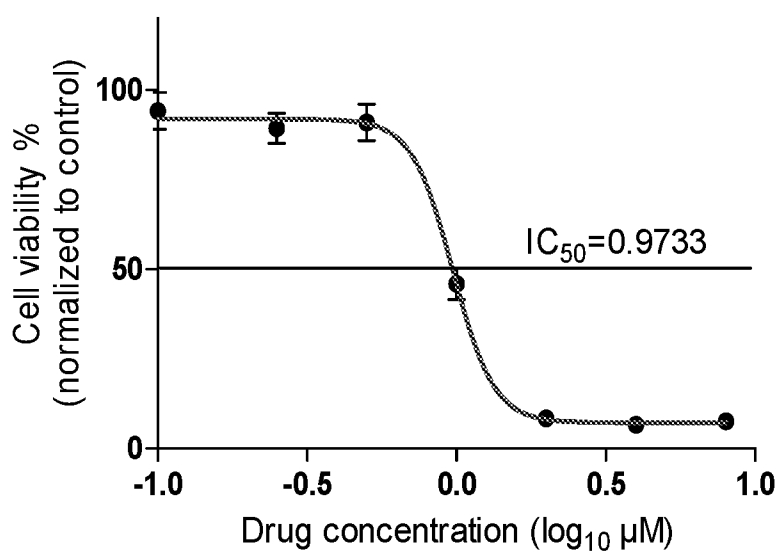
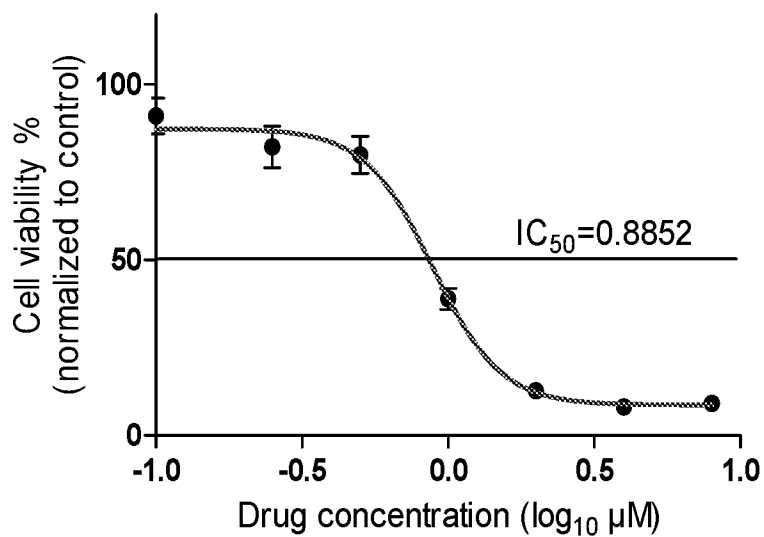
FIG. 4H



10/13

FIG. 5A**FIG. 5B**

LNCaP

**FIG. 5C**LNCaP^{RANKL}

11/13

FIG. 5D

C4-2B

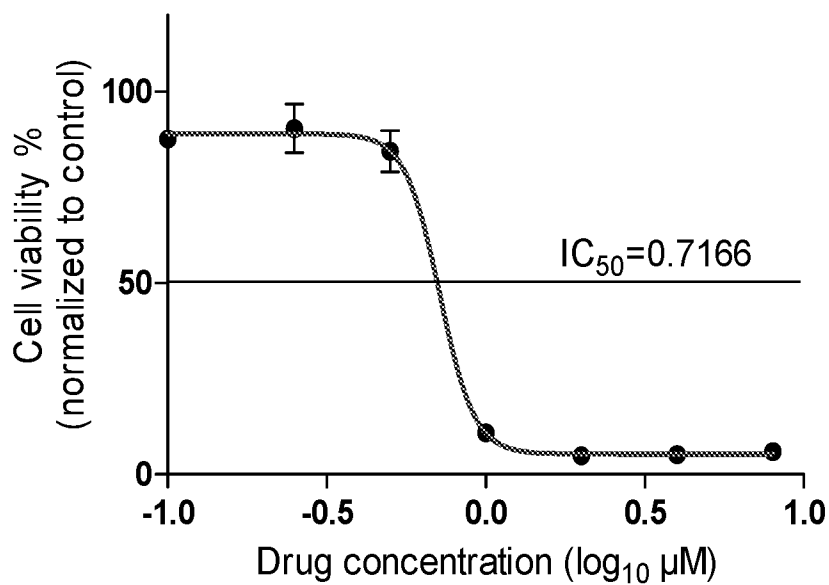


FIG. 5E

PC3

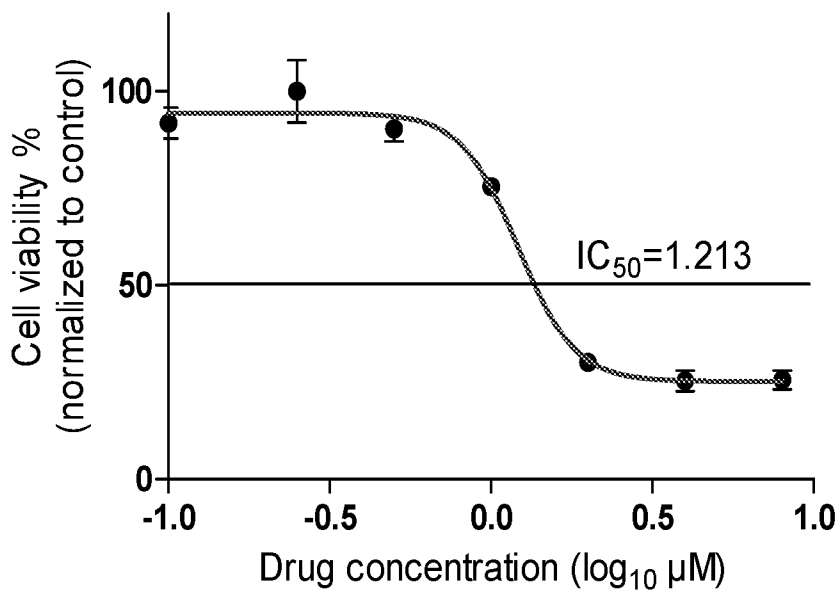


FIG. 5 F

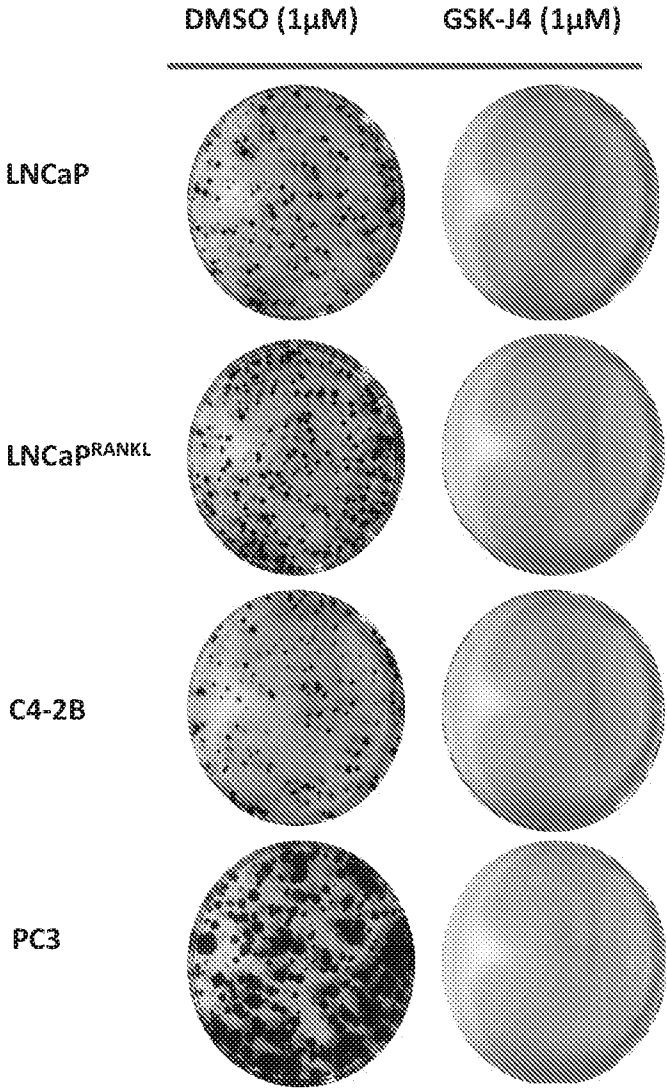
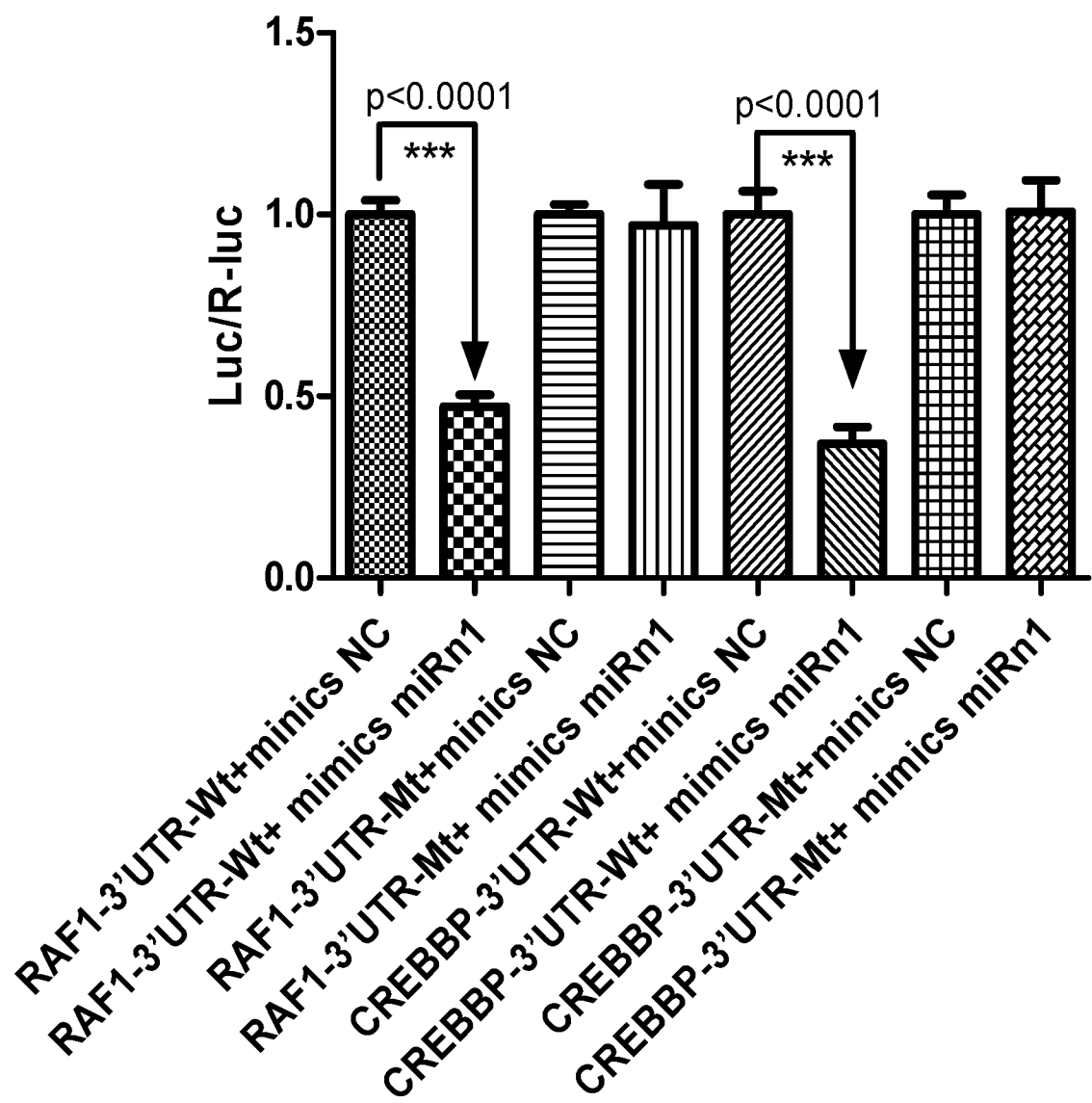


FIG. 6



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/31324

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/31324

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-20, drawn to methods for treating cancer; inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells; promoting cellular differentiation, inducing terminal differentiation of a cell, or inducing apoptosis of a cell; and determining whether a subject has cancer. The methods will be searched to the extent that the miRNA encompasses miR-n1. It is believed that claims 1-20, limited to miR-n1 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass methods wherein the miRNA encompasses miR-n1. Additional miRNA will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected miRNA(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be methods wherein the miRNA encompasses miR-n2, i.e. claims 1-20 limited to miR-n2.
*****Continued in Supplemental Box*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/31324

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7052, A61K 31/7105, A61P 35/00, C12N 15/11

CPC - C12N 15/113, C12N 2310/113, A61K 31/7088, C12N 2310/141

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2013/0018087 A1 (BOLL et al.) 17 January 2013 (17.01.2013); abstract, para [0027], [0028], [0095], [0311], [[0368]	1-20
A	US 2006/0183128 A1 (BERLIN et al.) 17 August 2006 (17.08.2006) para [0063]; SEQ ID NO: 205	1-20
A	US 2009/0170089 A1 (Lewin et al.) 2 July 2009 (02.07.2009) para [0064]; SEQ ID NO: 205	1-20
A	Genbank Submission JX944780 "Homo sapiens Unann-2 miRNA, complete sequence" (13 December 2012), [Retrieved on 11 September 2017], Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/JX944780 > Entire document	1-20
A	Genbank Submission AL954865 "Human DNA sequence from clone DAQB-314F7 on chromosome 6, complete sequence" (13 December 2012), [Retrieved on 11 September 2017], Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/AL954865 > Entire document	1-20
A	Genbank Submission CR936883 "Human DNA sequence from clone DAMC-4K22 on chromosome 6, complete sequence" (13 December 2012) [retrieved on 11 September 2017], Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/CR936883 > Entire document	1-20
A	Genbank Submission BX248109.8 "Human DNA sequence from clone DASS-381E5 on chromosome 6, complete sequence" (13 December 2012) [retrieved on 11 September 2017], Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/BX248109 > Entire document	1-20

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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Date of the actual completion of the international search

11 September 2017

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/31324

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Genbank Submission AL662871 "Human DNA sequence from clone CH501-322C17 on chromosome 6, complete sequence" (24 January 2013) [retrieved on 11 September 2017], Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/AL662871 > Entire document	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/31324

Continuation of Box No. III (Observations where unity of invention is lacking):

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the specific miRNA recited therein. Each invention requires a miRNA, not required by any of the other inventions.

Common Technical Features

The feature shared by the inventions listed as Group I+ is the methods of claims 1, 6, 10, 14.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2013/0018087 A1 to Boll et al. (hereinafter 'Boll').

Boll teaches [claim 1] a method of treating cancer in a subject in need thereof, comprising: administering a miRNA to the subject in need thereof to treat the cancer (abstract "The invention inter alia relates to new pharmaceutical compositions comprising miRNAs mi R-130a, mi R-203 and miR-205, and their use for the treatment of cancer, in particular prostate cancer"; claim 1 "A method for treating hormone-refractory prostate cancer comprising administering to a subject in need of said treating an effective amount of an isolated nucleic acid coding for a miRNA").

Boll teaches [claim 6] a method of inhibiting growth, proliferation, migration, invasion and/or metastasis of cancer cells comprising: administering a miRNA to a subject in need thereof to inhibit growth of cancer cells (para [0095] "Importantly, reconstitution of 130a, 203 and/or 205 in the LNCaP PCa cell line resulted in marked morphology change, growth arrest, and apoptosis. The inventors therefore propose that miR-130a, miR-203, and/or miR-205 act as tumor suppressor genes in prostate cancer by inter alia targeting androgen receptor transactivating proteins and negatively regulating cell survival").

Boll teaches [claim 10] a method of promoting cellular differentiation comprising: contacting an miRNA to the cell to promote cellular differentiation (abstract "The invention inter alia relates to new pharmaceutical compositions comprising miRNAs mi R-130a, mi R-203 and miR-205, and their use for the treatment of cancer, in particular prostate cancer.; para [0090] "MicroRNAs (miRNAs) are small non-coding RNAs that regulate expression of their targets either by translational repression, mRNA deadenylation or direct cleavage. miRNAs have crucial functions for basic cellular processes like differentiation or apoptosis, normal cellular development, but also play important roles in the pathogenesis, especially cancer").

Boll teaches [claim 14] a method, comprising:
subjecting a biological sample from a subject to analysis for miRNA expression level (para [0027] "Another object of the present invention relates to a method for detecting, grading and/or prognosing cancer, preferably prostate cancer, and most preferably hormone-refractory prostate cancer, comprising the step of determining in a sample from a subject the expression level(s) of miRNA 130a and/or miRNA 203 and/or miRNA 205");
comparing the miRNA expression level to a reference value (para [0028] "In a preferred embodiment, said method further comprises the step of comparing said expression level(s) determined in the sample from the subject to the expression level(s) of the respective compound(s) determined in a control"); and
determining the subject has cancer or cancer with metastatic potential if the miRNA expression level is lower than the reference value or determining that the subject does not have cancer if the miRNA expression level is not lower than the reference value (para [0368] "In a preferred embodiment, the control is a sample from a healthy subject. In a preferred embodiment a lower amount of miRNA 130a and/or miRNA 203 and/or miRNA 205 determined in the sample from the subject in comparison to the control indicates the presence of cancer in the subject").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

The inventions listed as Group I+ therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.