(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 September 2012 (13.09.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/122239 A1

(51) International Patent Classification:
A61K 31/7088 (2006.01) *C12N 5/10* (2006.01)

(21) International Application Number:
PCT/US2012/028016

(22) International Filing Date:
7 March 2012 (07.03.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/449,854 7 March 2011 (07.03.2011) US

(71) Applicant *(for all designated States except US)*: **THE OHIO STATE UNIVERSITY** [US/US]; 1524 North High Street, Columbus, OH 43201 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **CROCE, Carlo, M.** [US/US]; 2140 Cambridge Blvd., 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, CL, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2012/122239 A1

(54) Title: MUTATOR ACTIVITY INDUCED BY MICRORNA-155 (*miR-155*) LINKS INFLAMMATION AND CANCER

(57) Abstract: Methods of reducing spontaneous mutation rate of a cell in a subject in need thereof by reducing endogenous levels of miR-155 are described.

TITLE

Mutator Activity Induced by microRNA-155 *(miR-155)* Links Inflammation and Cancer

Inventor: Carlo M. Croce

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Application Number 61/449,854 filed March 7, 2011, the entire disclosure of which is expressly incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. CA123541, awarded by National Institutes of Health. The government has certain rights in this invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted via EFS-web and is hereby incorporated by reference in its entirety. The ASCII copy, created on March 6, 2012, is named 604_52873_SEQ_LIST_11137.txt, and is 34,668 bytes in size.

TECHNICAL FIELD AND INDUSTRIAL APPLICABILITY OF THE INVENTION

[0004] This invention relates generally to the field of molecular biology. Certain aspects of the invention include application in diagnostics, therapeutics, and prognostics of cancers and leukemias associated disorders.

BACKGROUND

[0005] There is no admission that the background art disclosed in this section legally constitutes prior art.

[0006] miRNAs repress gene expression by inhibiting mRNA translation or by promoting mRNA degradation and are considered to be master regulators of various processes, ranging from proliferation to apoptosis. Both loss and gain of miRNA function contribute to cancer development through the upregulation and silencing, respectively, of different target genes.

[0007] Chronic and persistent inflammation contributes to cancer development. Infection driven inflammation is involved in the pathogenesis of about 15-20% of human tumors. Tumor-infiltrating leukocytes, such as monocytes/macrophages, T lymphocytes, and neutrophils, are prime regulators of cancer inflammation. Furthermore, even tumors that are not epidemiologically linked to pathogens are characterized by the presence of an inflammatory component in their microenvironment.

SUMMARY

[0008] The present invention is based, at least in part, on the following information and discoveries, as described herein.

[0009] In a first broad aspect, there is provided herein a method for modulating WEE1 kinase expression levels in a target cell comprising: administering a microRNA-155 (miR-155) oligonucleotide to the target cell.

[0010] In another broad aspect, there is provided herein a method of modulating mutation of a target cell in a subject comprising: administering a miR-155 oligonucleotide to a target cell cells in the subject; and, measuring mutation of the target cell, wherein the target cell is a cancer cell or a precancerous cell.

[0011] In another broad aspect, there is provided herein a method of reducing spontaneous mutation rate of a cell in a subject in need thereof, comprising reducing endogenous levels of miR-155.

[0012] In another broad aspect, there is provided herein a method of reducing spontaneous mutation rate of an inflammation-related cancer cell in a subject in need thereof, comprising reducing endogenous levels of miR-155.

[0013] In another broad aspect, there is provided herein a method of slowing or inhibiting cell proliferation in a cancer cell or cancer cell population comprising: contacting the cell or cell population with a miR-155 antisense compound comprising a miR-155 oligonucleotide is complementary to a sequence at least 90% identical to mature microRNA-155, thereby slowing or inhibiting mutation of the cell or cell population.

[0014] In another broad aspect, there is provided herein a method of treating or preventing a miR-155 associated cancer, comprising: identifying a subject having, or suspected of having the miR-155 cancer; and, administering to the target cell a miR-155 oligonucleotide.

[0015] In another broad aspect, there is provided herein a method of treating or preventing an miR-155 associated -cancer comprising: identifying a subject having, or suspected of having the miR-155 associated cancer, and administering to the subject a miR-155 antisense compound comprising a miR-155 oligonucleotide having complementary at least 90% identical to mature microRNA-155.

[0016] In another broad aspect, there is provided herein a method of modulating the expression of one or more genes in a target cell, the genes being selected from: APC, adenomatous polyposis coli; *FADD,* Fas (TNFRSF6)-associated via death domain; FOXO3, forkhead box O3; KGF, keratinocyte growth factor; *HIVEP2,* HIV type I enhancerbinding protein *2;* MYO10, myosin X; RHOA, Ras homolog gene family, member A; *RIP1,* receptorinteracting protein kinase *1; SHIP1,* inositol polyphosphate-5-phosphatase; SMAD1/5, SMAD family member 1/5; SOCS1, suppressor of cytokine signaling *1; TP53INP,* Tumor protein 53-induced nuclear protein 1, comprising:

contacting the target cell with a miR-155 oligonucleotide.

[0017] In certain embodiments, the miR-155 oligonucleotide comprises an antisense miR-155 oligonucleotide.

[0018] In certain embodiments, the miR-155 oligonucleotide comprises a miR-155 antisense compound.

[0019] In certain embodiments, the miR-155 oligonucleotide comprises a miR-155 antagonist compound.

[0020] In certain embodiments, the miR-155 oligonucleotide is selected from the group consisting of a mature miR-155 oligonucleotide, a pre-miR-155 oligonucleotide, and a miR-155 seed sequence.

[0021] In certain embodiments, the miR-155 antisense compound comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to a sequence at least 80% identical to mature sequence of the modified oligonucleotide is complementary to a sequence at least 80% identical to mature miR-155, pre-miR-155, a miR-155 seed sequence, or a sequence fully complementary to the sequence of mature miR-155, pre-miR-155, or miR-155.

[0022] In certain embodiments, administering a miR-155 oligonucleotide comprises: administering an antisense miR-155 expression vector to a target cell; and expressing an antisense miR-155 in the target cell.

[0023] In certain embodiments, administering a miR-155 oligonucleotide comprises: administering a miR-155 expression vector to a target cell; and expressing a miR-155 in the target cell.

[0024] In certain embodiments, the miRNA-155 expression vector comprises a nucleic acid sequence encoding a miRNA-155 operably linked to a promoter.

[0025] In certain embodiments, the target cell is a cancer cell.

[0026] In certain embodiments, the target cell is a breast cancer or precancerous cell.

[0027] In certain embodiments, the target cell is a colon cancer or precancerous cell.

[0028] In certain embodiments, the target cell is a gastric cancer or precancerous cell

[0029] In certain embodiments, the target cell is a lung cancer or precancerous cell.

[0030] In certain embodiments, the modulation comprises decreasing expression of the one or more genes.

[0031] In certain embodiments, the modified oligonucleotide has no more than two mismatches to the nucleobase sequence of mature miR-155.

[0032] In certain embodiments, the modulation comprises decreasing expression of the one or more genes.

[0033] In certain embodiments, the method comprisesg contacting the cell with an antisense miR-155 inhibitory RNA (155-I).

[0034] In certain embodiments, the cell is contacted with the antisense miR-155 inhibitory RNA (155-I) in an amount sufficient to increase WEE1 levels.

[0035] In another broad aspect, there is provided herein a method of reducing spontaneous mutation rate of an inflammation-related cancer cell in a subject in need thereof, comprising contacting the cell with an antisense miR-155 inhibitory RNA (155-I).

[0036] In certain embodiments, the cell is contacted with the antisense miR-155 inhibitory RNA (155-I) in an amount sufficient to increase WEE1 levels.

[0037] In certain embodiments, the method comprises preventing the onset of an inflammatory-related cancer by modulating the up-regulation of miR-155 in a subject in need thereof.

[0038] In certain embodiments, the subject is human.

[0039] In another broad aspect, there is provided herein a composition useful for reducing spontaneous mutation rate of a cell in a subject in need thereof, comprising an antisense miR-155.

[0040] In another broad aspect, there is provided herein a method of identifying an agent that can be used to inhibit an inflammatory-related cancer comprising:

a) contacting miR-155 with an agent to be assessed;

b) contacting one or more target genes of miR-155 with an agent to be assessed; or

c) contacting a combination thereof, wherein if the agent inhibits expression of miR-155, enhances expression of the target genes, or performs a combination thereof, then the agent can be used to inhibit proliferation of the inflammatory-related cancer.

[0041] Other systems, methods, features, and advantages of the present invention will be or will become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present invention, and be protected by the accompanying claims.

BRIEF DESCRIPTION OF THE FIGURES

[0042] The patent or application file contains 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 Patent Office upon request and payment of the necessary fee.

[0043] **Figs. 1A-1B**. The average mutation rate in SW620 and MBA-MD-231 cells increases with the rate of miR-155 expression.

[0044] **Fig. 1A** and **Fig. 1B**. Average mutation rates of 6-TG–resistant colonies from SW620 (**Fig. 1A**) and MDA-MB-231 clones (**Fig. 1B**) stably expressing miR-155 and mock treated (Control) or treated with doxycycline (indicated clones) starting 48 h before 6-TG selection. *P = 0.065; **P < 0.006 (Student t tests).

[0045] **Figs. 2A-2D**. Proinflammatory environment increases the mutation rates in MDA-MB-

231 cells and the frequency of mutant colonies in T47D cells.

[0046] **Fig. 2A**. The relative up-regulation of miR-155 in the indicated cell lines either mock treated or treated with LSMCM for 48 h were determined using qRT-PCR. The figure gives the ratios LPS-stimulated/unstimulated. Values represent mean ± SD (n = 3).

[0047] **Fig. 2B**. The relative up-regulation of miR-155 in MDA-MB-231 cells treated as indicated was determined using qRT-PCR.

[0048] **Fig. 2C**. The mutation rates (MR) in MDA-MB-231 cells were estimated by calculating the slopes of the curves following mock and TNF/LPS treatment (n = 4 estimations of mutation frequency).

[0049] **Fig. 2D**. Ratios of 6-TG–resistant colonies in T47D cells treated with LSMCM for 48 h versus control mock-treated cells.

[0050] **Figs. 3A-3D**. Elevated miR-155 levels increase the rate of proliferation by targeting WEE1 transcripts.

[0051] **Fig. 3A**. Phenotypes of 6-TG-resistant HCT116 colonies following 48-h mock treatment (– doxycycline) or 48-h doxycycline treatment (+ doxycycline). HCT116 cells were transiently infected with pRetroX-Tight-PurmiR-155 and Tet-On constructs before doxycycline treatment.

[0052] **Fig. 3B**. Cells from MDA-MB-231 clone 19B were stained with CFSE before induction of miR-155 expression by doxycycline treatment. The proliferation rate was analyzed by flow cytometry 4 and 5 d later. The experiment was repeated two times with similar results.

[0053] **Fig. 3C**. The levels of WEE1 in T47D cells transfected with premiR-Control, premiR-155, or antisense miR-155 inhibitory RNA (155-I) and subsequently either mock treated or treated with LSMCM for 48 h were determined by Western blotting.

[0054] **Fig. 3D**. The levels of WEE1 in primary B cells isolated from the spleen of Eμ-miR-155 transgenic mice after transfection with premiR-Control or antisense miR-155 inhibitory RNA (155-I).

[0055] **Fig. 4**. The levels of microRNA-155 (miR-155) expression vary with the clones and the cell lines. SW620 and MDA-MB-231 clones stably transfected with a pRetroX-tight-Pur construct expressing mature miR-155 were mock treated or treated with doxycycline for 48 h. The relative levels of miR-155 were determined subsequently using quantitative RT-PCR. **Fig. 4** shows the ratios of the values for mock-treated/doxycycline-treated cells. Values represent mean ± SD (n = 3).

[0056] **Fig. 5**. Effects of increasing doses of doxycycline on the expression of miR-155. HCT116 cells were transiently transfected with a pRetroX-Tight-Pur construct expressing miR-155 precursor (premiR-155) or miR-155 mature form (miR-155) before 48-h treatment with the indicated doses of doxycycline (ng/mL).

[0057] **Fig. 6**. miR-155 targets the WEE1 3′ UTR. T47D cells transfected with a reporter construct containing the 3′ UTR of WEE1 downstream of the luciferase coding region were treated

with an unstimulated macrophage-conditioned medium (– LPS) or with LSMCM (+ LPS) or were transfected with premiR-Control or premiR-155. Results were normalized to Renilla luciferase. Values represent mean ± SD (n = 5).

[0058] **Fig. 7**. Schematic representation showing that the up-regulation of miR-155 over a prolonged period as a consequence of chronic inflammation or the deregulation of endogenous genetic circuitries in cancer or other diseases may lead to higher mutation rates in vivo. It was found that the targeting of WEE1 by miR-155 would further extend DNA damage. The up-regulation of miR-155 also down-regulates tumor-suppressor factors and other factors controlling cell homeostasis (**Fig. 10 - Table 2** and **Fig. 7**). Taken together, these effects can shorten the process of malignant transformation and favor cancer progression.

[0059] **Fig. 8**. Schematic representation summarizing the experimental design of **Fig. 2C**. HAT, 100 μM hypoxanthine, 400 nM aminopterin, 16 μM thymidine.

[0060] **Fig. 9**. Table 1 - Mutations found in HPRT cDNAs prepared from 6-thioguanine (6-TG)-resistant colonies of T47D, HCT116 and MDA-MB-231 cells.

[0061] **Fig. 10**. Table 2 - Transcripts encoding factors related to DNA replication and maintenance whose levels decrease significantly following treatment of T47D and MDA-MB-231 cells.

[0062] **Fig. 11.** Table S1 - Effects of miR-155 overexpression on the frequency of 6-thioguanine (6-TG)-resistant colonies and the average mutation rate. Mutations found in *HPRT* cDNAs prepared from 6-TG–resistant colonies of human HCT116 colon cancer cells and from human T47D and MDA-MB-231 breast cancer cells after exposure to LPS-stimulated macrophage-conditioned medium or doxycycline-induced overexpression of miR-155 microRNA.

[0063] **Fig. 12.** Table S2 – Mutations found in hypoxanthine phosphoribosyltransferase (HPRT) cDNAs prepared from 6-GT-resistant colonies of human HCT116 colon cancer cells and from human T47D and MDA-MB-231 breast cancer cells after exposure to LPS-stimulated macrophage-conditioned medium or doxycycline-induce overexpression of miR-155 microRNA. Mutations found in hypoxanthine phosphoribosyltransferase *(HPRT)* cDNA prepared from 6-TG–resistant colonies of human HCT116 colon cancer cells and from human T47D and MDA-MB-231 breast cancer cells after exposure to LPS-stimulated macrophage-conditioned medium (LSMCM) or doxycycline-induced overexpression of miR-155 microRNA. The length of *HPRT* transcribed region was 1,415 nt. The *HPRT* region analyzed was nucleotides 123–1,110. [a]Mock, unstimulated macrophage-conditioned medium. [b]LSMCM, LPS-stimulated macrophage-conditioned medium. [c]HPRT coding region, nucleotides 168–824.

[0064] **Fig.13**. Table S3 – Validated targets of miR-155 microRNA that play a role as tumor suppressors or regulators of cell homeostasis: APC, adenomatous polyposis coli; *BACH1,* BTB and CNC homology 1, basic leucine zipper transcription factor *1; CUTL1,* cut-like homeobox 1; *FADD,* Fas (TNFRSF6)-associated via death domain; *JARID2,* jumonji, AT-rich interactive domain 2;

FOXO3, forkhead box O3; KGF, keratinocyte growth factor; *HIVEP2,* HIV type I enhancer-binding protein *2;* MYO10, myosin X; RHOA, Ras homolog gene family, member A; *RIP1,* receptorinteracting protein kinase *1; SHIP1,* inositol polyphosphate-5-phosphatase; SMAD1/5, SMAD family member 1/5; SOCS1, suppressor of cytokine signaling *1; TP53INP,* Tumor protein 53-induced nuclear protein 1.

DETAILED DESCRIPTION

[0065] Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

[0066]The present invention provides research tools, diagnostic methods, and therapeutical methods and compositions using the knowledge derived from this discovery. The invention is industrially applicable for the purpose of sensitizing tumor cells to drug-inducing apoptosis and also to inhibit tumor cell survival, proliferation and invasive capabilities.

[0067] *Terms*

[0068] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not intended to limit the scope of the current teachings. In this application, the use of the singular includes the plural unless specifically stated otherwise. In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided.

[0069] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes V, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

[0070] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0071] Also, the use of "comprise", "contain", and "include", or modifications of those root words, for example but not limited to, "comprises", "contained", and "including", are not intended to be limiting. The term "and/or" means that the terms before and after can be taken together or separately. For illustration purposes, but not as a limitation, "X and/or Y" can mean "X" or "Y" or "X and Y".

[0072] It is understood that a miRNA is derived from genomic sequences or a gene. In this respect, the term "gene" is used for simplicity to refer to the genomic sequence encoding the

precursor miRNA for a given miRNA. However, embodiments of the invention may involve genomic sequences of a miRNA that are involved in its expression, such as a promoter or other regulatory sequences.

[0073] The terms "miR," "mir" and "miRNA" generally refer to microRNA, a class of small RNA molecules that are capable of modulating RNA translation (see, Zeng and Cullen, RNA, 9(1):112-123, 2003; Kidner and Martienssen Trends Genet, 19(1):13-6, 2003; Dennis C, Nature, 420(6917):732, 2002; Couzin J, Science 298(5602):2296-7, 2002, each of which is incorporated by reference herein).

[0074] MiRNA nucleic acid" generally refers to RNA or DNA that encodes a miR as defined above, or is complementary to a nucleic acid sequence encoding a miR, or hybridizes to such RNA or DNA and remains stably bound to it under appropriate stringency conditions. Particularly included are genomic DNA, cDNA, mRNA, miRNA and antisense molecules, pri-miRNA, pre-miRNA, mature miRNA, miRNA seed sequence; also included are nucleic acids based on alternative backbones or including alternative bases. MiRNA nucleic acids can be derived from natural sources or synthesized.

[0075] The term "miRNA" generally refers to a single-stranded molecule, but in specific embodiments, molecules implemented in the invention will also encompass a region or an additional strand that is partially (between 10 and 50% complementary across length of strand), substantially (greater than 50% but less than 100% complementary across length of strand) or fully complementary to another region of the same single-stranded molecule or to another nucleic acid. Thus, nucleic acids may encompass a molecule that comprises one or more complementary or self-complementary strand(s) or "complement(s)" of a particular sequence comprising a molecule. For example, precursor miRNA may have a self-complementary region, which is up to 100% complementary miRNA probes of the invention can be or be at least 60, 65, 70, 75, 80, 85, 90, 95, or 100% complementary to their target.

[0076] MicroRNAs are generally 21-23 nucleotides in length. MicroRNAs are processed from primary transcripts known as pri-miRNA to short stem-loop structures called precursor (pre)-miRNA and finally to functional, mature microRNA. Mature microRNA molecules are partially complementary to one or more messenger RNA molecules, and their primary function is to down-regulate gene expression. MicroRNAs regulate gene expression through the RNAi pathway.

[0077] "MicroRNA seed sequence," "miRNA seed sequence," "seed region" and "seed portion" are used to refer to nucleotides 2-7 or 2-8 of the mature miRNA sequence. The miRNA seed sequence is typically located at the 5' end of the miRNA.

[0078] The terms “miRNA-155” and “miR-155” are used interchangeably and, unless otherwise indicated, refer to microRNA-155, including miR-155, pri-miR-155, pre-miR-155, mature miR-155, miRNA-155 seed sequence, sequences comprising a miRNA-155 seed sequence, and variants thereof.

[0079] The terms “low miR- expression” and “high miR- expression” are relative terms that refer to the level of miR/s found in a sample. In some embodiments, low and high miR- expression are determined by comparison of miR/s levels in a group of cancerous samples and control or non-cancerous samples. Low and high expression can then be assigned to each sample based on whether the expression of a miR in a sample is above (high) or below (low) the average or median miR expression level. For individual samples, high or low miR expression can be determined by comparison of the sample to a control or reference sample known to have high or low expression, or by comparison to a standard value. Low and high miR expression can include expression of either the precursor or mature forms of miR, or both.

[0080] The term "expression vector" generally refers to a nucleic acid construct that can be generated recombinantly or synthetically. An expression vector generally includes a series of specified nucleic acid elements that enable transcription of a particular gene in a host cell. Generally, the gene expression is placed under the control of certain regulatory elements, such as constitutive or inducible promoters.

[0081] The term "operably linked" is used to describe the connection between regulatory elements and a gene or its coding region. That is, gene expression is typically placed under the control of certain regulatory elements, for example, without limitation, constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. A gene or coding region is the to be "operably linked to" or "operatively linked to" or "operably associated with" the regulatory elements, meaning that the gene or coding region is controlled or influenced by the regulatory element.

[0082] The term "combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, ACB, CBA, BCA, BAC, or CAB.

[0083] The terms “anticancer agent” and “anticancer drug” generally refer to any therapeutic agents (e.g., chemotherapeutic compounds and/or molecular therapeutic compounds), antisense therapies, radiation therapies, or surgical interventions, used in the treatment of hyperproliferative disease.

[0084] The term “adjunctive therapy” generally refers to a treatment used in combination with a primary treatment to improve the effects of the primary treatment.

[0085] The term “clinical outcome” generally refers to the health status of a subject following treatment for a disease or disorder, or in the absence of treatment. Clinical outcomes include, but are not limited to, an increase in the length of time until death, a decrease in the length of time until death, an increase in the chance of survival, an increase in the risk of death, survival, disease-free survival, chronic disease, metastasis, advanced or aggressive disease, disease recurrence, death, and favorable or poor response to therapy.

[0086] The term "control" generally refers to a sample or standard used for comparison with an experimental sample, such as a tumor sample obtained from a subject. In some embodiments, the control is a sample obtained from a healthy subject or a non-cancerous sample obtained from a subject diagnosed. In some embodiments, the control is non-cancerous cell/tissue sample obtained from the same subject. In some embodiments, the control is a historical control or standard value (i.e., a previously tested control sample or group of samples that represent baseline or normal values, such as the level in a non-cancerous sample). In other embodiments, the control is a sample obtained from a healthy subject, such as a donor. Cancerous samples and non-cancerous tissue samples can be obtained according to any method known in the art.

[0087] The term "cytokines" generally refers to proteins produced by a wide variety of hematopoietic and non-hematopoietic cells that affect the behavior of other cells. Cytokines are important for both the innate and adaptive immune responses.

[0088] The term "decrease in survival" generally refers to a decrease in the length of time before death of a subject, or an increase in the risk of death for the subject.

[0089] The term "detecting the level of miR expression" generally refers to quantifying the amount of such miR present in a sample. Detecting expression of a miR, or any microRNA, can be achieved using any method known in the art or described herein, such as by qRT-PCR. Detecting expression of a miR includes detecting expression of either a mature form of the miR or a precursor form that is correlated with the miR expression. For example, miRNA detection methods involve sequence specific detection, such as by RT-PCR. miR-specific primers and probes can be designed using the precursor and mature miR nucleic acid sequences, which are known in the art and include modifications which do not change the function of the sequences.

[0090] The term "normal cell" generally refers to a cell that is not undergoing abnormal growth or division. Normal cells are non-cancerous and are not part of any hyperproliferative disease or disorder.

[0091] The term "anti-neoplastic agent" generally refers to any compound that retards the proliferation, growth, or spread of a targeted (e.g., malignant) neoplasm.

[0092] The terms "prevent," "preventing" and "prevention" generally refer to a decrease in the occurrence of pathological cells (e.g., hyperproliferative or neoplastic cells) in an animal. The prevention may be complete, e.g., the total absence of pathological cells in a subject. The prevention may also be partial, such that the occurrence of pathological cells in a subject is less than that which would have occurred without the present invention. "Preventing" a disease generally refers to inhibiting the full development of a disease.

[0093] The terms "treating" and/or "ameliorating a disease" generally refer to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. "Ameliorating" generally refers to the reduction in the number or severity of signs or symptoms of a disease.

[0094] The term "subject" includes human and non-human animals. The preferred subject for treatment is a human. "Subject" and "subject" are used interchangeably herein.

[0095] The term "therapeutic" generally is a generic term that includes both diagnosis and treatment.

[0096] The term "therapeutic agent" generally refers to a chemical compound, small molecule, or other composition, such as an antisense compound, protein, peptide, small molecule, nucleic acid. antibody, protease inhibitor, hormone, chemokine or cytokine, capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject. For example, therapeutic agents include agents that prevent or inhibit development or metastasis. As used herein, a "candidate agent" is a compound selected for screening to determine if it can function as a therapeutic agent. "Incubating" includes a sufficient amount of time for an agent to interact with a cell or tissue. "Contacting" includes incubating an agent in solid or in liquid form with a cell or tissue. "Treating" a cell or tissue with an agent includes contacting or incubating the agent with the cell or tissue.

[0097] The term "therapeutically effective amount" generally refers to that amount of the therapeutic agent sufficient to result in amelioration of one or more symptoms of a disorder, or prevent advancement of a disorder, or cause regression of the disorder. For example, with respect to the treatment of cancer, in one embodiment, a therapeutically effective amount will refer to the amount of a therapeutic agent that decreases the rate of tumor growth, decreases tumor mass, decreases the number of metastases, increases time to tumor progression, or increases survival time by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%.

[0098] A "therapeutically effective amount" can be a quantity of a specified pharmaceutical or therapeutic agent sufficient to achieve a desired effect in a subject, or in a cell, being treated with the agent. For example, this can be the amount of a therapeutic agent that alters the expression of miR/s, and thereby prevents, treats or ameliorates the disease or disorder in a subject. The effective amount of the agent will be dependent on several factors, including, but not limited to the subject or cells being treated, and the manner of administration of the therapeutic composition.

[0099] The term "pharmaceutically acceptable vehicles" generally refers to such pharmaceutically acceptable carriers (vehicles) as would be generally used. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds, molecules or agents. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a

vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

[00100] The term "pharmaceutically acceptable salt" generally refers to any salt (e.g., obtained by reaction with an acid or a base) of a compound of the present invention that is physiologically tolerated in the target animal (e.g., a mammal). Salts of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, sulfonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Examples of bases include, but are not limited to, alkali metal (e.g., sodium) hydroxides, alkaline earth metal (e.g., magnesium) hydroxides, ammonia, and the like. Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, chloride, bromide, iodide, 2-hydroxyethanesulfonate, lactate, maleate, mesylate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like. For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound

[00101] *miR-155 Nucleic Acid Molecules*

[00102] Nucleic acid molecules that encode miR-155 are used in various embodiments of the present invention. miR-155 sequences for mature miR-155, pre-miR-155 can be used in some embodiments. In other embodiments cDNAs encoding mature miR-155 and pre-miR-155 can be used. Nucleic acid molecules encoding pri-miR-155 can also be used in some embodiments. A miRNA sequence may comprise from about 6 to about 99 or more nucleotides. In some embodiments, a miRNA sequence comprises about the first 6 to about the first 22 nucleotides of a pre-miRNA-155. Isolated or purified polynucleotides having at least 6 nucleotides (i.e., a

hybridizable portion) of a miR-155 coding sequence or its complement are used in some embodiments. In other embodiments, miR-155 polynucleotides preferably comprise at least 22 (continuous) nucleotides, or a full-length miR-155 coding sequence.

[00103] In some embodiments, nucleic acids are used that are capable of blocking the activity of a miRNA (anti-miRNA or anti-miR). Such nucleic acids include, for example, antisense miR-155. For example, a "miR-155 antagonist" means an agent designed to interfere with or inhibit the activity of miRNA-155.

[00104] In certain embodiments, the miR -155 antagonist can be comprised of an antisense compound targeted to a miRNA. For example, the miR-155 antagonist comprises can be comprised of a small molecule, or the like that interferes with or inhibits the activity of an miRNA.

[00105] In certain embodiments, the miR -155 antagonist can be comprised of a modified oligonucleotide having a nucleobase sequence that is complementary to the nucleobase sequence of a miRNA, or a precursor thereof.

[00106] In certain embodiments, the anti-miR is an antisense miR-155 nucleic acid comprising a total of about 5 to about 100 or more, more preferably about 10 to about 60 nucleotides, and has a sequence that is preferably complementary to at least the seed region of miR-155. In particularly preferred embodiments, an anti-miRNA may comprise a total of at least about 5, to about 26 nucleotides. In some embodiments, the sequence of the anti-miRNA can comprise at least 5 nucleotides that are substantially complementary to the 5' region of a miR-155, at least 5 nucleotides that are substantially complementary to the 3' region of a miR-155, at least 4-7 nucleotides that are substantially complementary to a miR-155 seed sequence, or at least 5-12 nucleotide that are substantially complementary to the flanking regions of a miR-155 seed sequence.

[00107] In some embodiments, an anti-miR-155 comprises the complement of a sequence of a miRNA. In other embodiments an anti-miR-155 comprises the complement of the seed sequence or is able to hybridize under stringent conditions to the seed sequence. Preferred molecules are those that are able to hybridize under stringent conditions to the complement of a cDNA encoding a mature miR-155.

[00108] It is to be understood that the methods described herein are not limited by the source of the miR-155 or anti-miR-155. The miR-155 can be from a human or non-human mammal, derived from any recombinant source, synthesized in vitro or by chemical synthesis. The nucleotide may be DNA or RNA and may exist in a double-stranded, single-stranded or partially double-stranded form, depending on the particular context. miR-155 and anti-miR-155 nucleic acids may be prepared by any conventional means typically used to prepare nucleic acids in large quantity. For example, nucleic acids may be chemically synthesized using commercially available reagents and synthesizers by methods that are well-known in the art and/or using automated synthesis methods.

[00109] It is also be understood that the methods described herein are not limited to naturally occurring miR-155 sequences; rather, mutants and variants of miR-155 sequences are also within the

contemplated scope. For example, nucleotide sequences that encode a mutant of a miR-155 that is a miR-155 with one or more substitutions, additions and/or deletions, and fragments of miR-155 as well as truncated versions of miR-155 maybe also be useful in the methods described herein.

[00110] It is also to be understood that, in certain embodiments, in order to increase the stability and/or optimize the delivery of the sense or antisense oligonucleotides, modified nucleotides or backbone modifications can be used. In some embodiments, a miR-155 or anti-miR-155 oligonucleotide can be modified to enhance delivery to target cells. Nucleic acid molecules encoding miR-155 and anti-miR-155 can be used in some embodiments to modulate function, activity and/or proliferation of immune cells.

[00111] *miR-155 Expression Vectors*

[00112] Expression vectors that contain a miR-155 or anti-miR-155 coding sequence can be used to deliver a miR-155 or anti-miR155 to target cells. In certain embodiments, expression vectors can contain a miR-155 sequence and/or anti-miR-155 sequence, optionally associated with a regulatory element that directs the expression of the coding sequence in a target cell. It is to be understood that the selection of particular vectors and/or expression control sequences to which the encoding sequence is operably linked generally depends (as is understood by those skilled in the art) on the particular functional properties desired; for example, the host cell to be transformed.

[00113] It is also to be understood that vectors useful with the methods described herein are preferably capable of directing replication in an appropriate host and of expression of a miR-155 or anti-miR-155 in a target cell.

[00114] It is also to be understood that a useful vector can include a selection gene whose expression confers a detectable marker such as a drug resistance. Non-limiting examples of selection genes include those vectors that encode proteins that confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients withheld from the media. It is also to be understood that the detectable marker can optionally be present on a separate plasmid and introduced by co-transfection.

[00115] It is also to be understood that expression control elements can be used to regulate the expression of an operably linked coding sequence. Non-limiting examples include: inducible promoters, constitutive promoters, enhancers, and other regulatory elements. In some embodiments an inducible promoter is used that is readily controlled, such as being responsive to a nutrient in the target cell's medium. In some embodiments, the promoter is the U6 promoter or CMV promoter. It is also to be understood that other methods, vectors, and target cells suitable for adaptation to the expression of miR-155 in target cells can be readily adapted to the specific circumstances.

[00116] *Delivery of Oligonucleotides and Expression Vectors to a Target Cell or Tissue*

[00117] In certain embodiments, a miR-155 or anti-miR-155 oligonucleotide is delivered to a target cell. In other embodiments, an expression vector encoding a miR-155 or anti-miR-155 is delivered to a target cell where the miR-155 or anti-miR-155 is expressed. It is to be understood

that different methods for delivery of oligonucleotides and expression vectors to target cells can be used.

[00118] In certain embodiments, the target cells may be present in a host, such as in a mammal, or may be in culture outside of a host. Thus, the delivery of miR-155 or anti-miR-155 to target cells in vivo, ex vivo and in vitro can accomplished in a suitable manner. In certain embodiments, a miR-155 or anti-miR-155 oligonucleotide is delivered to a target organ or tissue. Target organs and tissues may include locations where cancer cells or precursors of such cells are known to be located and may include, for example, solid cancers such as breast, colon, gastric and lung cancers.

[00119] In certain embodiments, cell development, function, proliferation and/or activity is modulated by delivering miR-155 or anti miR-155.

[00120] In certain embodiments, the mutation of a cell can be modulated (e.g., suppressed) by administering a miRNA-155 or anti-miR-155 oligonucleotide to the B cells. The numbers and/or activity of the cells can be modulated by administering a miRNA-155 or anti-miR-155 oligonucleotide to the cancer cells or to pre-cancerous cells.

[00121] In certain embodiments, the immune function and/or development of the cells can be modulated by delivering miR-155 or anti-miR-155 to the cells.

[00122] It is to be understood that the delivery of oligonucleotides and/or expression vectors to a target cell can be accomplished using different methods. In certain embodiments, a transfection agent can be used. In general, a transfection agent (e.g., a transfection reagent and/or delivery vehicle) can be a compound or compounds that bind(s) to or complex(es) with oligonucleotides and polynucleotides, and enhances their entry into cells. Non-limiting examples of useful transfection reagents include: cationic liposomes and lipids, polyamines, calcium phosphate precipitates, polycations, histone proteins, polyethylenimine, polylysine, and polyampholyte complexes. Another delivery method can include electroporating miRNA/s into a cell without inducing significant cell death. In addition, miRNAs can be transfected at different concentrations.

[00123] Non-limiting examples of useful reagents for delivery of miRNA, anti-miRNA and expression vectors include: protein and polymer complexes (polyplexes), lipids and liposomes (lipoplexes), combinations of polymers and lipids (lipopolyplexes), and multilayered and recharged particles. Transfection agents may also condense nucleic acids. Transfection agents may also be used to associate functional groups with a polynucleotide. Functional groups can include cell targeting moieties, cell receptor ligands, nuclear localization signals, compounds that enhance release of contents from endosomes or other intracellular vesicles (such as membrane active compounds), and other compounds that alter the behavior or interactions of the compound or complex to which they are attached (interaction modifiers).

[00124] In certain embodiments, miR-155 or anti-miR-155 nucleic acids and a transfection reagent can be delivered systematically such as by injection. In other embodiments, they may be injected into particular areas comprising target cells, such as particular organs, for example a solid

cancer tissue. The skilled artisan will be able to select and use an appropriate system for delivering miRNA-155, anti-miRNA-155 or an expression vector to target cells in vivo, ex vivo and/or in vitro without undue experimentation.

[00125] *General Description*

[00126] Described herein are the effects of *miR-155* overexpression and proinflammatory environment on the frequency of spontaneous hypoxanthine phosphoribosyltransferase *(HPRT)* mutations that can be detected based on the resistance to 6-thioguanine (6-TG). Both *miR-155* overexpression and inflammatory environment increased the frequency of *HPRT* mutations and down-regulated WEE1, a kinase that blocks cell-cycle progression. The increased frequency of *HPRT* mutation was only modestly attributable to defects in mismatch repair machinery. This result shows that *miR-155* enhances the mutation rate by simultaneously targeting different genes that suppress mutations and decreasing the efficiency of DNA safeguard mechanisms by targeting of cell-cycle regulators such as WEE1.

[00127] By simultaneously targeting tumor suppressor genes and inducing a mutator phenotype, *miR-155* allows the selection of gene alterations required for tumor development and progression. The drugs reducing endogenous *miR-155* levels are thus useful in the treatment of inflammation-related cancers.

[00128] Described herein are results showing that the mutator activity of *miR-155* and that of the miR-155-linked inflammatory environment are key mechanisms connecting inflammation and cancer. Also described herein are results that show that *miR-155* and inflammatory stimuli increase the spontaneous mutation rate.

[00129] Also provided are methods to treat suppress mutation rates in subject in need of such treatment, comprising administering a pharmaceutically-effective amount of a miR-155 composition herein.

[00130] Also provided are methods to treat cancer in a subject in need of such treatment, comprising administering a pharmaceutically-effective amount of an anti-sense miRNA, wherein the antisense miRNA is antisense to miRNA-155.

[00131] Also provided are methods for inducing apoptosis of rapidly mutating cells, comprising introducing an apoptosis-effective amount of a composition as described herein. In certain embodiments, the method comprises introducing an apoptosis-effective amount of an anti-sense miRNA, wherein the antisense miRNA is antisense to miR-155.

[00132] Also provided are methods for identifying pharmaceutically-useful compositions, comprising: introducing an anti-sense miRNA-155 to a cell culture; introducing a test composition to the cell culture; and identifying test compositions which induce apoptosis as pharmaceutically-useful compositions.

[00133] The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be

understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

[00134] *EXAMPLE I*

[00135] *Results*

[00136] *Overexpression of MiR-155 Results in Enhanced Mutation Rate.*

[00137] Human miR-155 resides in the non-coding BIC transcript (EMBL:AF402776), located on chromosome 21. miR-155 targets core components of the DNA mismatch repair (MMR) machinery, among other mutator pathways, suggesting that elevated levels of miR-155 might enhance the rate of spontaneous mutations.

[00138] To measure the mutation rate, hypoxanthine phosphoribosyltransferase (HPRT) locus was used as the method for estimating mutation rate. The HPRT enzyme catalyzes the conversion of guanine into guanine monophosphate and hypoxanthine into inosine monophosphate in the purine salvage pathway.

[00139] The loss of HPRT function confers resistance to 6-thioguanine (6-TG), because 6-TG becomes cytotoxic only after phosphoribosylation by HPRT. This resistance can be used to identify cells that have acquired mutations at the HPRT locus. Because the acquired mutations are thought to occur randomly, the HPRT gene can be used as a reporter gene, and the frequency of mutation at the HPRT locus can be used as an estimate of global genomic instability. To measure the effects of miR-155 on mutation rate, the inventor first developed stable clones of SW620 colorectal adenocarcinoma cells and MDA-MB-231 breast adenocarcinoma cells expressing mature miR-155 under the control of the Tet-On inducible system. Incubation of SW620 clones 8A, 22C, and 23A with doxycycline increased miR-155 expression by 2.94 ± 0.23-, 5.58 ± 0.43-, and 8.10 ± 0.65-fold, respectively (mean ± SD) (**Fig. 4** and **Fig. 11 - Table S1**).

[00140] Similarly, doxycycline treatment increased miR-155 expression by 12.01 ± 2.34-, 26.42 ± 1.11-, and 32.07 ± 3.27-fold in MDA-MB-231 clones 9C, 2B, and 19B, respectively. The cell growth-adjusted HPRT mutation rate, estimated based on a modified version of fluctuation analysis, increased with miR-155 levels in both SW620 and MDA-MB-231 cell clones (**Fig. 1A** and **Fig. 1B**). Constant elevated expression of miR-155 the enhanced mutation rate by up to 3.39-fold in SW620 clones and up to 3.47-fold in MDA-MB-231 clones (**Fig. 11 - Table S1**).

[00141] Moreover, HCT116 colorectal carcinoma cells transiently overexpressing miR-155 by 19.57 ± 0.62-fold under the control of Tet-On inducible system (**Fig. 5**) showed a 2.81-fold higher mutation rate (**Fig. 11 - Table S1**). These results establish a direct link between mutation rate and miR-155 levels.

[00142] The basal spontaneous cell growth-adjusted mutation rates of SW620 and MDA-MB-

231 cells was comparable ($0.75 \pm 0.27 \times 10^{-7}$ and 1.28×10^{-7} mutations per cell, respectively) and were ~440-fold lower than the spontaneous mutation rate of HCT116 cells (560×10^{-7} mutations per cell) (**Fig. 11 - Table S1**), because HCT116 cells contain a deletion of the hMLH1 MMR gene. The deletion of the hMLH1 MMR gene decreased the 155-induced mutator activity only partially, showing that miR-155-induced mutator activity is not very sensitive to the basal level of mutation rate (i.e., to the integrity of the DNA safeguarding machinery) and that miR-155 targets additional transcripts implicated in DNA repair and/or genome stability.

[00143] *Inflammatory Stimuli Up-Regulate miR-155 in Breast Cancer Cells*.

[00144] Human cell lines were screened for the effects of proinflammatory environment on miR-155 expression. Colon cancer cell lines (SW480, SW620, HCT15, HCT116, and RKO) were included because a fraction of colorectal cancers appear linked to the inflammatory environment. Breast (MDA-MB-231, T47D, 453, 436, and MCF7) and lung cancer (A459) cell lines were included because miR-155 is up-regulated in these types of cancers, and four other cell lines were included for comparison.

[00145] Cells were treated overnight with the supernatant of LPS stimulated human THP-1 monocytic cells, namely LPS-stimulated macrophage-conditioned medium (LSMCM), which contains many inflammatory cytokines such as TNF, IL-6, IL-8, and IL1-β. Based on quantitative RT-PCR (qRT-PCR) analyses, miR-155 expression in colon and lung cancer cell lines was affected only slightly by LSMCM (**Fig. 2A**).

[00146] In contrast, miR-155 levels increased by 9-, 17-, and 21-fold in MDA-MB-231, BC-453, and T47D breast cancer cell lines, respectively. The inventor also analyzed the expression of miR-155a, a microRNA that is up-regulated in certain tumors and in LPS-challenged THP-1 cells, since it is now believed by the inventor herein that miR-155a controls the termination of the immune response. In sharp contrast with miR-155, the highest miR-155a levels were found in HCT15 and HCT116 colon cell lines (not shown), indicating that the up-regulation of miR-155 and miR-155a by inflammatory stimuli occurs independently in the above cancer cell lines and likely is tissue specific.

[00147] Because both TNF and LPS can induce miR-155, the inventor analyzed the effects of these two molecules in MDA-MB-231 cells. It was found that stimulation with either TNF or LPS or with both increased miR-155 expression (**Fig. 2B**). The inventor therefore used TNF/ LPS to mimic the effects of a proinflammatory environment.

[00148] *Inflammatory Stimuli Enhance the Mutation Rate*.

[00149] In MDA-MB-231 cells without stimulation, the mutation rate was calculated to be 0.69×10^{-7} mutations per cell per generation, a value similar to that previously found in SW480 cells (0.75×10^{-7}). The estimated mutation rate was based on the average mutant frequency and population doubling. Of note, both MDAMB-231 and SW480 cells have intact DNA-repair machinery, unlike HCT116 cells. Mutant frequencies already had became significantly different ($P = 0.038$) 3 d after treatment, with mutation rate increasing by 2.52-fold to 1.73×10^{-7} mutations per

cell per generation (**Fig. 2C**) in TNF/LPS-treated MDA-MB-231 cells vs. untreated control cells. The treatment lowered the rate of cell proliferation, likely because TNF induces growth arrest in breast cancer cells. Accordingly, one or two LSMCM stimulations of T47D cells increased the frequency of 6-TG-resistant colonies by 50% and 150%, respectively (**Fig. 2D**).

[00150] Thus, proinflammatory signals resulting in the up-regulation of miR-155 expression induce a significant, although moderate, mutator phenotype, which might be enhanced with chronic inflammation.

[00151] *Characterization of HPRT Mutants*.

[00152] The inventor then analyzed HPRT mutations found in cDNAs prepared from RNA extracted from T47D, HCT116, and MDA-MB-231 6-TG–resistant colonies to determine the mutation signature (**Fig. 11 - Table S1** and **Fig. 12 - Table S2**).

[00153] HPRT mutations from doxycycline-treated HCT116 cells displayed single base deletions or insertions of the type generally found in DNA MMR-deficient cells. The majority displayed a frameshift, transition, and transversion mutation signature consistent with an MMR defect. There also was an increase in insertions and exon deletions with miR-155 overexpression; this increase generally has been ascribed to altered recombination repair. In contrast, deletion mutations consistent with recombination repair defects accounted for the majority of HPRT mutations in LSMCM-stimulated T47D cells and doxycycline treated MDA-MB-231 cells, regardless of conditions (**Fig. 11 - Table S1** and **Fig. 12 - Table S2**). These results are consistent with a role for recombination repair, exemplified by breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) mutations, in these breast tumor cell lines. Of note, these types of mutations have been found previously in several T-cell leukemic cell lines. However, the inventor noted a modest increase in transitions and transversions consistent with decreased MMR in these cells.

[00154] *Overexpression of miR-155 Enhances Cell Proliferation.*

[00155] Remarkably, miR-155 up-regulation increased the size of HCT116 (**Fig. 3A**) and MDA-MB-231 HPRT mutant colonies and allowed them to appear earlier during the selection process. Based on a forward scatter comparison, larger colonies of MDA-MB-231 clone 19B, that presented a 32-fold up-regulation of miR-155 after doxycycline treatment, did not arise from the presence of larger cells (not shown).

[00156] In contrast, carboxyfluorescein succinimidyl ester (CFSE) staining suggested that these cells underwent at least one extra round of cell division within 4–5 d as compared with untreated cells (**Fig. 3B**). These results correlate with reports showing that miR-155 promotes proliferation in transgenic mice. Thus, the larger size of HPRT mutant colonies overexpressing miR-155 probably arises from enhanced cell proliferation.

[00157] *MiR-155 and Inflammatory Environment Down-Regulate WEE1, a Cell-Cycle Inhibitor*.

[00158] While not wishing to be bound by theory, the inventor herein now believes that miR-155 enhances cell proliferation by targeting cell-cycle regulators. Indeed, in T47D cells, both

LSMCM treatment and miR-155 overexpression reduced the levels of WEE1, a kinase that catalyzes the inhibitory tyrosine phosphorylation of Cdc2/cyclin B, blocking cell-cycle progression at the G2/M phase (**Fig. 3C**). In contrast, an antisense miR-155 inhibitory RNA (155-I) increased WEE1 accumulation. Both LSMCM and miR-155 overexpression also reduced the expression of a luciferase reporter construct containing the WEE1 3′ UTR (**Fig. 6**).

[00159] Accordingly, 155-I increased Wee1 levels in primary B cells isolated from Eμ-miR-155 transgenic mice that overexpress miR-155 in B-cell lineage, thus confirming that Wee1 is a bona fide miR-155 target. Taken together, these results show that inflammatory stimuli down-regulate WEE1 through up-regulation of miR-155. Because WEE1 depletion rapidly induces DNA damage in newly replicated DNA, these results show that miR-155 overexpression may shorten the period required for selection of cancer-associated mutations. Furthermore, Affymetrix microarrays revealed that transcripts coding for several factors controlling cell cycle, DNA repair, and genome stability were affected by LSMCM in both T47D and MDA-MB-231 cell lines (**Fig. 10 - Table 2**). This result shows that the ability of inflammatory stimuli to induce defective checkpoints and genomic instability, similar to miR-155, might contribute to tumorigenesis.

[00160] *Discussion*

[00161] In this example, the mutator activity of miR-155 and of the miR-155-related proinflammatory environment were analyzed. Cells in which inflammatory stimuli resulted in the up-regulation of miR-155 showed a two- to threefold increase in the mutation rate as deduced by HPRT assay.

[00162] Furthermore, inducible expression of miR-155 resulted in a similar increase in mutation rate, showing that the up-regulation of the mutation rate by the inflammatory stimuli is miR-155 dependent. The mutation rate was not increased in cells in which inflammatory stimuli up-regulated only miR-155, another microRNA implicated in the innate immune response (data not shown.

[00163] Although miR-155 levels in MDA-MB-231 cells were increased constantly by 12- and 32-fold during doxycycline treatment, they increased transiently by only plus or minus fourfold after LPS/TNF treatment (**Fig. 1B** and **Fig. 2B**).

[00164] Nevertheless, the mutation rate increased by 1.56- to 3.47-fold after doxycycline treatment and by 2.52-fold after TNF/LPS treatment (**Fig. 1B** and **Fig. 2C**). This result shows that increased miR-155 levels resulting from chronic inflammation, autoimmune diseases, or the deregulation of endogenous genetic circuitries with the onset of cancer may produce a significant mutator phenotype. These results also show that other inflammatory signaling pathways may work in synergy or in parallel with miR-155.

[00165] Of note, miR-155 targets tumor suppressor genes such as Fas-associated via death domain (FADD), Jumonji AT-rich interactive domain 2 (JARID2), and Src homology 2-containing inositol phosphatase-1 (SHIP1) (**Fig. 13 - Table S3**). In addition, other microRNAs with mutator activity are up-regulated by LPS signaling. The increased mutation rate in HCT116 cells that lack

the hMLHl DNA repair enzyme showed that this increase occurs through miR-155 targeting of other transcripts involved in DNA repair, recombination, or cell-cycle checkpoints. Because mutations accumulate during the S phase, when the replication of the DNA takes place right before the G2/M check point, the inventor looked for transcripts that are predicted targets of miR-155 and act as inhibitors of G2/M transition because their reduced expression might be associated with an increased mutation rate. In addition, these transcripts can act as targets of LPS/TNF signaling. It is to be understood that the inventor first concentrated on WEE1 kinase, because it fulfilled all these criteria.

[00166] In T47D cells, overexpression of miR-155 or treatment with LSMCM resulted in the down-regulation of WEE1 expression. By targeting WEE1 and consequently facilitating G2/M transition, miR-155 allows cells that have not yet repaired the DNA to proceed to mitosis, resulting in accumulated mutations. Akt kinase also is known to function as a G2/M initiator and to inactivate WEE1 by phosphorylation, thus promoting the cell-cycle transition. Akt is implicated in LPS signaling by modulating the levels of miR-155, among other microRNAs. While not wishing to be bound by theory, the inventor herein now believes that oncogenic Akt and onco-inflammatory miR-155 cross talk at the level of WEE1 during inflammation. The inventor considers that the increased mutation rate associated with inflammatory signals is a combinatorial effect of miR-155 targeting of WEE1 and other DNA repair enzymes that are down-regulated by LPS and are either direct or indirect targets of miR-155. It is believed that cancer results from the accumulation of mutations in somatic cells, and this example shows that, by increasing the mutation rate, the inflammatory miR-155 is a key player in inflammatory-induced cancers in general.

[00167] The control of cell-cycle progression and DNA repair in eukaryotes are highly conserved. However, in the event of an infection the cells must respond quickly by producing cytokines, chemokines, and other inflammatory components of the immune defense. During this robust response, it is possible that the DNA repair machinery and cell-cycle checkpoints are put on hold. At this stage the up-regulation of miR-155 by inflammatory stimuli to clear the antigen quickly also results in an increased mutation rate. Furthermore, regardless of the primary cause of a mutation, there is a high probability that, in the event of an infection, the mutation will be fixed.

[00168] While not wishing to be bound by theory, the inventor herein now we believes that simultaneous miR-155–driven suppression of a number of tumor suppressor genes combined with a mutator phenotype allows the shortening of the series of steps required for tumorigenesis and represents a model for cancer pathogenesis (**Fig. 7**).

[00169] Thus, the up-regulation of miR-155 by chronic inflammation appears to indicate at least one of the missing links between cancer and inflammation.

[00170] *Materials and Methods*

[00171] *Cell Culture, Transfection, and Treatment.*

[00172] Cells were grown following standard procedures. T47D cells were transfected using

lipofectamine (Invitrogen). Unstimulated LSMCM were prepared from the supernatant of human THP-1 monocytic cells mock stimulated or stimulated with Salmonella enteritidis derived LPS (100 ng/mL, Sigma) for 6 h. THP-1 cells subsequently were centrifuged, and the supernatant was filtrated to eliminate any remaining cells.

[00173] T47D cells then were cultivated in the presence of unstimulated medium or LSMCM for 48 h. When needed, a second stimulation was conducted in the same way, after the cells had been allowed to recover 4 d in regular medium. TNF was obtained from Invitrogen. The B-cell line was established by purifying B cells from the spleen of an Eμ-miR-155 transgenic mouse using the isolation kit from R & D Systems. B cells subsequently were cultured for 2 wk in 100 ng/mL RPMI/15% FBS/LPS and for 3 more weeks without LPS. They were electroporated using the Amaxa kit (Lonza).

[00174] *Retroviral Infection*.

[00175] The Retro-X Tet-Advanced System (Clontech) was used according to the manufacturer's instruction. Clones stably expressing miR-155 were prepared from MDA-MB-231 cells and SW620 cells following manufacturers' instructions. In brief, cells were infected first with the pRetroX-tight-Pur-miR-155 response virus. Colonies resistant for puromycin then were infected with the pRetroX-Tet-On Advanced regulator virus and selected for resistance to both puromycin and Geneticin. Throughout the selection process, cells were grown in medium containing Tet-FBS (Clontech) that does not contain any tetracycline residue. A fraction of double-resistant clones then was treated with 500 ng/mL doxycycline for 2 d before miR-155 expression was analyzed by qRT-PCR. HCT116 cells were transiently infected with a viral suspension containing both the pRetroX-Tet-On Advanced regulator vector and the pRetroX-tight-Pur response vector containing the construct of interest and then were left to recover for 2 d in regular medium before the addition of doxycycline.

[00176] *Preparation of Expression Constructs*.

[00177] The WEE1 reporter construct was prepared by inserting the 3′ UTR of human WEE1, prealably amplified by PCR from HEK-293 cells' genomic DNA, downstream of the Luciferase gene in the XbaI site of the pGL3-Control vector (Promega). The mature miR-155 and miR-155 precursor (premiR-155) were cloned in the pRetroX-tight-Pur vector following digestion by NotI and EcoRI of double-strand DNAs prepared by reannealing the following primers:

miR-155 mature:

V155MatForward:

5′-ATAGCGGCCGCTTAATGCTAATCGTGATAGGGGTGAATTCGCG -3′ **[SEQ ID NO:1]** and

V155-MatReverse:

5′-CGCGAATTCACCCCTATCACGATTAGCATTAAGCGGCCGCTAT-3′ **[SEQ ID NO:2]**;

premiR-155:

V155PreForward:

5′ATAGCGGCCGCCTGTTAATGCTAATCGTGATAGGGGTTTTTGCCTCCAACTGACTCCT ACATATTAGCATTAACAGGAATTCGCG-3′ **[SEQ ID NO:3]**

and

V155PreReverse:

5′CGCGAATTCCTGTTAATGCTAATATGTAGGAGTCAGTTGGAGGCAAAAACCCCTATCA CGATTAGCATTAACAGGCGGCCGCTAT-3′ **[SEQ ID NO:4]**.

[00178] *Selection of 6-TG–Resistant Colonies*.

[00179] To eliminate any preexisting HPRT mutants, cells were grown in 100 μM hypoxanthine, 400 nM aminopterin, and 16 μM thymidine (HAT medium) Sigma) for 3 d. The MDA-MB-231 cells used for the experiment reported in **Fig. 2C** were cleansed in HAT medium for 15 d. After three washes, cells were resuspended and incubated in regular medium for another 3 d. T47D, HCT116, SW620, or MDA-MB-231 cells then were treated with macrophage-conditioned medium or doxycycline as required. Two days later, HCT116 cells were plated in 96-well round-bottomed plates (1,000 cells per well), and T47D, SW620, or MDA-MB-231 cells were plated in 48-well plates (106 cells per plate) in selection medium containing 30 μM 6-TG. HPRT mutants then were selected based on their resistance to 6-TG. During the selection process, cells containing the retroviral constructs were constantly stimulated with doxycycline. After 2–3 wk of selection on 6-TG medium (with 6-TG–containing medium changed every 3 d), plates were stained with crystal violet to allow the visualization and counting of 6-TG–resistant colonies.

[00180] *Estimation of Mutation Rates*.

[00181] For the experiments with SW620 and MDA-MB-231 stable clones (**Fig. 1** and **Fig. 11 - Table S1**), mutation rates were adjusted for cell growth and were estimated based on a modified version of fluctuation analysis. The cell growth-adjusted mutation rate was analyzed based on the formula $r = f \times \tau/t$, where "f" is the mutation frequency (mutations per cell), "τ" is 1/cell division rate (in cell divisions per day), and "t" is the length of miR-155 induction (in days). For the experiments with MDA-MB-231 cells (**Fig. 2C**), the estimated mutation rate was based on the average mutant frequency and population doubling according to the schema shown in **Fig. 8**.

[00182] Mutant frequency and population doubling were estimated at each of the steps shown thereafter (i.e., right after HAT cleansing, 3 d after HAT cleansing, 3 d after mock (control) or TNF/LPS treatment, and 3 d after the end of the treatment). Cells were plated in 6-TG-supplemented medium at a density of 1.5×10^6 cells per 10-cm dish. Additionally, plating efficiency (PE) at the time of selection was determined by plating 500 cells per 10-cm dish in triplicate in RPMI medium without hypoxanthine. Cells were incubated for 14–20 d, and colonies were visualized by staining with 0.5% crystal violet in 4% paraformaldehyde (Sigma). Mutant frequency (MF) then was determined as follows: $MF = a/(60 \times 10^6 \times [b/1.5 \times 10^3])$, where "a" is the total number of 6-TG–resistant colonies, and "b" is the total number of colonies on all three plates. PE and the exact number of cells subcultured were used to calculate population doubling (PD) as

follows: PD = (ln[total number of cells] – ln[number of cells plated × PE])/ln2. Mutation rate was estimated by plotting the observed mutant frequencies as a function of PD and calculating the slope by linear regression. This slope yields the mutation rate (mutations per cell per generation).

[00183] *Analysis of HPRT cDNA Mutations*.

[00184] The 6-TG–resistant colonies from miR-155-Off and miR-155-On infected cells were selected randomly as representative mutant clones. Clones were expanded for 1 wk before RNA extraction. Total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor from Applied Biosystems. HPRT cDNAs (nucleotides 123–1,110) were amplified subsequently by PCR using the Advantage 2 Polymerase Mix from Clontech, with the forward primer 5′-GCGCGCCGGCCGGCTCCGTT-3′ **[SEQ ID NO:5]** and the reverse primer 5′-GGCGATGTCAATAGGACTCCAGATG-3′ **[SEQ ID NO:6]**.

[00185] In most cases, the PCR products were cloned in the TOPO vector (Invitrogen) and subsequently sequenced following plasmid purification. In other cases, the PCR products were purified using the PCR purification kit from Qiagen and were directly sequenced at the sequencing facility at Ohio State University using the primers 5′-GCCGGCCGGCTCCGTTATGG-3′ **[SEQ ID NO:7]** and 5′-ATGTCAATAGGACTCCAGATG-3′ **[SEQ ID NO:8]**.

[00186] *Isolation of RNAs and qRT-PCR*.

[00187] RNA was extracted with TRIzol (Invitrogen) and subsequently subjected to DNase digestion (Turbo-DNase; Ambion). MiR-155 qRT-PCR was performed using TaqMan MicroRNA Assays (Applied Biosystems). Values were normalized using RNU-44. Real-time PCR was run in triplicate from three different cDNAs.

[00188] *FACS Analysis*.

[00189] CFSE was purchased from Molecular Probes/Invitrogen. CFSE staining was carried out using manufacturer's protocol. Cells were fixed in 1% paraformaldehyde before analyses. Flow cytometry analyses were performed at the corresponding facility of Ohio State University. Data were analyzed using the software program FlowJo (Tree Star, Inc.).

[00190] *Western Blots*.

[00191] Cells were lysed 48 h after transfection or electroporation. Anti-WEE1 and anti–α-tubulin antibodies were from Cell Signaling Technology.

[00192] *Affymetrix Microarray Analyses*.

[00193] RNAs extracted with TRIzol (Invitrogen) were subsequently subjected to DNase digestion (Turbo-DNase; Ambion). Affymetrix microarray analyses were done at the Ohio State University microarray facility.

[00194] *Luciferase Assays*.

[00195] Cells plated in 12-well plates (1 × 106 cells per plate) were transfected with 0.4 μg of DNA (pGL3-control vector or WEE1 reporter constructs; Promega), 20 ng of Renilla luciferase control vector (pRL-TK; Promega), and 50 nM of either a premiR control (premiR Precursor

Molecule-Negative Control #1; Ambion), premiR-155 (miR-155 precursor; Ambion), or 155-I (an antisense miR-155 inhibitory RNA; Ambion). Assays were performed 48 h after transfection using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

[00196] While the invention has been described with reference to various and preferred embodiments, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the essential scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof.

CLAIMS

What is claimed is:

1. A method for modulating WEE1 kinase expression levels in a target cell, comprising: administering a microRNA-155 (miR-155) oligonucleotide to the target cell.

2. A method of modulating mutation of a target cell in a subject, comprising: administering a miR-155 oligonucleotide to a target cell cells in the subject; and, measuring mutation of the target cell, wherein the target cell is a cancer cell or a precancerous cell.

3. A method of reducing spontaneous mutation rate of a cell in a subject in need thereof, comprising: reducing endogenous levels of miR-155.

4. A method of reducing spontaneous mutation rate of an inflammation-related cancer cell in a subject in need thereof, comprising reducing endogenous levels of miR-155.

5. A method of slowing or inhibiting cell proliferation in a cancer cell or cancer cell population comprising: contacting the cell or cell population with a miR-155 antisense compound comprising a miR-155 oligonucleotide is complementary to a sequence at least 90% identical to mature microRNA-155, thereby slowing or inhibiting mutation of the cell or cell population,

6. A method of treating or preventing a miR-155 associated cancer, comprising: identifying a subject having, or suspected of having the miR-155 cancer; and, administering to the target cell a miR-155 oligonucleotide.

7. A method of treating or preventing an miR-155 associated -cancer comprising: identifying a subject having, or suspected of having the miR-155 associated cancer, and administering to the subject a miR-155 antisense compound comprising a miR-155 oligonucleotide having complementary at least 90% identical to mature microRNA-155.

8. A method of modulating the expression of one or more genes in a target cell, the genes being selected from: APC, adenomatous polyposis coli; *FADD,* Fas (TNFRSF6)-associated via death domain; FOXO3, forkhead box O3; KGF, keratinocyte growth factor; *HIVEP2,* HIV type I enhancerbinding protein *2;* MYO10, myosin X; RHOA, Ras homolog gene family, member A; *RIP1,* receptorinteracting protein kinase *1; SHIP1,* inositol polyphosphate-5-phosphatase; SMAD1/5, SMAD family member 1/5; SOCS1, suppressor of cytokine signaling *1; TP53INP,*

Tumor protein 53-induced nuclear protein 1, comprising:

contacting the target cell with a miR-155 oligonucleotide.

9. The method of any one of the Claims herein, wherein the miR-155 oligonucleotide comprises an antisense miR-155 oligonucleotide.

10. The method of any one of the Claims herein, wherein the miR-155 oligonucleotide comprises a miR-155 antisense compound.

11. The method of any one of the Claims herein, wherein the miR-155 oligonucleotide comprises a miR-155 antagonist compound.

12. The method of any one of the Claims herein, wherein the miR-155 oligonucleotide is selected from the group consisting of a mature miR-155 oligonucleotide, a pre-miR-155 oligonucleotide, and a miR-155 seed sequence.

13. The method of any one of the Claims herein, wherein the miR-155 antisense compound comprises a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to a sequence at least 80% identical to mature sequence of the modified oligonucleotide is complementary to a sequence at least 80% identical to mature miR-155, pre-miR-155, a miR-155 seed sequence, or a sequence fully complementary to the sequence of mature miR-155, pre-miR-155, or miR-155.

14. The method of any one of the Claims herein, wherein administering a miR-155 oligonucleotide comprises: administering an antisense miR-155 expression vector to a target cell; and expressing an antisense miR-155 in the target cell.

15. The method of any one of the Claims herein, wherein administering a miR-155 oligonucleotide comprises: administering a miR-155 expression vector to a target cell; and expressing a miR-155 in the target cell.

16. The method of the preceding Claim, wherein the miRNA-155 expression vector comprises a nucleic acid sequence encoding a miRNA-155 operably linked to a promoter.

17. The method of any one of the Claims herein, wherein the target cell is a cancer cell.

18. The method of any one of the Claims herein, wherein the target cell is a breast

cancer or precancerous cell.

19. The method of any one of the Claims herein, wherein the target cell is a colon cancer or precancerous cell.

20. The method of any one of the Claims herein, wherein the target cell is a gastric cancer or precancerous cell.

21. The method of any one of the Claims herein, wherein the target cell is a lung cancer or precancerous cell.

22. The method of any one of the Claims herein, wherein the modulation comprises decreasing expression of the one or more genes.

23. The method of any one of the Claims herein, wherein the modified oligonucleotide has no more than two mismatches to the nucleobase sequence of mature miR-155.

24. The method of the preceding Claim, wherein the modulation comprises decreasing expression of the one or more genes.

25. The method of any one of the Claims herein, comprising contacting the cell with an antisense miR-155 inhibitory RNA (155-I).

26. The method of any one of the Claims herein, wherein the cell is contacted with the antisense miR-155 inhibitory RNA (155-I) in an amount sufficient to increase WEE1 levels.

27. A method of reducing spontaneous mutation rate of an inflammation-related cancer cell in a subject in need thereof, comprising contacting the cell with an antisense miR-155 inhibitory RNA (155-I).

28. The method of claim 27, wherein the cell is contacted with the antisense miR-155 inhibitory RNA (155-I) in an amount sufficient to increase WEE1 levels.

29. A method of preventing the onset of an inflammatory-related cancer, comprising modulating the up-regulation of miR-155 in a subject in need thereof.

30. The method of any one of the Claims herein, wherein the subject is human.

31. A composition useful for reducing spontaneous mutation rate of a cell in a subject in

need thereof, comprising an antisense miR-155.

32. A method of identifying an agent that can be used to inhibit an inflammatory-related cancer comprising:

a) contacting miR-155 with an agent to be assessed;

b) contacting one or more target genes of miR-155 with an agent to be assessed; or

c) contacting a combination thereof, wherein if the agent inhibits expression of miR-155, enhances expression of the target genes, or performs a combination thereof, then the agent can be used to inhibit proliferation of the inflammatory-related cancer.

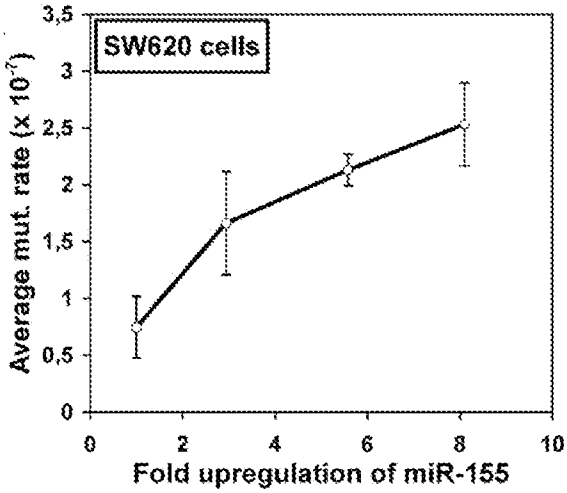

Figure 1A

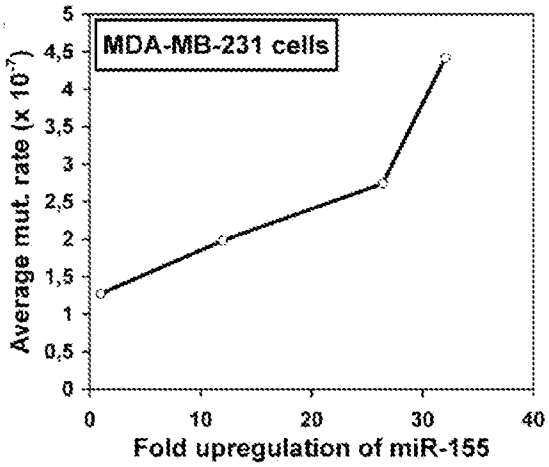

Figure 1B

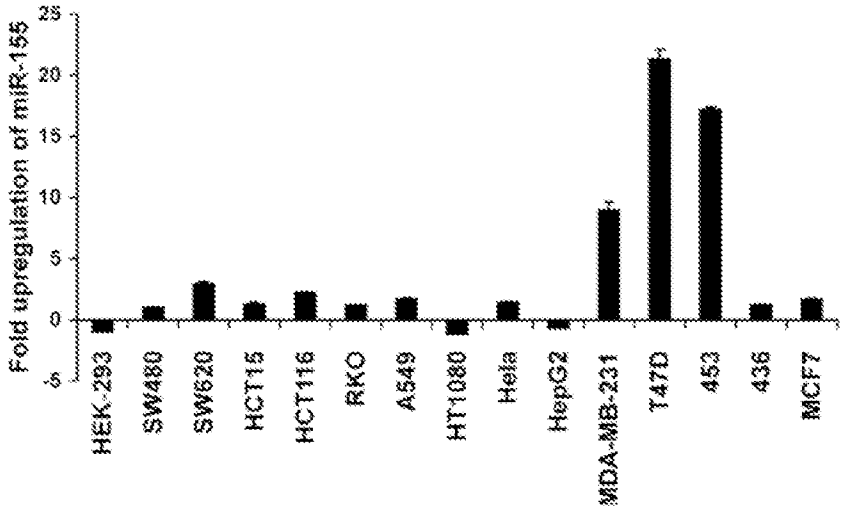

Figure 2A

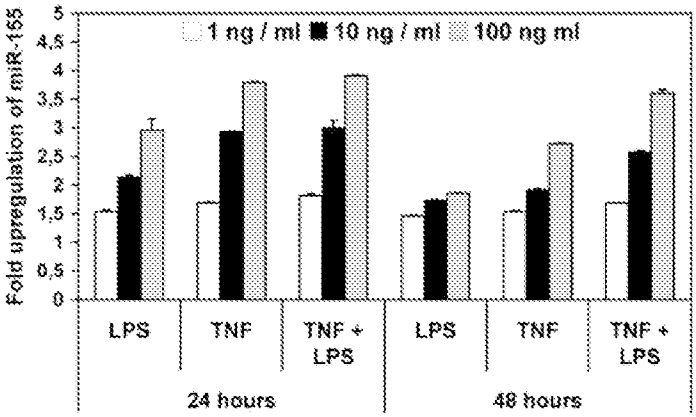

Figure 2B

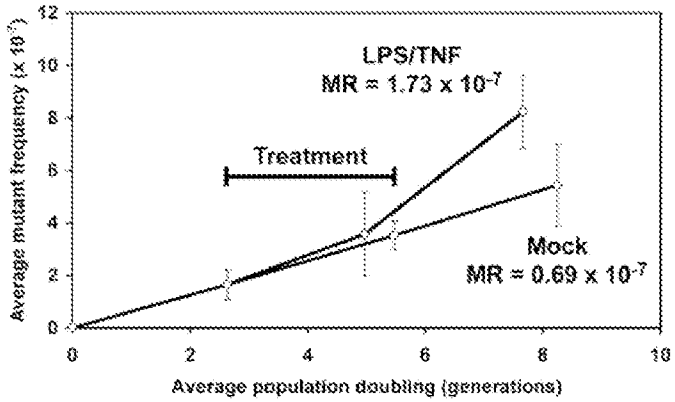

Figure 2C

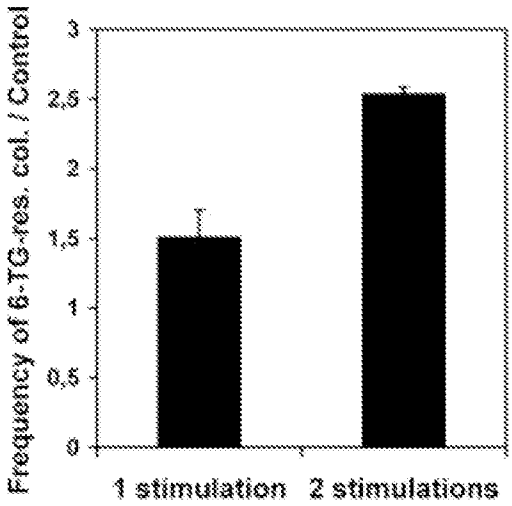

Figure 2D

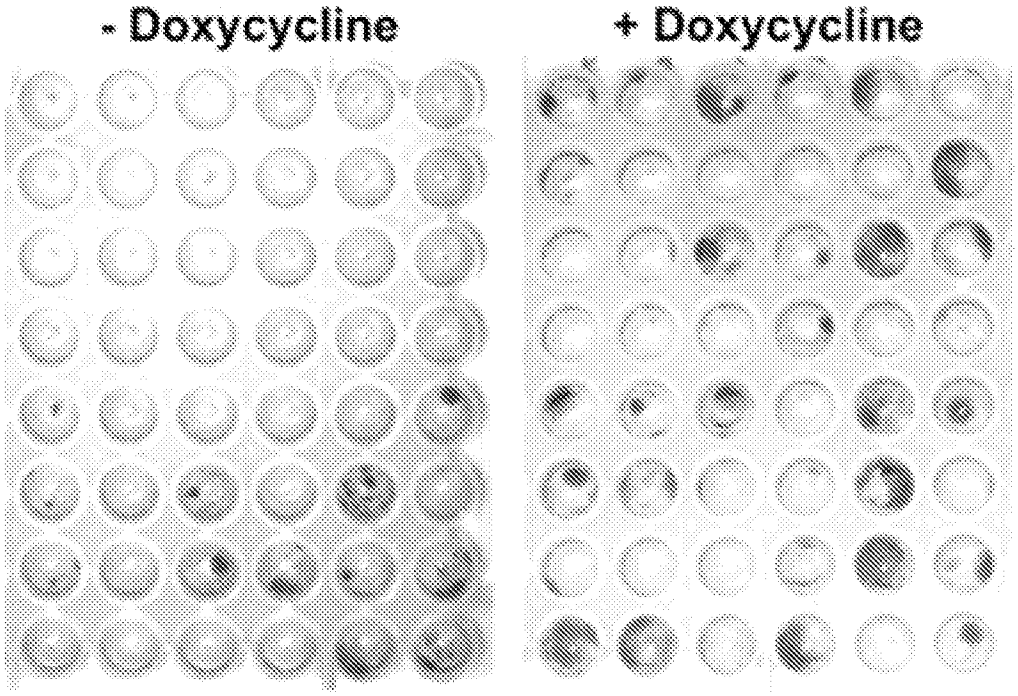

Figure 3A

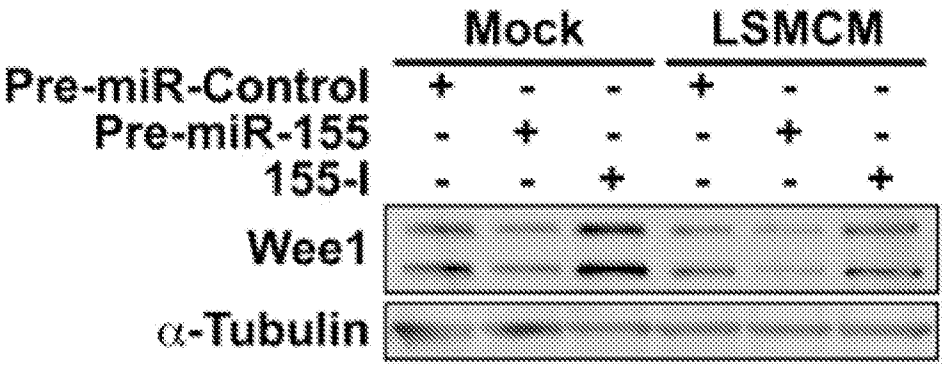

Figure 3C

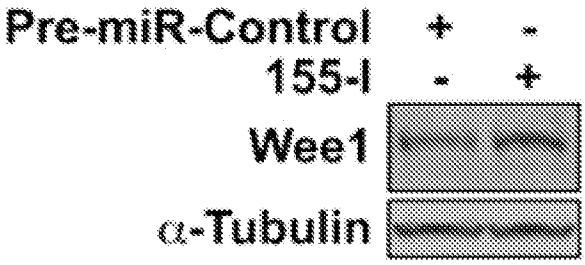

Figure 3D

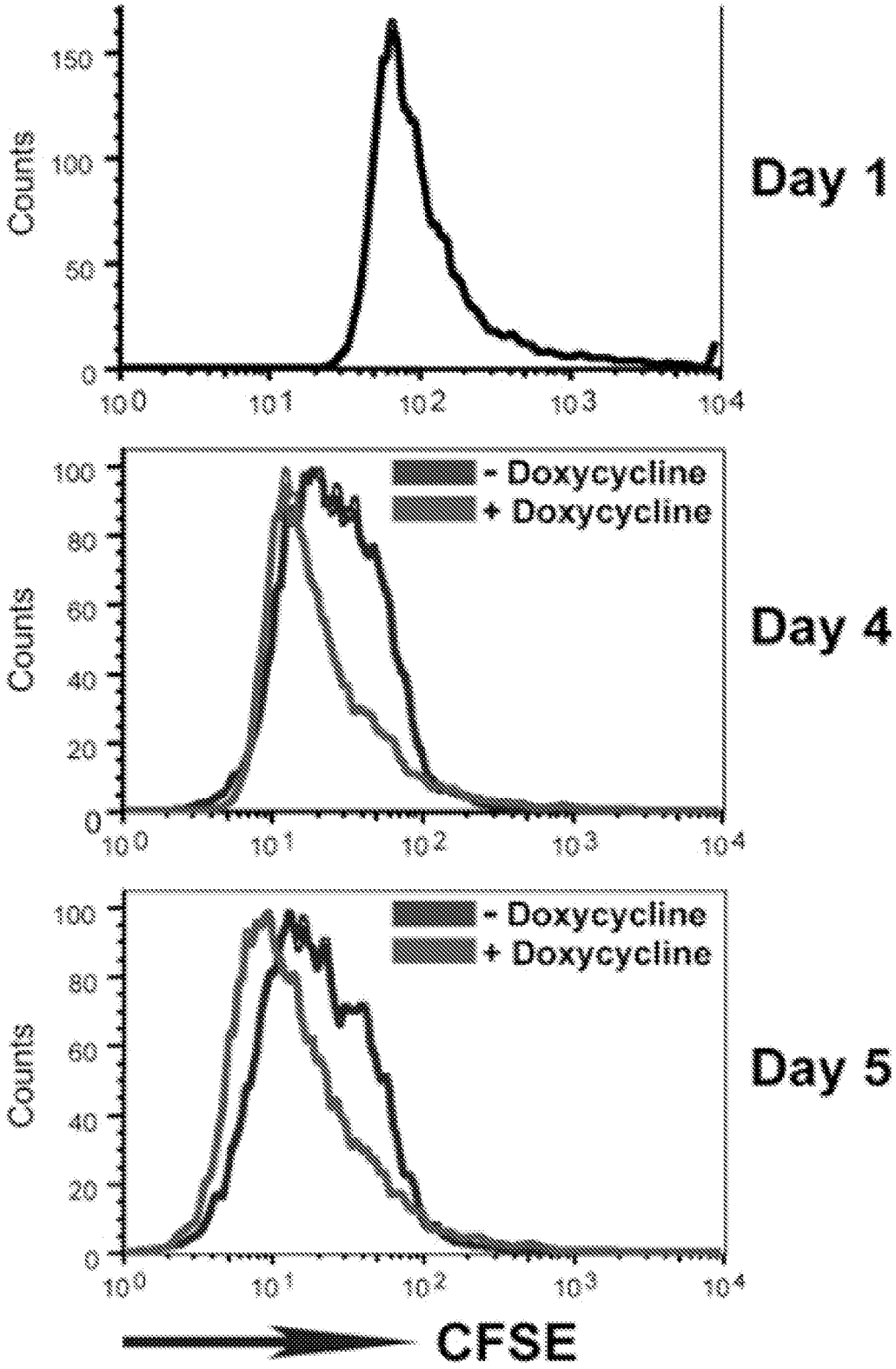
Counts
150
100
50
0
10^0
10^1
10^2
10^3
10^4
Day 1
Counts
100
80
60
40
20
0
- Doxycycline
+ Doxycycline
Day 4
Counts
100
80
60
40
20
0
- Doxycycline
+ Doxycycline
Day 5
CFSE

Figure 3B

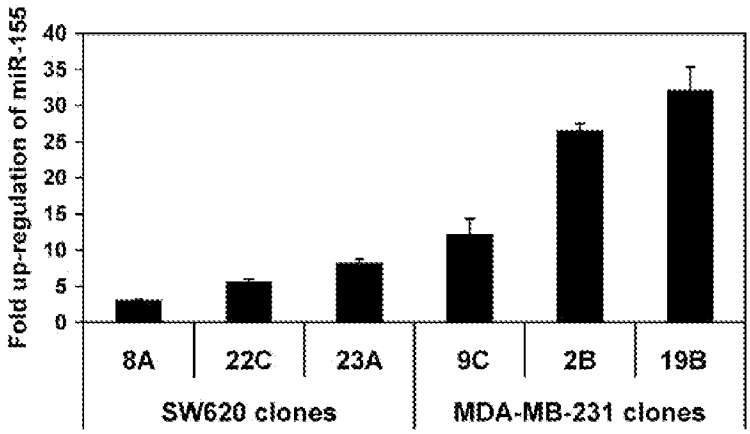

Figure 4

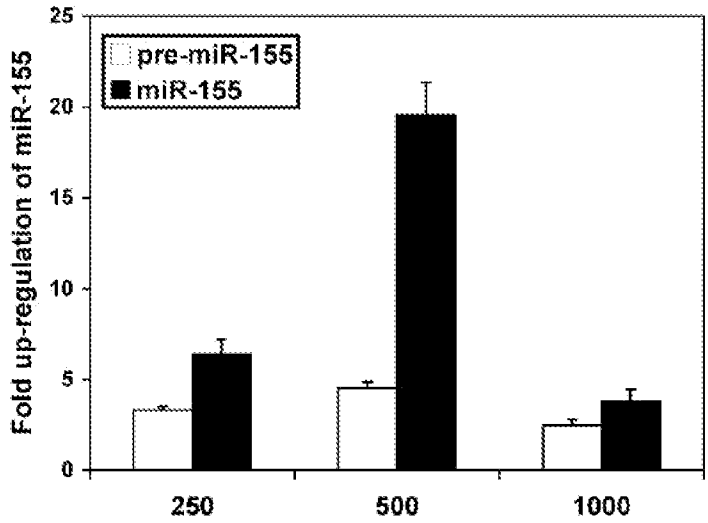

Figure 5

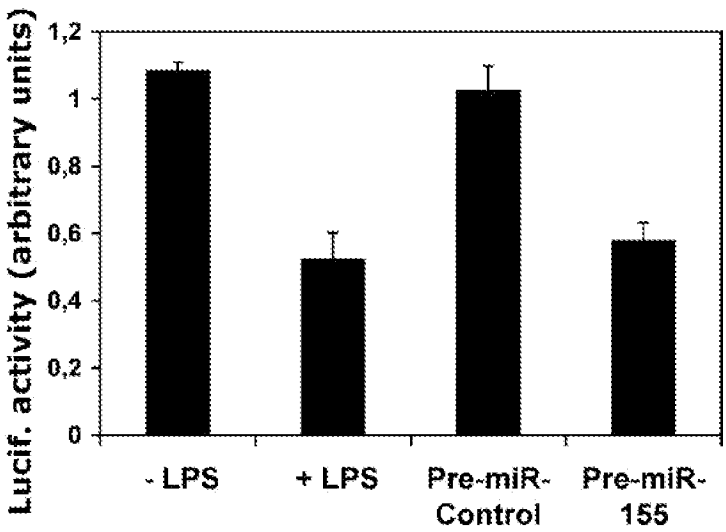

Figure 6

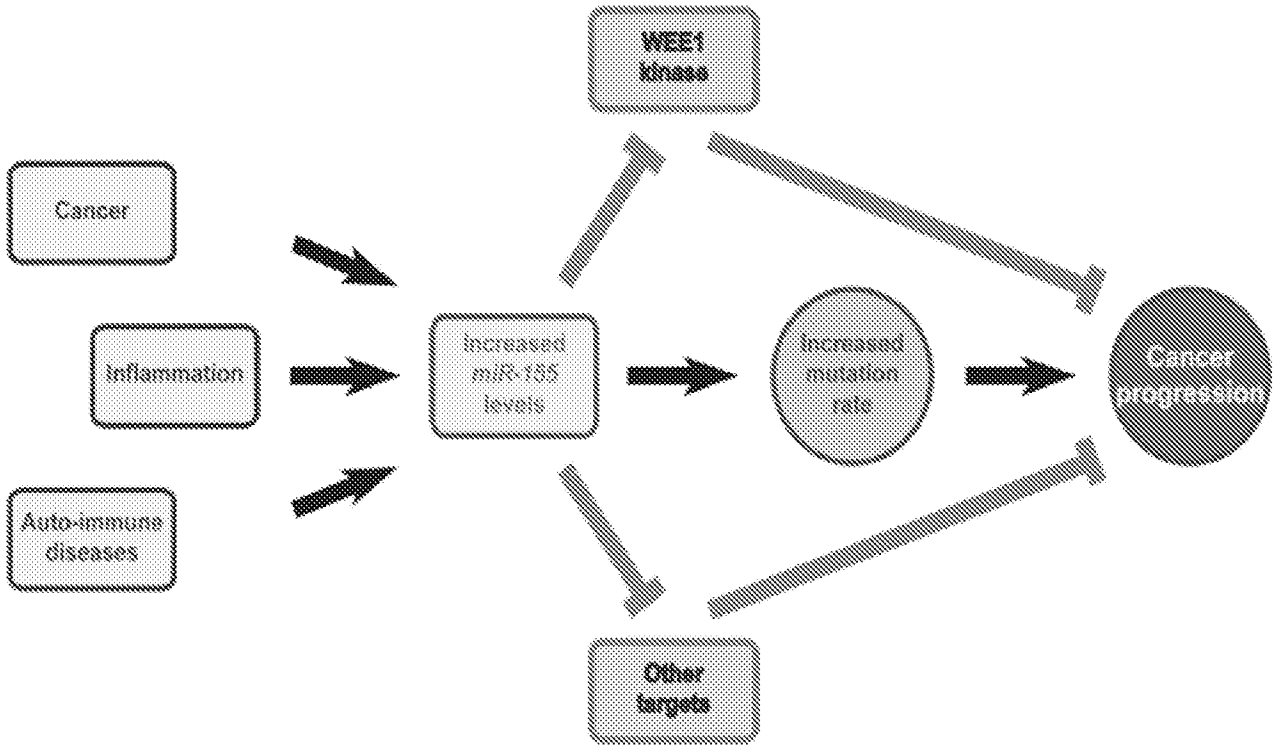

Figure 7

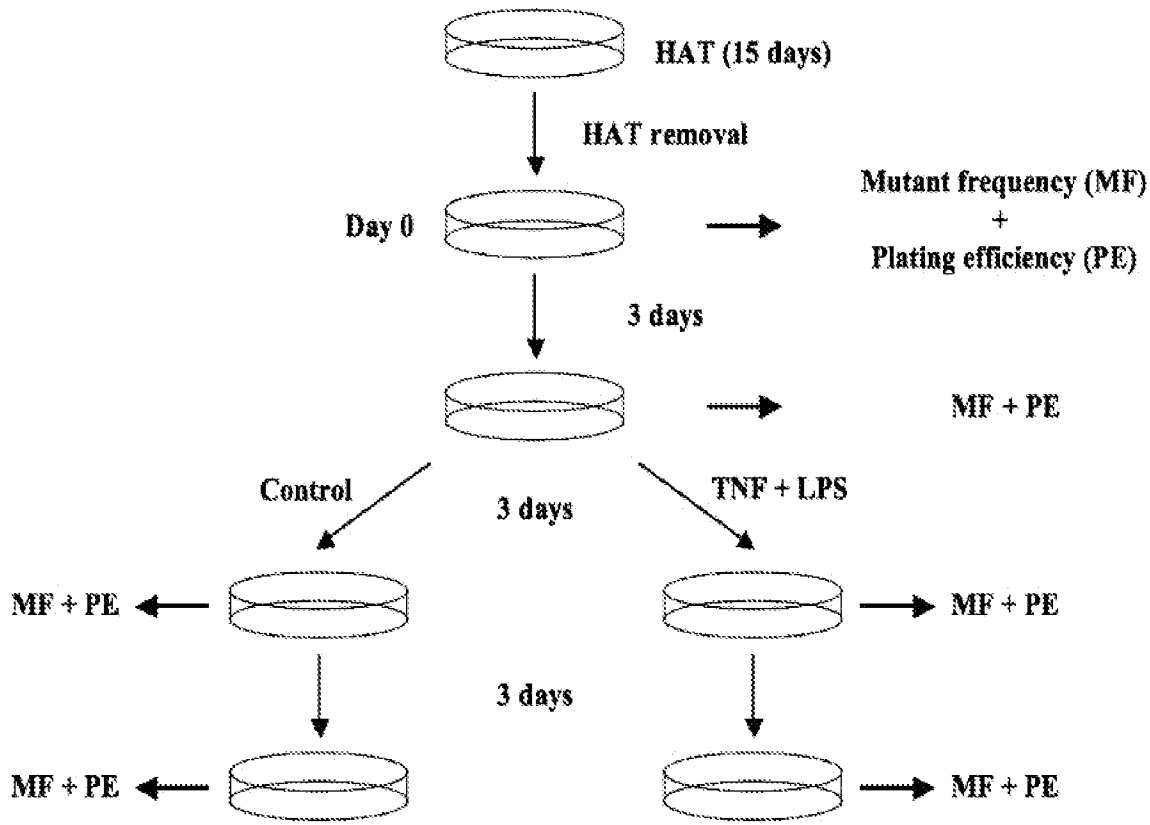

Figure 8

Table 1. Mutations found in HPRT cDNAs prepared from 6-TG-resistant colonies of T47D, HCT116, and MDA-MB-231 cells

Cell lines	Treatment	Number of clones analyzed	Clones without mutation in the coding sequence	Clones with frameshift mutation(s)	Clones with deletion(s)	Clones with only a single deletion	Clones with one or more exons lacking	Clones with other types of deletions	Clones with insertion(s)	Clones with transition(s)	Clones with transversion(s)
T47D	Mock	24	0	12	14	17	24 Del ex. 3: 11 Del ex. 2+3: 9 Del ex. 4+5: 4	4 Del 1: 7	1 Ins 1: 1	4 Transitions: 5	0
T47D	LSMCM	40	0	20	40	25	38 Del l ex. 3: 14 Del ex. 2+3: 16 Del ex. 4 + 5: 5 Del ex. 2+3+4: 1 Del ex. 6+7+8: 1 Del ex. 5: 1	11 Del 17: 3 Del 1: 21	3 Ins 1: 3	7 Transitions: 7	1 Transitions: 3
HCT116	– Doxycycline	14	1	13	13	0	0	13 Del 2: 1 Del 1: 12	4 Ins 1: 5	0 Transitions: 7	2 Transversions: 3
HCT116	– Doxycycline	14	1	13	13	0	0	13 Del 2: 1 Del 1: 12	4 Ins 1: 5	0	2 Transversions: 2
HCT116	+ Doxycycline	25	4	18	11	0	2 Del ex. 3: 1 Del ex. 2+3: 1	9 Del 1: 16	13 Ins 1: 17	6 Transitions: 6	6 Transversions: 8
MDA-MB-231	– Doxycycline	11	3	8	10	5	8 Del ex. 2+3: 1 Del ex. 7: 8	3 Del 1: 5	0	1 Transitions: 1	0
MDA-MB-231	+ Doxycycline	24	2	17	21	11	17 Del ex. 2: 1 Del ex. 2 + 3: 1 Del ex. 7: 16	9 Del 1: 17	0	4 Transitions: 6	2 Transversions: 2

Ex., exon; LSMCM, LPS-stimulated macrophage-conditioned medium; Mock, unstimulated macrophage-conditioned medium.

Figure 9

Table 2. Transcripts encoding factors related to DNA replication and maintenance whose levels decrease significantly following treatment of T47D and MDA-MB-231 cells

Symbol	Gene name	Fold change
RFC5	Replication factor C (activator 1) 5, 36.5kDa	0.72
NEIL3	Nei endonuclease VIII-like 3 (*E. coli*)	0.71
AURKB	Aurora kinase B	0.71
GTSE1	G-2 and S-phase expressed 1	0.64
RAD54L	RAD54-like (*S. cerevisiae*)	0.75
ARL3	ADP ribosylation factor-like 3	0.81
BCCIP	BRCA2 and CDKN1A interacting protein	0.77
SKP2	S-phase kinase-associated protein 2 (p45)	0.65
RDM1	RAD52 motif 1	0.75
PARP2	Poly (ADP ribose) polymerase 2	0.86
RAD54B	RAD54 homolog B (*S. cerevisiae*)	0.67
ERCC6L	Excision repair cross-complementing rodent repair deficiency, complementation group 6-like	0.61
DCTPP1	dCTP pyrophosphatase 1	0.78
DDB2	Damage-specific DNA binding protein 2, 48kDa	0.84
CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.64
AK3L1	Adenylate kinase 3-like 1	0.84
APRT	Adenine phosphoribosyltransferase	0.84
DDB2	Damage-specific DNA binding protein 2, 48kD	0.84
BRCC3	BRCA1/BRCA2-containing complex, subunit 3	0.83
TOP2A	Topoisomerase (DNA) II alpha 170kD	0.77
CDT1	Chromatin licensing and DNA replication factor 1	0.74
RECQL4	RecQ protein-like	0.72
PARP1	Poly (ADP ribose) polymerase 1	0.86

After Affymetrix microarray analyses, comparisons were done between two pools of cells treated with either unstimulated macrophage-conditioned medium or with LPS-stimulated macrophage-conditioned medium. The two pools contained four independent replicates from both T47D and MDA-MB-231 cell lines. Transcripts are arranged according to the decreasing values of P (all $<1 \times 10^{-7}$).

Figure 10

Table S1

Cell lines	Clones or experiments	Doxycycline (ng/ml)	Number of plated cells	Fold *miR-155* up-regulation ± standard deviation	Number of 6-TG-resistant colonies	Average mutation rate	Fold increase of the average mutation rate
HCT116 cells		0	0.1×10^7		112	560×10^{-7}	
		500	0.1×10^7	19.57 ± 0.62	352	1570×10^{-7}	2.81
SW620 clone 8A	Experiment 1	0	6×10^7		12		
		500	6×10^7		20		
	Experiment 2	0	6×10^7		10		
		500	6×10^7		14		
	Experiment 3	0	6×10^7		11		
		500	6×10^7		25		
	Total	0	18×10^7		33		
		500	18×10^7	2.94 ± 0.23	59	1.66×10^{-7}	
SW620 clone 22C	Experiment 1	0	3.5×10^7		7		
		500	3.5×10^7		14		
	Experiment 2	0	3.5×10^7		7		
		500	3.5×10^7		15		
	Experiment 3	0	3.5×10^7		5		
		500	3.5×10^7		16		
	Total	0	10.5×10^7		19	0.75×10^{-7}	
		500	10.5×10^7	5.58 ± 0.43	45	2.13×10^{-7}	2.86
SW620 clone 23A	Experiment 1	0	2×10^7		2		
		500	2×10^7		12		
	Experiment 2	0	2×10^7		2		
		500	2×10^7		9		
	Experiment 3	0	2×10^7		1		
		500	2×10^7		10		
	Total	0	6×10^7		5	0.75×10^{-7}	
		500	6×10^7	8.10 ± 0.65	31	2.53×10^{-7}	3.39
MDA-MB-231	Clone 9C	0	4×10^7		11	1.28×10^{-7}	
		500	4×10^7	12.01 ± 2.34	16	1.98×10^{-7}	1.56
MDA-MB-231	Clone 2B	0	2.5×10^7		6	1.28×10^{-7}	
		500	2.5×10^7	26.42 ± 1.11	15	2.74×10^{-7}	2.15
MDA-MB-231	Clone 19B	0	2×10^7		5	1.28×10^{-7}	
		500	2×10^7	32.07 ± 3.27	20	4.42×10^{-7}	3.47

Figure 11

Table 83

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding[c] / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
T47D	Mock[a]	del 184 (frameshift)	no		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	8
		del 184 (frameshift)	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				transition	505	4	coding	GGG-G(A>G)C-ATA	10	Asp > Gly	
		del 184 (frameshift)	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				transition	556	5	coding	AAT-G(T>C)C-TTG	11	Val > Ala	
		del 184 (frameshift)	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				del 1	1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		del 291 (no frameshift)	no		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	8
		del 291	yes		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				del 1	195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		del 84 (no frameshift)	no		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	

Figure 12

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding[a] / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				transition	227	2	coding	CCTT-GA(T>C)-TTAT	15	Asp <-> Asp	
				transition	407	3	coding	GCTG-GA(T>C)-TACA	16	Asp <-> Asp	
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1
				del 1	948, 949, 950 or 951	9	3'-untranslated	AGAA-TTT(T)-ATCT	17	none	
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1
				del 1	170 or 171	1	coding	CGTT-ATG(G)-CGAC	18	frameshift (initiator codon)	
				del 1	185	1	coding	CAGC-CC(T)-GGCG	19	frameshift	
				ins 1	344, 345 or 346	3	coding	GAGG-CC(C>ATCA	20	frameshift	
				transition	968	9	3'-untranslated	TACT-TT(A>G)-GAAA	21	none	
T47D	LSMCM[8]	del 184 (frameshift)	no		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	11
		del 184	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				del 1	1038, 1039 or 1040	9	3'-untranslated	CTTT-GG(G)-CGGATC	22	none	
		del 184	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				3 transversions	153, 157 and 161	1	5'-untranslated	CGCG--(C>G)-CGG-(C>G)-CGG-(C>G)-TCCG	23	none	
				transition	168	1	coding	CGTT-(A>G)-TGGC	24	lack of initiator codon	
				ins 1	925, 926, 927, 928, 929 or 930	9	3'-untranslated	GAGC-TTTT(T)-GCAT	25	none	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding* / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
		del 184	yes		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				transition	787	9	coding	TTGT-G(T>C)C-ATTA	26	Val > Ala	
		del 291 (no frameshift)	no		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	13
		del 291	yes		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				ins 1	1100, 1101, 1102 or 1103	9	3'-untranslated	GAGTG-AAA(A)-CATT	27	none	
		del 291	yes		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	GAAACA-T(T)-GAAC	28	none	
		del 291	yes		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
				transition	497	4	coding	CCAG-TC(A>G)-ACAG	29	Ser <-> Ser	
		del 84 (no frameshift)	no		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1
				del 1	1045 or 1046	9	3'-untranslated	GCGG-A(T)-GTTG	30	none	
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
				del 1	1079, 1080 or 1081	9	3'-untranslated	TCTT-AA(A)-CCAC	31	none	
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC...GTGGAA)-GATATA	14 & 117	loss of function	1

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding[c] / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 1	145, 146 or 147	1	5'-untranslated	TCAG-CC(C)-GCGC	32	none	
				del 1	152	1	5'-untranslated	GCGC-(G)-CCGG	33	none	
				del 1	175, 176 or 177	1	coding	GCGA-CC(C)-GCAG	34	frameshift	
				del 1	208 or 209	2	coding	TGAT-GA(A)-CCAG	35	frameshift	
				del 1	219	2	coding	TTAT-(G)ACCT	36	frameshift	
				ins 1	257, 258 or 259	2	coding	CTGA-GG(G)-ATTT	37	frameshift	
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC... GTGGAA)-GATATA	14 & 117	loss of function	1
				del 1	168	1	coding	CGTT-(A)-TGGC	38	lack of initiator codon	
		del 84	yes		486-569	whole 4 + 5	coding	TATTGT-(AATGAC... GTGGAA)-GATATA	14 & 117	loss of function	1
				transition	378	1	coding	GGGG-(G>A)GC-TATA	39	Gly > Ser	
		del 357 (no frameshift)	yes		195-551	2 + 3 + 4	coding	GTCGTG-(ATTAGT... GGAAAG)-AATGTC	40 & 118	loss of function	1
				transition	787	9	coding	TTGT-GT(>C)C-ATTA	26	Val > Ala	
		del 207 (no frameshift)			570-776	6 + 7 + 8	coding	GTGGAA-(GATATA... TTGAAT)-CATGTT	41 & 119	loss of function	1
		del 18 (no frameshift)	yes		552-569	whole 5	coding	GGAAAG-(AATGTC... GTGGAA)-GATATA	42 & 120	loss of function	1
				del 1	145, 146 or 147	1	5'-untranslated	TCAG-CC(C)-GCGC	32	none	
				del 1	208 or 209	2	coding	GATG-A(A)-CCAG	43	frameshift	
		del 17 (frameshift)	yes		595-611	6	coding	AACAA-(TGCAGAC ...TTCC)-TTGGT	44 & 121	frameshift	1
				del 1	1079, 1080 or 1081	9	3'-untranslated	TCTT-AA(A)-CCAC	31	none	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 1	1093 or 1094	9	3'-untranslated	ACTA-T(T)-GAGT	45	none	
		del 17 (frameshift)	yes		595-611	6	coding	AACAA-(TGCAGAC ...TTCC)-TTGGT	44 & 121	frameshift	1
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		del 17 (frameshift)	yes		595-611	6	coding	AACAA-(TGCAGAC ...TTCC)-TTGGT	44 & 121	frameshift	1
				del 1	170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	
				del 1	175, 176 or 177	1	coding	GCGA-CC(C)-GCAG	34	frameshift	
				transition	307	3	coding	GACTG-(A>G)-ACGTC	47	Glu > Gly	
		del 1	yes		166 or 167	1	5'-untranslated	TCCG-T(T)-TGC	48	none	1
				del 1	175, 176 or 177	1	coding	GCGA-CC(C)-GCAG	34	frameshift	
				del 1	260, 261 or 262	2	coding	GAGGA-TT(T)-GGAAA	49	frameshift	
				transition	315	3	coding	GTCTT-(G>A)-CTCGAG	50	Ala > Thr	
HCT116	Doxycycline	del 1	yes		145, 146 or 147	1	5'-untranslated	GTCAG-CC(C)-GCGCG	51	none	1
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 1	yes		145, 146 or 147	1	5'-untranslated	GTCAG-CC(C)-GCGCG	51	none	1
				del 1	153 or 154	1	5'-untranslated	GCGCG--C(C)-GGCCG	53	none	
				del 2	602, 603 or 604	6	coding	CAGAC-T(TT)-GCTTT	54	frameshift	
		del 1	yes		152	1	5'-untranslated	CGCGC-(G)-CCGGC	55	none	1
				del 1	170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding' / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 1	yes		152	1	5'-untranslated	CGCGC-(G)-CCGGC	55	none	1
				del 1	155 or 156	1	5'-untranslated	GCGCC-G(G)-CCGGC	56	none	
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 1	yes		153 or 154	1	5'-untranslated	GCGCG--C(C)-GGCCG	53	none	1
				del 1	157 or 158	1	5'-untranslated	GCCGG-C(C)-GGCTC	57	none	
				del 1	170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	
		del 1	yes		155 or 156	1	5'-untranslated	GCGCC-G(G)-CCGGC	56	none	1
				del 1	170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 1	yes		170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	1
				del 1	174, 175 or 176	1	coding	GGCGA-CC(C)-GCAGC	58	frameshift	
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
				del 1	196 or 197	2	coding	CGTGA-T(T)-AGTGAT	59	frameshift	
		del 1	yes		182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	1
				del 1	196 or 197	2	coding	CGTGA-T(T)-AGTGAT	59	frameshift	
		del 1	yes		182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	1
				transversion	188	1	coding	GCCCT-GG(C>A)-GTGAT	60	Gly <-> Gly	
				del 1	191	1	coding	GGCGT-(C)-GTGAT	61	frameshift	
				del 1	196 or 197	2	coding	CGTGA-T(T)-AGTGAT	59	frameshift	
		del 1	yes		170 or 171	1	coding	GTTAT-G(G)-CGACC	46	frameshift	1

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 1	182, 183 or 184	1	coding	CCGCAG-CCC)-TGGCG	52	frameshift	
				del 1	196 or 197	2	coding	CGTGA-T(T)-AGTGAT	59	frameshift	
				del 1	227, 228 or 229	2	coding	CTTGA-TT(T)-ATTTG	62	frameshift	
				del 1	231, 232, 233 or 234	2	coding	GATTTA-TTT(T)-GCATA	63	frameshift	
		ins 1	yes		186, 187 or 188	1	coding	GCCCT-GG(G)-CGTCG	64	frameshift	1
				del 1	190	1	coding	TGGCG-(T)-CGTGAT	65	frameshift	
				del 1	227, 228 or 229	2	coding	CTTGA-TT(T)-ATTTG	62	frameshift	
				del 1	231, 232, 233 or 234	2	coding	GATTTA-TTT(T)-GCATA	63	frameshift	
		del 1	yes		188	1	coding	CCTGG-(C)-GTCGT	66	frameshift	1
				del 1	263 or 264	2	coding	GATTT-G(G)-AAAGG	67	frameshift	
				ins 1	374, 375, 376, 377, 378 or 379	3	coding	CTCAA-GGGGGG(G)-CTATA	68	frameshift	
				transversion	596	6	coding	ACA-AT(G>C)-CAGAC	69	Met > Ile	
				ins 1	601	6	coding	GCAGA-(G)-CTTTG	70	frameshift	
		del 1	yes		260, 261 or 262	2	coding	GAGGA-TT(T)-GGAAA	49	frameshift	1
				ins 1	296, 297 or 298	2	coding	ATTAT-GG(G)-ACAGG	71	frameshift	
		ins 1	no		1030 , 1031 or 1032	9	3'-untranslated	TATCAG-TT(T)-CCCTT	72	none	1
HCT116	+ Doxycycline	del 1	yes		145, 146 or 147	1	5'-untranslated	GTCAG-CCC)-GCGCG	51	none	1
				transversion	182	1	coding	CCCGC-AGC>G)-CCTGG	73	Ser > Arg	
				del 1	196 or 197	2	coding	CGTGA-T(T)-AGTGAT	59	frameshift	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
		del 1	yes		153 or 154	1	5'-untranslated	GCGCG-C(C)-GGCCG	74	none	1
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 1	yes		166 or 167	1	5'-untranslated	GCTCCG-T(T)-ATGGC	75	none	1
				del 1	181	1	coding	GGGCA-(G)-CCCTG	76	frameshift	
				transition	657	7	coding	GCTTG-(C>T)TG-GTGAA	77	Leu <-> Leu	
		del 1	yes		169	1	coding	CCGTTA-(T)-GCGCAC	78	lack of initiator codon	1
				del 1	174, 175 or 176	1	coding	GGCGA-CC(C)-GCAGC	58	frameshift	
				del 1	215 or 216	2	coding	CCAGG-T(T)-TATGAC	79	frameshift	
				transition	657	7	coding	GCTTG-(C>T)TG-GTGAA>	77	Leu <-> Leu	
		del 1	yes		170 or 171	1	coding	GTTAT-GG)-CGACC	46	frameshift	1
				del 1	182, 183 or 184	1	coding	CCGCAG-CC(C)-TGGCG	52	frameshift	
		del 291 (no frameshift)	yes		195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT... TATTGT)-AATGAC	9 & 116	loss of function	1
				transversion	197	2	coding	TCGTG-AT(T>A)-AGTGAT	80	Ile <-> Ile	
		ins 1	no		235	2	coding	TATTTT-(C)-GCATA	81	frameshift	4
		ins 1	yes		235	2	coding	TATTTT-(C)-GCATA	81	frameshift	2
				transversion	400	3	coding	GCTGAC-CCT>A)C-CTGGAT	82	Leu > Gln	
				ins 1	407, 408 or 409	3	coding	CTGGA-TT(T)-ACATC	83	frameshift	
		ins 1	yes		235	2	coding	TATTTT-(C)-GCATA	81	frameshift	1
				ins 1	406 or 407	3	coding	GCTGG-A(A)-TTACA	84	frameshift	
		ins 1	yes		235	2	coding	TATTTT-(C)-GCATA	81	frameshift	1
				ins 1	410 or 411	3	coding	GATTA-C(C)-ATCAA	85	frameshift	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
		ins 1	yes		235	2	coding	TATTTT-(C)-GCATA	81	frameshift	1
				transversion	536	4	coding	TCTC-TC(A>C)-ACTTT	86	Ser <-> Ser	
		ins 1	no		239 or 240	2	coding	TGCAT-A(A)-CCTAA	87	frameshift	1
		ins 1	no		240, 241 or 242	2	coding	GCATA-CC(C)-TAATC	88	frameshift	1
		del 184 (frameshift)	no		302-485	whole 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	1
		tranversion	yes		409	3	coding	TGGAT-T(A>T)C-ATCAA	89	Tyr > Phe	1
				transition	606	6	coding	GACTTTG-(C>T)TT-TCCTT	90	Leu > Phe	
		ins 1	yes		410	3	coding	GATTA-C(C)-ATCAA	85	frameshift	1
				transition	657	7	coding	GCTTG-(C>T)TG-GTGAA	77	Leu <-> Leu	
		transition	yes		476	3	coding	GACTG-AA(G>A)-AGCTA	91	Lys <-> Lys	1
				ins 1	659, 660 or 661	7	coding	TTGCT-GG(G)-TGAAA	92	frameshift	
				ins 1	662 or 663	7	coding	CTGGT-G(G)-AAAAG	93	frameshift	
				transition	667	7	coding	TGAAA-A(G>A)G-ACCCC	94	Arg > Lys	
				transversion	674	7	coding	GGACC-CC(A>C)-CGAAG	95	Pro <-> Pro	
				transversion	CCCCA-C(G>C)A-AGTGT	7	coding	CCCCA-C(G>C)A-AGTGT	96	Arg > Pro	
		no mutation in CDS			del 1	196 or 197	1				2
		no mutation in CDS	yes								1
				del 1	157 or 158	1	5'-untranslated	GCCGG-C(C)-GGCTC	57	none	
				del 1	1057 or 1058	9	3'-untranslated	TTAAC-C(C)-GTAAA	97	none	
		no mutation in CDS	yes								1

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding' / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 1	157 or 158	1	5'-untranslated	GCCGG-C(C)-GGCTC	57	none	
				transition	866	9	3'-untranslated	GTCCGA(T>C)TGACA	98	none	
MDA-MB-231	- Doxycycline	del 47 (frameshift)	no		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	5
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	1
				del 291 (no frameshift)	195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	1
				transition	365	3	coding	CCCTC-TG(T>C)-GTGCT	100	Cys <-> Cys	
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	1
				del 1	1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	
		del 1	yes		1038, 1039 or 1040	9	3'-untranslated	CCTTT-GG(G)-CGGAT	101	none	1
				del 1	1072 or 1073	9	3'-untranslated	AAAAA-T(T)-CTCTT	102	none	
		del 1	yes		1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	1
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		no mutation									1
MDA-MB-231	+ Doxycycline	del 47 (frameshift)	no		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	12
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	1

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding* / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
				del 107 (frameshift)		whole 2	coding	GTCGTG-(ATTAGT...GGACAG)-GACTGA	103 & 123	loss of function	
				del 291 (no frameshift)	195-485	whole 2 + 3	coding	GTCGTG-(ATTAGT...TATTGT)-AATGAC	9 & 116	loss of function	
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	1
				transition	220	2	coding	GGTTAT-G(A>G)C-CTTGAT	104	Asp > Gly	
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
				del 1	1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	
				del 1	1079, 1080 or 1081	9	3'-untranslated	TCTT-AA(A)-CCAC	31	none	
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	2
				del 1	1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		del 47 (frameshift)	yes		653-699	whole 7	coding	CGCAAG-(CTTGCT...CAGACT)-TTGTTG	99 & 122	loss of function	2
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	
		transversion	yes		311	3	coding	CTGAA-CG(T>A)-CTTGC	105	Arg <-> Arg	1
		transition			349	3	coding	GCCAT-C(A>G)C-ATTGT	106	His > Arg	1
				transition	357	3	coding	TTGTA-(G>A)CC-CTCTG	107	Ala > Thr	
				transversion	747	8	coding	CCCTT-(G>T)AC-TATAA	108	Asp > Tyr	

Figure 12 cont.

Cellules	Treatment	Mutation type	Associated mutations	Associated mutation type	Nt positions	Exons	Coding' / Untranslated region	Sequence context	SEQ ID NO:	Expected phenotypic change	Number of occurrences
		transition	no		357	3	coding	TTGTA-(G>A)CC-CTCTG	107	Ala > Thr	1
		transition	yes		458	3	coding	TGACT-GT(A>G)-GATTT	109	Val <> Val	1
				del 1	992, 993 or 994	9	3'-untranslated	TTCCT-AA(A)-CTGTT	110	none	1
		transition	yes		570	6	coding	TGGAA-(G>A)AT-ATAAT	111	Asp > Asn	1
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AATG-AAAAA(A)-TTCT	13	none	1
				del 1	1082 or 1083	9	3'-untranslated	TTAAA-C(C)-ACAGC	112	none	1
		del 1	yes		1061, 1062 or 1063	9	3'-untranslated	TGT-AA(A)-TGA	12	none	1
				del 1	1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AAATG-AAAAA(A)-TTCT	113	none	
				del 1	1100, 1101 or 1102	9	3'-untranslated	GAGTG-AA(A)-CATTG	114	none	
		del 1	yes		1066, 1067, 1068, 1069, 1070 or 1071	9	3'-untranslated	AAATG-AAAAA(A)-TTCTCT	115	none	1
				del 1	1100, 1101 or 1102	9	3'-untranslated	GAGTG-AA(A)-CATTG	114	none	1

Figure 12 cont.

Table S3	
Factors	**Main function**
APC	Predicted target of *miR-155* (www.targetscan.org). APC is a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway. It is also involved in cell migration and adhesion, transcriptional activation, and apoptosis.
BACH1	Involved in DNA replication checkpoint control.
CUTL1 / CUX1	Dual function, acts as an oncogene or tumour suppressor gene depending on the cellular context. Together with E2F1 they regulate coordinated expression of the mitotic complex genes Ect2, MgcRacGAP, and MKLP1 in S phase.
FADD	Adaptor molecule that interacts with various cell surface receptors and mediates cell apoptotic signals.
FOXO3	Triggers apoptosis through expression of many genes involved in cell death.
JARID2	Negatively regulates cell proliferation and survival, also part of methyltransferase complex.
KGF / FGF-7	Epithelial specific growth factor, it is down regulation by *miR-155* results in increased cell migration.
RhoA	Ectopic expression of miR-155 reduced RhoA protein and disrupted tight junction formation in mammary cells.
RIP1	Induces necrotic cell death downstream of TNF signaling.
SHIP1 / INPP5D	Negative regulator of cell proliferation and survival. It is down regulation by *miR-155* results in activation of Akt.
SMAD1	BMP signalling cascade. It is targeting by *miR-155* results in BMP induction of the cyclin-dependent kinase inhibitor p21 and it reverses BMP-mediated cell growth inhibition.
SMAD5	Same as SMAD1
HIVEP2	Same as SMAD1
MYO10	Same as SMAD1
SOCS1	Negative regulator of cytokine signalling. It is down regulation by *miR-155* results in constitutive activation of STAT3 through the JAK pathway.
TP53INP	Proapoptotic stress-induced p53 target gene.

Figure 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/28016

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/7088, C12N 5/10(2012.01)
USPC - 435/375; 435/455
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)
USPC: 435/375; 435/455

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/375; 435/455; 514/44A; 514/44R (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PubWEST (USPT, EPAB, JPAB, PGPB); Google Scholar
Search terms: miR-155, antagomir-155, antagonist miR-155, WEE1 kinase, SHIP1, FOXO3, KGF, FADD, SMAD1, MYO10, administer, transfect

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUTZ et al., Down-Regulation of Wee1 Kinase by a Specific Subset of microRNA in Human Sporadic Pituitary Adenomas. J Clin Endocrinol Metab, October 2010, Vol 95, No 10, Pages E181-E191. Especially pg E188 fig 4 A,B, D; pg E188 col 2 para 2.	1
X --- Y	O'CONNELL et al., Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Nat Acad Sci, 28 April 2009, Vol 106, No 17, Pages 7113-7118. Especially pg 7113 col 2 para 4 continued to pg 7114 col 1 para 1	8 --- 9
Y	MARTIN et al., MicroRNA-155 regulates human angiotensin II type 1 receptor expression in fibroblasts. J Biol Chem, 7 July 2006, Vol 281, No 27, Pages 18277-18284. Especially pg 18282 col 1 para 2 continued to pg 18282 col 2 para 1; pg 18282 fig 5 A, C.	9

☐ Further documents are listed in the continuation of Box C. ☐

* 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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2012 (18.07.2012)	03 AUG 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/28016

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.: 10-26 and 30
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:

Group I: Claims 1, 8, and 9 drawn to a method of modulating the expression of one or more genes in a target cell by administering a microRNA-155 (miR-155) oligonucleotide

Group II: Claims 2, 6, and 9, drawn to a method of modulating mutation of a cell or treating a cancer by administering a miR-155 oligonucleotide

Group III: Claims 3-5, 7, 9, 27-29, and 31, drawn to a method of reducing the spontaneous mutation rate of a cell by reducing endogenous levels of miR-155, as for example by administering a miR-155 anti-sense compound, and to such compounds

Group IV: Claim 32, drawn to a method of identifying an agent that can be used to inhibit an inflammatory-related cancer

---please see continuationon extra sheet---

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, 8, 9

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 12/28016

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

The inventions listed as Groups I-IV 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:

The shared technical feature of the inventions listed as Groups I-IV is miR-155. This shared technical feature fails to provide a contribution over the prior art, as evidenced by the article entitled "MicroRNAs in Immune Regulation - Opportunities for Cancer Immunotherapy" by Okada et al. (published in Int J Biochem Cell Biol, August 2010 , Vol 42, No 8, pages 1256-1261; hereinafter 'Okada'), which discloses miR-155 (abstract; p 1259, left col, para 4). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

The shared technical feature of the inventions listed as Groups II-IV is the association of miR-155 with cancer and the administration of an agent that modulates miR-155 to treat cancer, especially inflammatory-related cancer. This shared technical feature fails to provide a contribution over Okada which further discloses that overexpression of miR-155 in hematopoietic cells induce malignancy or myeloproliferative disorder, and is associated with human acute myeloid leukemia (p 1259, left col, para 4). Okada further teaches that miR-155 also plays key roles in both innate and adaptive immune responses (p 1259, left col, para 4) and that transgenic expression of miR-155 could reverse cancer-induced skewing towards Th2 polarization and promote effective Th1 type anti-tumor immune responses (p 1259, left col, para 4). Okada concludes that modulation of miRs in tumor cells may represent a viable approach to cancer therapy (p 1260, left col, para 1). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

The shared technical feature of the inventions listed as Groups I and IV is a target gene of miR-155. This shared technical feature also fails to provide a contribution over Okada. Okada discloses that the Sh2 domain-containing inositol-5-phosphatase(SHIP)1 gene is directly regulated by miR-155 (p 1259, left col, para 4). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

Further, the special technical feature of the inventions listed as Group I is the modulation of expression of a miR-155 target gene. This special technical feature is not shared by the inventions of Groups II-IV. The special technical feature of the inventions listed as Group III is a miR-155 anti-sense compound. This special technical feature is not shared by the inventions of Groups I-II and IV. The special technical feature of the inventions listed as Group IV is the method steps for screening for a therapeutic agent. This special technical feature is not shared by the inventions of Groups I-III.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. Without a shared special technical feature, the inventions of Groups I-IV lack unity with one another.