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(57) **ABSTRACT**

The present invention relates to a method for modulating the expression and/or activity of an epidermal growth factor receptor (EGFR) ligand in a cell or tissue, the method comprising contacting the cell or tissue with a miR-7 miRNA, a pre-cursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA.

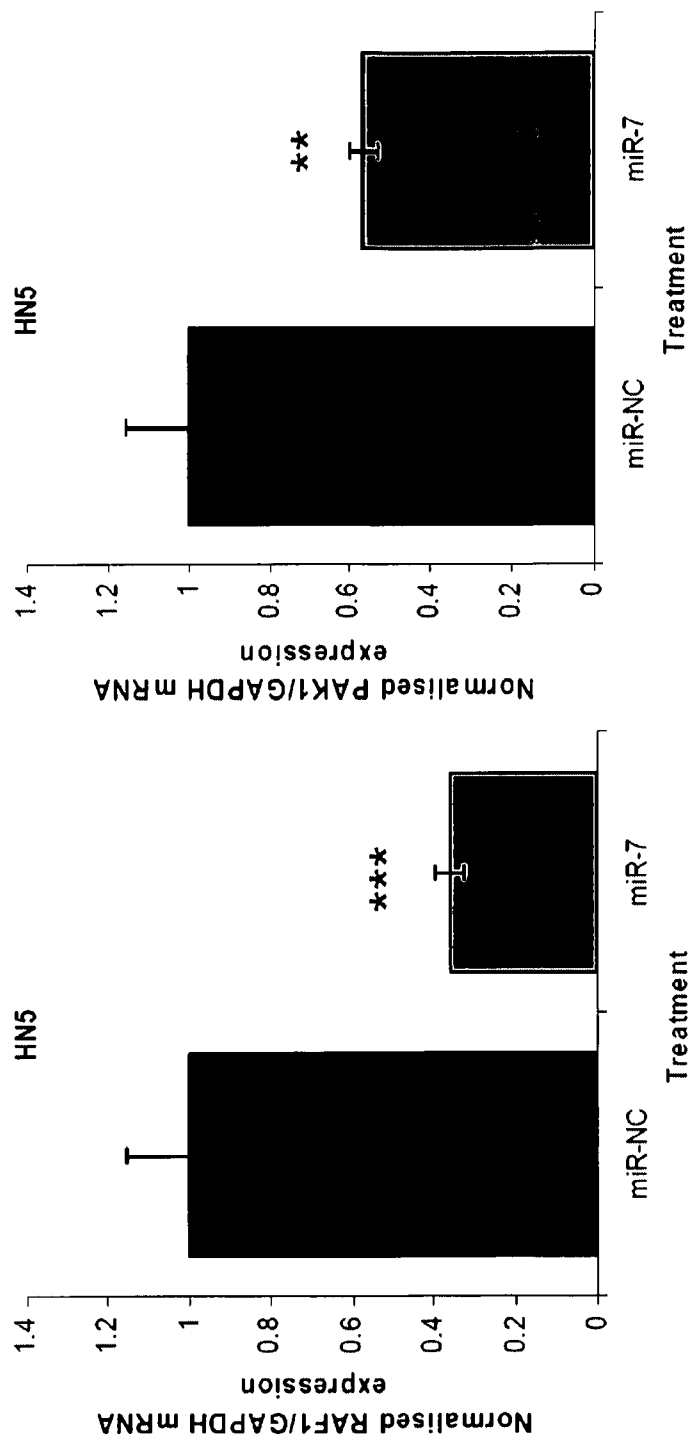


FIGURE 1

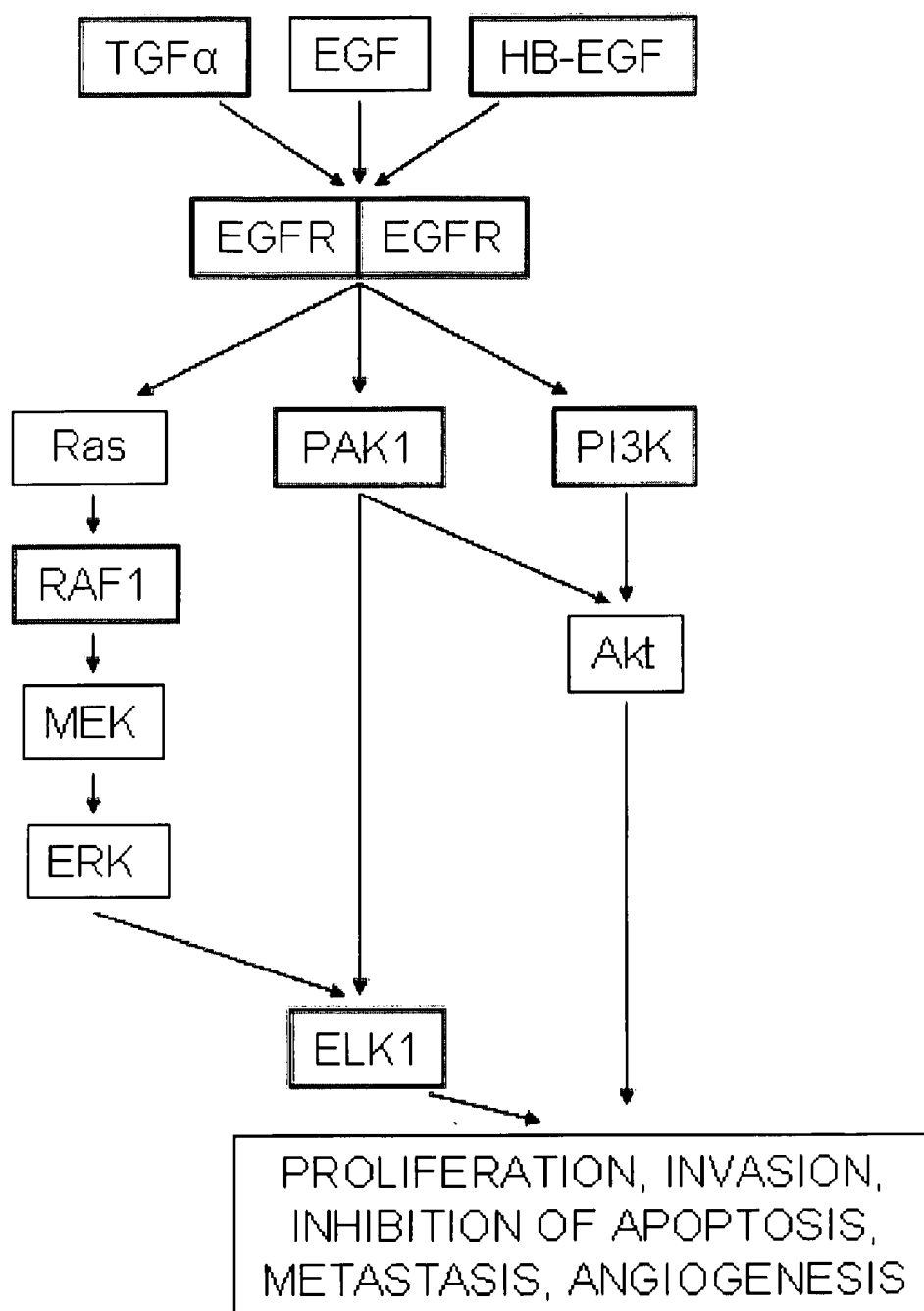


FIGURE 2

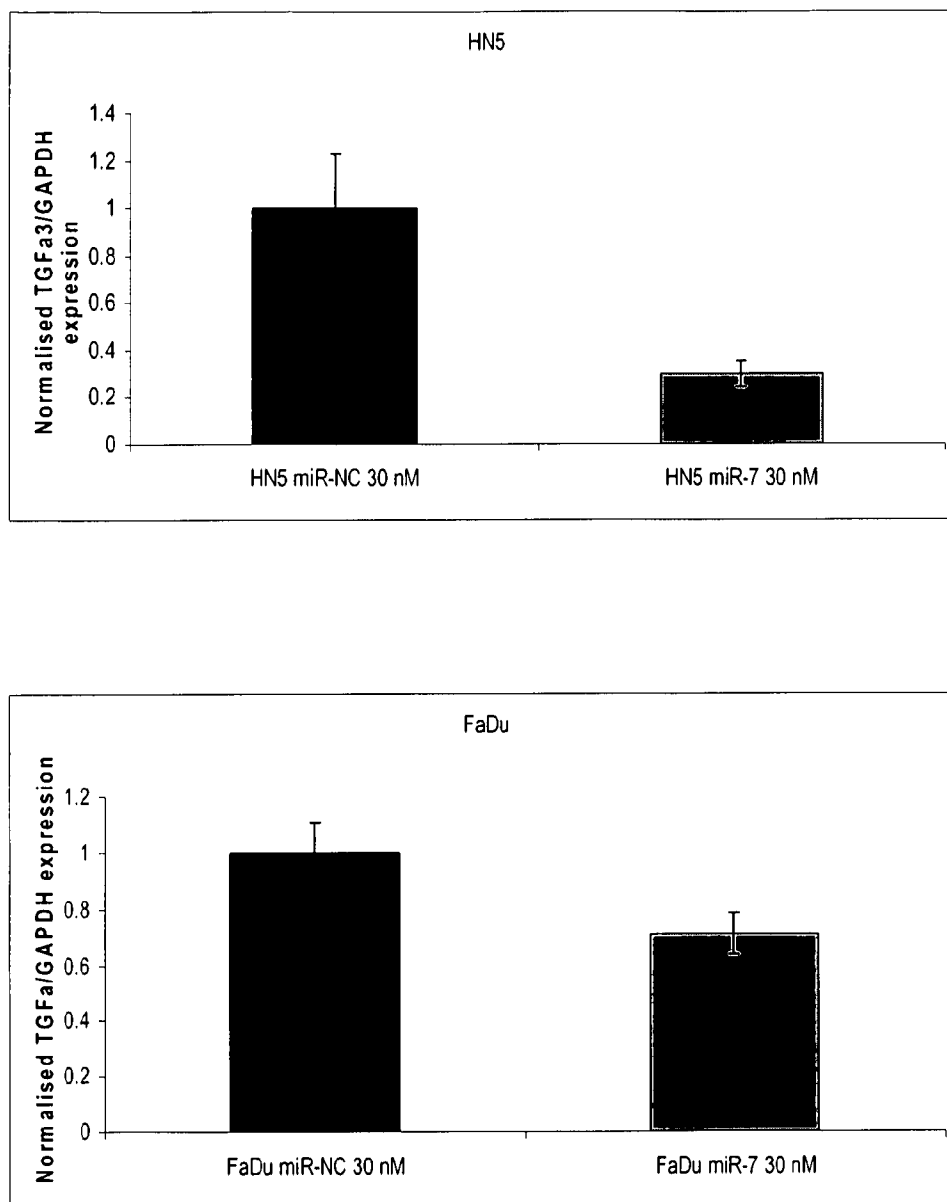


FIGURE 3

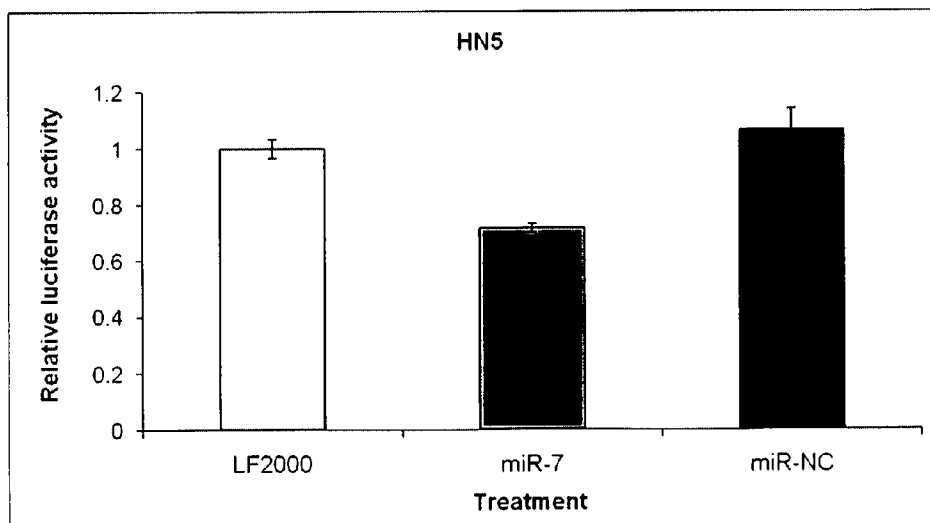
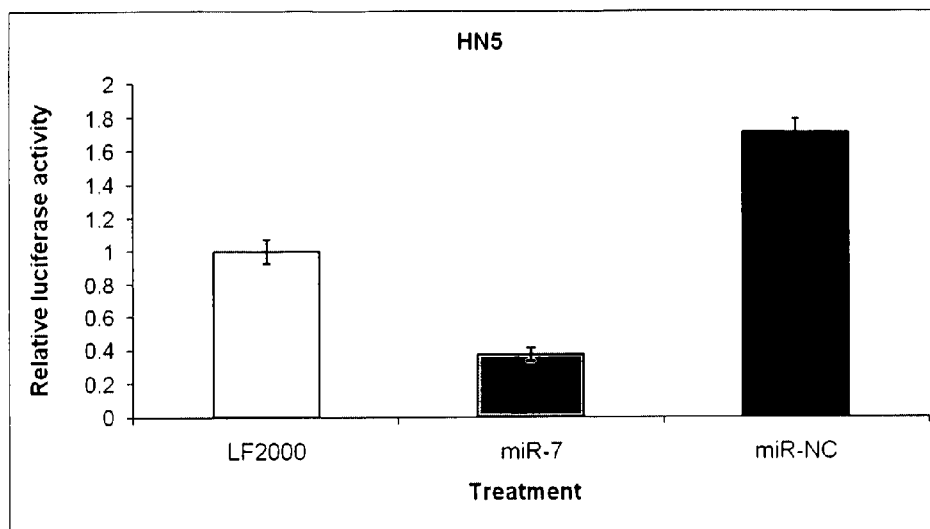


FIGURE 4

MODULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR LIGANDS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of Australian Provisional Patent Application No. 2009905758 filed 24 Nov. 2009, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates generally to methods for modulating the activity and/or expression of epidermal growth factor receptor (EGFR) ligands such as transforming growth factor- α (TGF α). In particular, the present invention relates to methods for modulating EGFR ligand expression and/or activity utilizing miRNA and to methods for treating conditions associated with dysregulated expression and/or activity of EGFR ligands.

BACKGROUND

[0003] The epidermal growth factor (EGF) family includes EGF, transforming growth factor- α (TGF α), heparin binding EGF-like growth factor (HB-EGF), amphiregulin (AR), epiregulin (EPR), betacellulin (BIC), epigen and the neuregulins (NRG)-1, NRG-2, NRG-3 and NRG-4. Members of the EGF family are ligands for the epidermal growth factor receptor (EGFR), a ligand activated receptor tyrosine kinase and member of the ErbB receptor family. EGFR ligands are important in many cellular signalling pathways and dysregulation of EGFR ligands is apparent in a number of diseases. For example, in non-small-cell lung cancer, increased plasma TGF α is associated with erlotinib resistance and increased amphiregulin is an indicator of poor prognosis. TGF α is involved in the stimulation and control of cell proliferation and differentiation and is produced in normal tissues by macrophages, hepatocytes, platelets and keratinocytes. TGF α is also produced by a number of carcinomas and upregulation of expression of TGF α has been found in many forms of cancer accordingly TGF α is a target of anticancer therapies.

[0004] EGFR is a target for anti-cancer therapies as it is over expressed in a large number of cancers. For example, more than 80% of all head and neck cancers (HNCs) overexpress EGFR. Signalling from EGFR results in activation of downstream phosphoinositide 3-kinase (PI3K)/Akt and Ras/Raf/MAPK pathways that promote tumour proliferation, invasion, metastasis, angiogenesis and apoptosis inhibition which all contribute to cancer progression and poor patient prognosis. However, clinical trials of tyrosine kinase inhibitors targeting EGFR, including gefitinib and erlotinib and the monoclonal antibody cetuximab, have shown only limited effectiveness in a range of cancers including HNCs. Similarly, anti-Akt agents also have limited therapeutic effectiveness.

[0005] microRNAs (miRNAs) are an abundant class of highly conserved, small (typically 21-25 nucleotides) endogenous non-protein-coding RNAs that negatively regulate gene expression. miRNAs bind specific 3'-untranslated regions (3'-UTRs) within messenger RNAs (mRNA) to induce mRNA cleavage or translational repression. Individual miRNAs typically bind incompletely to their cognate target messenger RNA (mRNA) and a unique miRNA may regulate the expression of multiple genes.

[0006] miRNAs are generated from RNA precursors (pri-miRNAs) that usually contain several hundred nucleotides transcribed from regions of non-coding DNA. Pri-miRNAs are processed in the nucleus by RNase III endonuclease to form stem-loop precursors (pre-miRNAs) of approximately 70 nucleotides. Pre-miRNAs are actively transported into the cytoplasm where they are further processed into short RNA duplexes, typically of 21-23 bp. The functional miRNA strand dissociates from its complementary non-functional strand and locates within the RNA-induced-silencing-complex (RISC). (Alternatively, RISC can directly load pre-miRNA hairpin structures.) miRNAs bind the 3'UTRs of target mRNAs and important in this binding is a so-called 'seed' region of approximately 6-7 nucleotides near the 5' end of the miRNA (typically nucleotide positions 2 to 8). The role of the 3' end is less clear. miRNA-induced regulation of gene expression is typically achieved by translational repression, either degrading proteins as they emerge from ribosomes or 'freezing' ribosomes, and/or promoting the movement of target mRNAs into sites of RNA destruction.

[0007] miRNAs are crucial to many normal cellular functions and are involved in processes such as stem cell division, embryonic development, cellular differentiation, inflammation and immunity. Increasingly, specific miRNAs, and expression patterns and altered regulation of expression of individual miRNAs, are also being implicated in a variety of disease conditions, including cancer. Some miRNAs are altered in cancer and may act as tumour suppressors or oncogenes. For example, let-7d (a member of the let-7 family of miRNAs) regulates RAS oncogene expression in normal head and neck tissue although let-7d expression is reduced in many head and neck cancers causing upregulation of RAS expression, increased tumour growth and reduced patient survival. In contrast, miR-184 expression is upregulated in tongue squamous cell carcinoma, leading to increased expression of the oncogene c-Myc, increased cell proliferation and tumour growth.

SUMMARY

[0008] In a first aspect the present invention provides a method for modulating the expression and/or activity of an epidermal growth factor receptor (EGFR) ligand in a cell or tissue, the method comprising contacting the cell or tissue with a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA.

[0009] The miR-7 miRNA may be hsa-miR-7 and may comprise the nucleotide sequence set forth in SEQ ID NO:1. The miR-7 miRNA precursor may be selected from hsa-miR-7-1, hsa-miR-7-2 and hsa-miR-7-3, and may comprise a sequence as set forth in any one of SEQ ID Nos:2 to 4.

[0010] Typically contacting the cell or tissue with the miRNA reduces or inhibits the expression and/or activity of the EGFR ligand. Contacting the cell or tissue with an antagonist of the miRNA may increase the expression and/or activity of the ligand.

[0011] Typically the 3' untranslated region of the mRNA encoding the EGFR ligand comprises one or more miRNA binding sites. Typically the miRNA binds to one or more of the sites. The binding sites may comprise sequences as set forth in any of SEQ ID Nos:6 to 11, or variants thereof.

[0012] The EGFR ligand may be selected from TGF α and HB-EGF. In a particular embodiment the EGFR ligand is TGF α . The mRNA encoding the TGF α may comprise a 3'

untranslated region comprising the sequence set forth in SEQ ID NO:12, or a variant thereof.

[0013] The miRNA or antagonist thereof may be contacted with the cell or tissue in vivo or ex vivo. The subject containing the cell or tissue, or from which the cell or tissue is derived, may suffer from, be predisposed to, or otherwise at risk of developing a disease or condition associated with dysregulated expression or activity of the EGFR ligand. The disease or condition may be associated with upregulated or elevated expression or activity of the EGFR ligand. The disease or condition may be a cancer. The cancer may be, for example, a head and neck cancer, a glioblastoma, pancreatic cancer, colon cancer, lung cancer including non small cell lung cancer, prostate cancer, breast cancer, liver cancer, neuroblastoma or melanoma.

[0014] In a second aspect the present invention provides a method for modulating the expression and/or activity of an epidermal growth factor receptor (EGFR) ligand in a cell or tissue, the method comprising contacting the cell or tissue with an agent capable of stimulating or enhancing the expression or activity of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA, whereby the miRNA the expression or activity of which is stimulated or enhanced modulates the expression and/or activity of the EGFR ligand.

[0015] In a third aspect the present invention provides a method for treating a disease or condition associated with dysregulated expression or activity of an EGFR ligand in a subject, comprising administering to the subject an effective amount of a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA, whereby the miRNA modulates the expression or activity of the EGFR ligand.

[0016] In a particular embodiment the disease or condition is associated with upregulated or elevated expression or activity of the EGFR ligand and the subject is administered an effective amount of a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA.

[0017] The miR-7 miRNA may comprise the nucleotide sequence as set forth in SEQ ID NO:1. The miR-7 miRNA precursor may comprise a sequence as set forth in any one of SEQ ID Nos:2 to 4.

[0018] Typically contacting the cell or tissue with the miRNA reduces or inhibits the expression or activity of the EGFR ligand. Contacting the cell or tissue with an antagonist of the miRNA may increase the expression or activity of the ligand.

[0019] Typically the 3' untranslated region of the mRNA encoding the EGFR ligand comprises one or more miRNA binding sites. Typically the miRNA binds to one or more of the sites. The binding sites may comprise sequences set forth in any of SEQ ID Nos:6 to 11, or variants thereof.

[0020] The EGFR ligand may be selected from TGF α and HB-EGF. In a particular embodiment the EGFR ligand is TGF α . The mRNA encoding the TGF α may comprise a 3' untranslated region comprising the sequence set forth in SEQ ID NO:12, or a variant thereof.

[0021] The disease or condition may be a cancer. The cancer may be, for example, a head and neck cancer, a glioblastoma, pancreatic cancer, colon cancer, lung cancer including non small cell lung cancer, prostate cancer, breast cancer, liver cancer, neuroblastoma or melanoma.

[0022] The miRNA may be co-administered with one or more additional therapeutic agents suitable for the treatment of the disease or condition. In particular embodiments the additional therapeutic agent is a tyrosine kinase inhibitor or monoclonal antibody, such as an inhibitor of EGFR. Co-administration may comprise simultaneous or sequential administration of the miRNA and the one or more additional agents. For simultaneous administration the miRNA and the one or more additional agents may be formulated in a single pharmaceutical composition together with pharmaceutically acceptable carriers, excipients or adjuvants.

[0023] In a fourth aspect the present invention provides a method for treating a disease or condition associated with upregulated or elevated expression or activity of an EGFR ligand in a subject, comprising administering to the subject an effective amount of an agent capable of stimulating or enhancing the expression or activity of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA, whereby the miRNA the expression or activity of which is stimulated or enhanced modulates the expression and/or activity of the EGFR ligand.

[0024] A fifth aspect of the present invention provides the use of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA for the manufacture of a medicament for the treatment of a disease, or condition associated with upregulated or elevated expression or activity of an EGFR ligand, whereby the miRNA modulates the expression or activity of the EGFR ligand.

[0025] A sixth aspect of the invention provides the use of an agent capable of stimulating or enhancing the expression or activity of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA, for the manufacture of a medicament for the treatment of a disease or condition associated with upregulated or elevated expression or activity of an EGFR ligand, whereby the miRNA the expression or activity of which is stimulated or enhanced modulates the expression and/or activity of the EGFR ligand.

[0026] A seventh aspect of the present invention provides a method for preventing or reducing tumour growth, cancer metastasis or reoccurrence in a subject, wherein the tumour or cancer is associated with upregulated or elevated expression or activity of an EGFR ligand, the method comprising administering to the subject an effective amount of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA, whereby the miRNA modulates the expression or activity of the EGFR ligand.

[0027] An eighth aspect of the present invention provides a method for preventing or reducing tumour growth, cancer metastasis or reoccurrence in a subject, wherein the tumour or cancer is associated with upregulated or elevated expression or activity of an EGFR ligand, the method comprising administering to the subject an effective amount of an agent capable of stimulating or enhancing the expression or activity of a miR-7 miRNA, a precursor or variant thereof, or a miRNA comprising a seed region comprising the sequence GGAAGA, whereby the miRNA the expression or activity of which is stimulated or enhanced modulates the expression and/or activity of the EGFR ligand.

[0028] A ninth aspect of the present invention provides a method for evaluating the efficacy of a treatment regime in a

subject suffering from a disease or condition associated with dysregulated expression or activity of an EGFR ligand, the method comprising:

[0029] (a) treating the subject with a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA, for a period sufficient to evaluate the efficacy of the regime;

[0030] (b) obtaining a biological sample from the subject;

[0031] (c) determining the level of expression and/or activity of the EGFR ligand in the sample;

[0032] (d) repeating steps (b) and (c) at least once over a period of time of treatment; and

[0033] (e) determining whether the expression and/or activity of the EGFR ligand change over the period of time,

[0034] wherein a change in the level of expression and/or activity of the EGFR ligand is indicative of the efficacy of the treatment regime.

In particular embodiments the EGFR ligand is TGF α .

BRIEF DESCRIPTION OF THE FIGURES

[0035] Embodiments of the invention are described and exemplified herein, by way of non-limiting example only, with reference to the following figures.

[0036] FIG. 1 shows the experimental validation of genes down-regulated by miR-7 by cDNA analysis. Quantitative RT-PCR of RAF1 and PAK1 mRNA expression in HN5 cells 24 h after transfection with 30 nM miR-7 or miR-NC precursor. RAF1 and PAK1 mRNA expression was normalised to GAPDH mRNA expression and is shown as a ratio of miR-NC-transfected cells (\pm SD) using the $2^{-\Delta\Delta CT}$ method. Bars represent mean mRNA expression (\pm SD) compared to miR-NC. Data representative of a single experiment. *** and ** indicate a significant difference from miR-NC treated cells ($p < 0.001$ and $p < 0.01$ respectively).

[0037] FIG. 2 shows a model of miR-7 (SEQ ID NO:1) action on EGFR signalling in HNC cells. Schematic model using cDNA microarray data showing miR-7 regulation of EGFR signalling via multiple targets. Genes found to be down-regulated by miR-7 as per the cDNA microarray are outlined in bold.

[0038] FIG. 3 shows normalised relative expression levels for TGF α mRNA as determined by quantitative RT-PCR in HN5 cells (A) and FaDu cells (B) in the presence of 30 nM miR-7 (SEQ ID NO:1) or miR-NC. Data are representative of three independent experiments. *** indicates a significant difference from miR-NC treated cells ($p < 0.001$).

[0039] FIG. 4A shows luciferase reporter assays to verify activity of miR-7 upon the consensus miR-7 target site. HN5 cells were transfected with consensus miR-7 target site firefly luciferase plasmid and 1 nM miR-7 or miR-NC precursor. Relative luciferase expression (firefly normalised to *Renilla*) values are shown as a ratio of vehicle (Lipofectamine 2000, LF) only. Bars represent standard deviation (SD). Data are representative of a single experiment. *** indicates a significant difference from vehicle (Lipofectamine 2000, LF)-treated reporter vector ($p < 0.001$).

[0040] FIG. 4B shows luciferase reporter assays to verify activity of miR-7 upon a miR-7 target site within the full-length wild-type TGF α 3'-UTR 24 h after transfection. HN5 cells were transfected with wild-type TGF α miR-7 target site number 5 3'-UTR firefly luciferase plasmid and 0.5 nM miR-7 or miR-NC precursor. Relative luciferase expression (firefly normalised to *Renilla*) values are shown as a ratio of vehicle

(Lipofectamine 2000, LF) only. Bars represent standard deviation (SD). Data are representative of a single experiment. ** indicates a significant difference from vehicle (Lipofectamine 2000, LF)-treated reporter vector ($p < 0.01$).

[0041] A listing of nucleotide sequences corresponding to the sequence identifiers referred to in the specification is provided. The nucleotide sequences of mature human miR-7, human miR-7 precursors and seed region are set forth in SEQ ID Nos:1 to 5. The sequence of a consensus miR-7 binding site is provided in SEQ ID NO:6. Predicted miR-7 binding sites within the human TGF α 3' untranslated region are set forth in SEQ ID Nos:7 to 11, while the 3' untranslated region of human TGF α 3' is provided in SEQ ID NO:12. SEQ ID Nos:13 to 23 provide sequences of oligonucleotides used in the present study as exemplified herein.

DEFINITIONS

[0042] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a nucleic acid molecule” includes a plurality of nucleic acid molecules, and a reference to “a cell” is a reference to one or more cells, and so forth.

[0043] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0044] In the context of this specification, the term “activity” as it pertains to a protein, polypeptide or polynucleotide means any cellular function, action, effect or influence exerted by the protein, polypeptide or polynucleotide, either by a nucleic acid sequence or fragment thereof, or by the protein or polypeptide itself or any fragment thereof.

[0045] It will be understood that as used herein the term “expression” may refer to expression of a polypeptide or protein, or to expression of a polynucleotide or gene, depending on the context. The polynucleotide may be coding or non-coding (e.g. miRNA). Expression of a polynucleotide may be determined, for example, by measuring the production of RNA transcript levels. Expression of a protein or polypeptide may be determined, for example, by immunoassay using an antibody(ies) that bind with the polypeptide.

[0046] The terms “modulate,” “modulation,” “modulating,” “modulator” and grammatical equivalents as used herein refer to the act of, and to agents described herein which are capable of, affecting directly or indirectly the activity and/or expression level of EGF receptor (EGFR) ligands such that the activity or expression is altered when compared to “wild-type” activity or expression i.e. activity or expression before contacting with an agent of the present invention. The term “indirectly” in the context of modulation of EGFR ligand activity or expression refers to the mode of action of an agent, wherein the effect is mediated via an intermediary molecule rather than through direct contact with EGFR ligand. In contrast, the term “directly” in the context of modulation of EGFR ligand activity or expression refers to an agent that interacts with the EGFR ligand or its mRNA by, for example, binding to the 3'-UTR.

[0047] In the context of this specification, the term “antagonist” refers to any agent capable of blocking or inhibiting the expression and/or activity of an EGFR ligand. Thus, the antagonist may operate to prevent transcription or post-tran-

scriptional processing of the EGFR ligand or otherwise inhibit the activity of the EGFR ligand in any way, via either direct or indirect action. The antagonist may for example be nucleic acid, peptide, any other suitable chemical compound or molecule or any combination of these. Additionally, it will be understood that in indirectly impairing the activity of the EGFR ligand, the antagonist may alter the activity and/or expression of other cellular molecules which may in turn act as regulators of the activity and/or expression of activity of the EGFR ligand itself. Similarly, the antagonist may alter the activity of molecules which are themselves subject to regulation or modulation by the EGFR ligand.

[0048] As used herein the term “oligonucleotide” refers to a single-stranded sequence of ribonucleotide or deoxyribonucleotide bases, known analogues of natural nucleotides, or mixtures thereof. An “oligonucleotide” comprises a nucleic-acid based molecule including DNA, RNA, PNA, LNA or any combination thereof. An oligonucleotide that predominantly comprises ribonucleotide bases, natural or non-natural, may be referred to as an RNA oligonucleotide. Oligonucleotides are typically short (for example less than 50 nucleotides in length) sequences that may be prepared by any suitable method, including, for example, direct chemical synthesis or cloning and restriction of appropriate sequences. “Antisense oligonucleotides” are oligonucleotides complementary to a specific DNA or RNA sequence. Typically in the context of the present invention an antisense oligonucleotide is an RNA oligonucleotide complementary to a specific miRNA. The antisense oligonucleotide binds to and silences or represses, partially or fully, the activity of its complementary miRNA. Not all bases in an antisense oligonucleotide need be complementary to the ‘target’ or miRNA sequence; the oligonucleotide need only contain sufficient complementary bases to enable the oligonucleotide to recognise the target. An oligonucleotide may also include additional bases. The antisense oligonucleotide sequence may be an unmodified ribonucleotide sequence or may be chemically modified or conjugated by a variety of means as described herein.

[0049] The term “polynucleotide” as used herein refers to a single- or double-stranded polymer of deoxyribonucleotide, ribonucleotide bases or known analogues of natural nucleotides, or mixtures thereof. A “polynucleotide” comprises a nucleic-acid based molecule including DNA, RNA, PNA, LNA or any combination thereof. The term includes reference to the specified sequence as well as to the sequence complementary thereto, unless otherwise indicated. Polynucleotides may be chemically modified by a variety of means known to those skilled in the art. Thus a “polynucleotide” comprises a nucleic-acid based molecule including DNA, RNA, PNA, LNA or any combination thereof.

[0050] The term “sequence identity” or “percentage of sequence identity” may be determined by comparing two optimally aligned sequences or subsequences over a comparison window or span, wherein the portion of the polynucleotide sequence in the comparison window may optionally comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.

[0051] As used herein the term “associated with” when used in the context of a disease or condition means that the disease or condition may result from, result in, be characterised by, or otherwise associated with abnormal EGF ligand levels. Thus, the association between the disease or condition

and the abnormal EGF ligand levels may be direct or indirect and may be temporally separated.

[0052] As used herein the terms “treating” and “treatment” and grammatical equivalents refer to any and all uses which remedy a condition or symptoms, prevent the establishment of a condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of a condition or disease or other undesirable symptoms in any way whatsoever. Thus the term “treating” is to be considered in its broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery. In conditions which display or are characterized by multiple symptoms, the treatment need not necessarily remedy, prevent, hinder, retard, or reverse all of said symptoms, but may prevent, hinder, retard, or reverse one or more of said symptoms.

[0053] As used herein the term “effective amount” includes within its meaning a non-toxic but sufficient amount or dose of an agent or compound to provide the desired effect. The exact amount or dose required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact “effective amount”. However, for any given case, an appropriate “effective amount” may be determined by one of ordinary skill in the art using only routine experimentation.

[0054] The term “subject” as used herein refers to mammals and includes humans, primates, livestock animals (e.g. sheep, pigs, cattle, horses, donkeys), laboratory test animals (e.g. mice, rabbits, rats, guinea pigs), performance and show animals (e.g. horses, livestock, dogs, cats), companion animals (e.g. dogs, cats) and captive wild animals. Preferably, the mammal is human or a laboratory test animal. Even more preferably, the mammal is a human.

DETAILED DESCRIPTION

[0055] It is to be understood at the outset, that the figures and examples provided herein are to exemplify and not to limit the invention and its various embodiments

[0056] As exemplified herein the inventors have for the first time identified ligands of the epidermal growth factor receptor (EGFR) that are targets of the miRNA miR-7 and are down-regulated in cancer cell lines by miR-7. Accordingly, provided in embodiments disclosed herein are methods and compositions for the modulation of the expression and/or activity of such EGFR ligands using miR-7, precursors and variants of miR-7, miRNA bearing the miR-7 seed region, and antagonists of such miRNA. In particular embodiments methods and compositions disclosed herein are used to treat diseases and conditions associated with dysregulation of EGFR ligand expression or activity, such as cancer.

[0057] Embodiments of the invention employ, unless otherwise indicated, conventional molecular biology and pharmacology known to, and within the ordinary skill of, those skilled the art. Such techniques are described in, for example, “Molecular Cloning: A Laboratory Manual”, 2nd Ed., (ed. by Sambrook, Fritsch and Maniatis) (Cold Spring Harbor Laboratory Press: 1989); “Nucleic Acid Hybridization”, (Hames & Higgins eds. 1984); “Oligonucleotide Synthesis”, (Gait ed., 1984); Remington’s Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton, Pa., USA.; “The Merck Index”, 12th Edition (1996), Therapeutic Category and Bio-

logical Activity Index,—and “Transcription & Translation”, (Hames & Higgins eds. 1984).

[0058] Reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as, an acknowledgment or admission or any form of suggestion that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates, miRNA

[0059] Micro RNAs (miRNAs) are small non-coding RNAs which function as, regulatory molecules in plants and animals to control gene expression by binding complementary sites on mRNA. Without wishing to be bound by any theory or hypothesis, the present invention is predicated on the inventors finding that the miRNA miR-7 specifically binds the 3'-UTR of mRNA encoding EGFR ligands, especially transforming growth factor- α (TGF- α). Moreover, the inventors have surprisingly discovered that increasing the expression of miR-7 in cancer cells that express or overexpress EGFR, such as head and neck cancer cells, results in a reduced level of EGFR ligand mRNA and protein expression, G1 phase cell cycle arrest and cell death.

[0060] miRNAs bind the 3'UTRs of target mRNAs and important in this binding is a so-called ‘seed’ region of approximately 6-7 nucleotides near the 5' end of the miRNA (typically nucleotide positions 2 to 8). Accordingly, embodiments of the present invention broadly contemplate contacting cells or tissue, or administering to subjects in need thereof, one or more miRNA, at least one of which comprises the seed region of miR-7. In particular embodiments this seed region comprises the sequence GGAAGA (SEQ ID NO:5).

[0061] In particular embodiments, miR-7 is employed. The nucleotide sequence of human miR-7 is provided in SEQ ID NO:1. Additional sequence information for the miR-7 miRNA can be found at <http://microrna.sanger.ac.uk/sequences/index.shtml>. Like most miRNAs, miR-7 is highly conserved between different species. Thus, whilst typically the miRNA may be derived from the species of the subject to be treated, or constitute a sequence identical to miRNA from that species, this need not be the case in view of, for example, the high level of sequence conservation of miRNA sequences between species.

[0062] Embodiments of the invention also contemplate the administration of miRNA variants of miR-7. Variants include nucleotide sequences that are substantially similar to sequences of miRNA disclosed herein. Variants include nucleotide sequences that are substantially similar to sequences of miRNA disclosed herein. In some embodiments, the variant miRNA to be administered comprises a sequence displaying at least 80% sequence identity to the sequence of miR-7 (SEQ ID NO:1). In some embodiments, the miRNA to be administered comprises a sequence displaying at least 90% sequence identity to SEQ ID NO:1. In other embodiments, the miRNA to be administered comprises a sequence displaying at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:1. Alternatively or in addition variants may comprise modifications, such as non-natural residues at one or more positions with respect to the miR-7 sequence.

[0063] Also contemplated is the administration of a precursor molecule of miR-7 or of a miRNA comprising a seed region comprising the sequence GGAAGA. miRNAs are generated from RNA precursors (pri-miRNAs) that usually

contain several hundred nucleotides transcribed from regions of non-coding DNA. Pri-miRNAs are processed in the nucleus by RNase III endonuclease to form stem-loop precursors (pre-miRNAs) of approximately 70 nucleotides. Pre-miRNAs are actively transported into the cytoplasm where they are further processed into short RNA duplexes, typically of 21-23 bp, one of which represents the functional miRNA strand. The administration of such pri-miRNA and pre-miRNA precursors is contemplated herein, wherein the pri-miRNA or pre-miRNA is cleaved and intracellularised to generate a functional miRNA.

[0064] In addition to the full-length miR-7 molecule, such as that shown in SEQ ID NO:1, the term “miR-7” also includes fragments of a miR-7 molecule provided the fragments are functional fragments. The term “fragment” of a miRNA molecule means a portion of the full-length molecule. The size of the fragment is limited only in that it must be a functional fragment, that is, able to modulate the expression of EGFR, modulate cell growth, and/or modulate cell differentiation. Typically, it will comprise at least the seed region sequence GGAAGA (SEQ ID NO:5).

[0065] Administration of the miRNA may be directly to a subject in need of treatment, or may be ex vivo administration to cells or tissue derived from the subject. The miRNAs to be administered may be synthetically produced or naturally derived from a cellular source.

[0066] Also contemplated by embodiments of the invention is the administration of miRNAs linked to an additional agent capable of delivering the miRNA to the desired site. The additional agent may itself be capable of inhibiting the activity and/or expression of an EGFR ligand. For example, miR-7 may be conjugated to an antibody directed to a cell type known to express an EGFR ligand in order to target the miR-7 to those cells. In some embodiments the link between the miRNA and the additional agent is a cleavable link. The presence of a cleavable link allows for cleavage of the miRNA from the additional agent for example after internalisation into cells expressing an EGFR ligand.

[0067] Also contemplated by embodiments of the invention is the administration of agents capable of stimulating or enhancing the expression or activity of miRNA described herein. Such agents may be proteinaceous, non-proteinaceous or nucleic acid-based and include, for example, molecules and compounds capable of binding to the regulatory sequences of miRNA genes to thereby induce or enhance the level of endogenous expression of the miRNA. Those skilled in the art will appreciate that the scope of the invention is not so limited and any agents capable of stimulating or enhancing miRNA expression or activity are contemplated and fall within the scope of the present disclosure.

EGFR Ligands

[0068] The epidermal growth factor receptor (EGFR) family includes distinct tyrosine kinases, EGFR/HER/ ErbB1, HER2/Neu/ErbB2, HER3/ErbB3 and HER4/ErbB4. These receptors are widely expressed and are activated by a family of at least twelve ligands that induce either homo- or hetero-dimerisation of the EGFR homologues. The ligands include members of the epidermal growth factor family such as EGF, transforming growth factor- α (TGF α), heparin binding EGF-like growth factor (HB-EGF), amphiregulin (AR), epiregulin (EPR), betacellulin (BTC), epigen and the neuregulins (NRG)-1, NRG-2, NRG-3 and NRG-4. In particular embodiments of the invention the EGFR ligand, the

expression or activity of which is to be modulated, is TGF α or HB-EGF, although the scope of the present disclosure is not so limited. More particularly: the EGFR ligand is TGF α .

[0069] In embodiments in which the subject is human, the 3' UTR of TGF α mRNA typically comprises the sequence provided in SEQ ID NO:12, or a variant thereof. Variants include nucleotide sequences that are substantially similar to the sequence of SEQ ID NO:12. In some embodiments, the variant 3' UTR comprises a sequence displaying at least 80% sequence identity to the sequence of SEQ ID NO:12. In some embodiments, the 3' UTR comprises a sequence displaying at least 90% sequence identity to SEQ ID NO:12. In other embodiments, the 3' UTR comprises a sequence displaying at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:12.

[0070] As described herein the 3' UTR of mRNA encoding EGFR ligands such as TGF α typically comprise one or more motifs or miR-7 target sites and particular embodiments of the invention contemplate, the direct binding of the miRNA employed to such sites in effecting the modulation of EGFR ligand expression and/or activity. The 3' UTR of the TGF α mRNA contains 5 miR-7 target sites. The miR-7 target sites in the human TGF α 3' UTR comprise the sequences shown in SEQ ID Nos:7 to 11. Variants of such target site sequences are also contemplated, including the generalised or consensus miR-7 target site shown in SEQ ID NO:6. As for variants of the 3' UTR sequence, miRNA target site variants may display at least about 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the miRNA target site sequences shown in SEQ ID Nos:6 to it

Antagonists

[0071] Embodiments of the invention also provide for the administration of antagonists of the miRNA described herein in circumstances where it is desirable to upregulate the expression and/or activity of the target EGFR ligand. Those skilled in the art will readily appreciate that suitable antagonists for use in accordance with embodiments disclosed herein may take a variety of forms. The antagonist may be an antisense construct comprising a nucleotide sequence specific to an miRNA described herein, or a portion thereof, wherein the antisense construct inhibits, at least partially, the expression or activity of the miRNA. By "specific" it is meant that the antisense construct is substantially specific for the miRNA, but not necessarily exclusively so. That is, while being specific for a particular miRNA sequence, the antisense construct may also cross-hybridise with other sequences, such as other miRNA sufficient to inhibit expression. Further, for example, the nucleotide sequence of an antisense construct according to the present invention may display less than 100% sequence identity with a particular miRNA and retain specificity thereto. It will be appreciated by those skilled in the art that suitable antisense constructs need not bind directly with the miRNA to which they are directed in order to effect the expression or activity of those miRNA. Binding of an antisense construct to its complementary cellular nucleotide sequence may interfere with transcription, RNA processing, transport, and/or stability of the miRNA to which it is specific. An antisense molecule may comprise DNA, RNA, LNA, PNA or any combination thereof.

[0072] Suitable antisense constructs for use in accordance with embodiments disclosed herein include, for example, antisense oligonucleotides, small interfering RNAs (siRNAs) and catalytic antisense nucleic acid constructs. Suitable anti-

sense oligonucleotides may be prepared by methods well known to those of skill in the art. Typically oligonucleotides will be chemically synthesized on automated synthesizers.

[0073] Those skilled in the art will readily appreciate that one or more base changes may be made such that less than 100% complementarity exists whilst the oligonucleotide retains specificity for its miRNA and retains antagonistic activity against this miRNA. Further, as described below, oligonucleotide sequences may include one or more chemical modifications without departing from the scope of the present invention.

[0074] Oligonucleotides in accordance with the invention may include modifications designed to improve their delivery into cells, their stability once inside a cell, and/or their binding to the appropriate miRNA target. For example, the oligonucleotide sequence may be modified by the addition of one or more phosphorothioate (for example phosphoromonothioate or phosphorodithioate) linkages between residues in the sequence, or the inclusion of one or morpholine rings into the backbone. Alternative non-phosphate linkages between residues include phosphonate, hydroxylamine, hydroxylhydrazinyl, amide and carbamate linkages (see, for example, United States Patent Application Publication No. 20060287260, Manoharan I., the disclosure of which is incorporated herein in its entirety), methylphosphonates, phosphorothiolates, phosphoramidates or boron derivatives. The nucleotide residues present in the oligonucleotide may be naturally occurring nucleotides or may be modified nucleotides. Suitable modified nucleotides include 2'-O-methyl nucleotides, such as 2'-O-methyl adenine, 2'-O-methyl-uracil, 2'-O-methyl-thymine, 2'-O-methyl-cytosine, 2'-O-methyl-guanine, 2'-O-methyl-2-amino-adenine; 2-amino-adenine, 2-amino-purine, inosine; propynyl nucleotides such as 5-propynyl uracil and 5-propynyl cytosine; 2-thio-thymidine; universal bases such as 5-nitro-indole; locked nucleic acid (LNA), and peptide nucleic acid (PNA). The ribose sugar moiety that occurs naturally in ribonucleosides may be replaced, for example with a hexose sugar, polycyclic heteroalkyl ring, or cyclohexenyl group as described in United States Patent Application Publication No. 20060035254, Manoharan et al., the disclosure of which is incorporated herein in its entirety. Alternatively, or in addition, the oligonucleotide sequence may be conjugated to one or more suitable chemical moieties at one or both ends. For example, the oligonucleotide may be conjugated to cholesterol via a suitable linkage such as a hydroxypropylol linkage at the 3' end.

[0075] Modified oligonucleotides with 'silencing' activity against specific miRNA ("antagomirs") are described in Krutzfeldt, J. et al., 2005, Nature 438:685-689, the disclosure of which is incorporated herein in its entirety are also antagonists of EGFR ligands. For example, an antagomir with sequence complementary to a miRNA specific for an EGFR ligand may bind to the miRNA and this interaction inhibits the miRNA's activity. The antagomir may be 100% complementary to, for example, a miR-7 molecule or may be less than 100% complementary provided that the antisense molecule is able to inhibit the function of miR-7. Antagomirs may comprise 2-O-methyl nucleotides, phosphorothioate linkages between residues at the 5' and 3' end, and a conjugated cholesterol moiety via a hydroxypropylol linkage at the 3' end. Embodiments as disclosed herein contemplate use of antagomirs modified in the manner described in Krutzfeldt et al. as well as modifications or variations thereof. The design of

oligonucleotides or antagomirs for use in accordance with embodiments disclosed herein is well within the capabilities of those skilled in the art.

[0076] An alternative antisense technology is RNA interference (RNAi), see, eg. Chuang et al. (2000) *PNAS USA* 97: 4985) may be used to antagonise EGFR ligands, according to known methods in the art (for example Fire et al. (1998) *Nature* 391: 806-811; Hammond, et al. (2001) *Nature Rev. Genet.* 2: 110-1119; Hammond et al. (2000) *Nature* 404: 293-296; Bernstein et al (2001) *Nature* 409: 363-366; Elbashir et al (2001) *Nature* 411: 494-498; WO 99/49029 and WO 01/70949, the disclosures of which are incorporated herein by reference), to inhibit the expression or activity of miRNA. RNAi refers to a means of selective post-transcriptional gene silencing by destruction of specific RNA by small interfering RNA molecules (siRNA). The siRNA is generated by cleavage of double stranded RNA, where one strand is identical to the message to be inactivated. Double-stranded RNA molecules may be synthesised in which one strand is identical to a specific region of the miRNA transcript and introduced directly. Alternatively corresponding dsDNA can be employed, which, once presented intracellularly is converted into dsRNA. Methods for the synthesis of suitable molecules for use in RNAi and for achieving post-transcriptional gene silencing are known to those of skill in the art.

[0077] A further means of inhibiting the expression or activity of miRNA includes introducing catalytic antisense nucleic acid constructs, such as DNazymes and ribozymes, which are capable of cleaving miRNA transcripts. Ribozymes, for example, are targeted to, and anneal with, a particular sequence by virtue of two regions of sequence complementarity to the target flanking the ribozyme catalytic site. After binding, the ribozyme cleaves the target in a site-specific manner. The design and testing of ribozymes which specifically recognise and cleave miRNA sequences can be achieved by techniques well known to those in the art (for example Lieber and Strauss, (1995) *Mol. Cell. Biol.* 15:540-551, the disclosure of which is incorporated herein by reference).

[0078] Antibodies against endogenous miRNAs may also be antagonists. The term "antibody" includes within its meaning anti-miR-7 polyclonal and monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-miR-7 antibody compositions with polypeptidic specificity, single chain anti-miRNA antibodies, and fragments of anti-miRNA antibodies. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Antibody fragments comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(abc)₂, and Fv fragments; diabodies, linear antibodies; single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

[0079] It will also be recognised by those skilled in the art that an antagonist in accordance with embodiments of the invention may effect a modulator or regulator of the expression or activity of a miRNA disclosed herein. Similarly, the antagonist may affect a target of a miRNA disclosed herein. Thus, antagonists may take any suitable form, depending on

the nature and identity of the molecule(s) to be effected, such as for example a small molecule inhibitor, peptide inhibitor or antibody.

Methods of Modulating EGFR Ligand Expression and/or Activity

[0080] In particular embodiments the present invention provides methods of modulating the expression of an EGFR ligand in a cell or tissue by contacting the cell or tissue with an miRNA as disclosed herein, or an antagonist of such an miRNA. Examples of cells which express EGFR ligands include cancer cells, lung cells, bone cells, blood cells, and skin cells. The cell may be isolated or purified from a subject, may be located in a sample from a subject, or may be located in or on a subject. Typically the EGFR ligand expression and/or activity is decreased in the cell or tissue following contact of the cell or tissue with a miRNA as disclosed herein compared to the level in a cell or tissue which has not been contacted with the miRNA. Similarly, the expression and/or activity of the EGFR ligand is typically increased in the cell or tissue following contact of the cell or tissue with an antagonist of a miRNA as disclosed herein compared to the level in a sample of a subject which has not been contacted with the antagonist.

[0081] Contacting the cell or tissue with the miRNA or antagonist may be achieved by any method known in the art. In some embodiments the cell has been isolated from the subject and combining the cell and the miRNA or antagonist thereof occurs ex vivo or in vitro. In other embodiments the cell has not been isolated from the subject and contacting the cell and the miRNA or antagonist thereof occurs in vivo. The miRNA or antagonist may be contacted with the cell directly, i.e. applied directly to a cell requiring modulation of EGFR ligand expression or activity, or alternatively may be combined with the cell indirectly, e.g. by injecting the molecule into the bloodstream of a subject, which then carries the molecule to the cell requiring modulation of EGFR ligand expression or activity. Further, a sample may be removed from a subject and combined with an miRNA or antagonist in vitro prior to returning at least a portion of the sample back to the subject. For example, the sample may be a blood sample which is removed from a subject and combined with the molecule prior to injecting at least a portion of the blood back into the subject.

[0082] In some embodiments the miRNA or antagonist thereof is contacted with a cell, wherein the endogenous levels of the miRNA are different as compared to the cell before contacting with the miRNA or antagonist. The term, "endogenous" as used in this context refers to the "naturally-occurring" levels of expression and/or activity of the relevant miRNA. In these embodiments, compounds or compositions can be contacted with cells such that the expression and/or activity of the miRNA are increased or decreased as compared to the "naturally-occurring" levels.

[0083] In some embodiments administration of polynucleotides (miRNA or nucleic-acid based antagonists thereof) is via a vector (e.g. viral)-based approach, or by administration of a polynucleotide in the form of a fusion protein where the polynucleotide is bound to a protamine-Fab antibody fragment which targets the polynucleotide to cells of interest, i.e. cells expressing EGFR ligands.

Diseases and Conditions

[0084] EGFR ligands are dysregulated in many conditions including cancer. Accordingly, methods and compositions

provided herein for modulating the expression and/or activity of an EGFR ligand using antagonists as described above are also applicable to the treatment or prevention of conditions associated with EGFR ligand dysregulation. Conditions to which methods and compositions of the invention are applicable include, but are not limited to cancer, renal disease, pulmonary disease, cardiac disease, skin disease or infectious disease. The term "cancer" as used herein refers to any malignant cell growth or tumour caused by abnormal and uncontrolled cell division.

[0085] The cancer may be any cancer in which the expression or activity of an EGFR ligand, such as TGF α , capable of being modulated by an miRNA as described herein (or antagonist thereof) is dysregulated. Typically such cancers will be associated with upregulated or elevated levels of expression or activity of the EGFR ligand relative to normal cells and tissues. Exemplary cancers include, but are not limited to liver, ovarian, colorectal, lung, small cell lung, breast, prostate, pancreatic, renal, colon, gastric, endometrial, stomach, oesophageal, and head and neck cancers, peritoneal carcinomatosis, lymphoma, sarcoma or secondary metastases thereof, glioblastoma, neuroblastoma, and melanoma.

Compositions and Routes of Administration

[0086] Embodiments of the present invention contemplate compositions for modulating the expression and/or activity of an EGFR ligand in a cell, tissue or subject and for treating or preventing a condition associated with dysregulation an EGFR ligand. Such compositions may be administered in any convenient or suitable route such as by parenteral (including, for example, intraarterial, intravenous, intramuscular, subcutaneous), oral, nasal, mucosal (including sublingual), intracavitary or topical routes. Thus compositions may be formulated in a variety of forms including solutions, suspensions, emulsions, and solid forms and are typically formulated so as to be suitable for the chosen route of administration, for example as capsules, tablets, caplets, elixirs for oral ingestion, in an aerosol form suitable for administration by inhalation (such as by intranasal inhalation or oral inhalation), ointment, cream, gel, jelly or lotion suitable for topical administration, or in an injectible formulation suitable for parenteral administration. The preferred route of administration will depend on a number of factors including the condition to be treated and the desired outcome. The most advantageous route for any given circumstance can be determined by those skilled in the art. For example, in circumstances where it is required that appropriate concentrations of the desired agent are delivered directly to the site in the body to be treated, administration may be regional rather than systemic. Regional administration provides the capability of delivering very high local concentrations of the desired agent to the required site and thus is suitable for achieving the desired therapeutic or preventative effect whilst avoiding exposure of other organs of the body to the compound and thereby potentially reducing side effects.

[0087] In general, suitable compositions may be prepared according to methods which are known to those of ordinary skill in the art and may include a pharmaceutically acceptable diluent, adjuvant and/or excipient. The diluents, adjuvants and excipients must be "acceptable" in terms of being compatible with the other ingredients of the composition, and not deleterious to the recipient thereof.

[0088] Examples of pharmaceutically acceptable diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrrolidone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 1% to 99.9% by weight of the compositions.

[0089] For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, medium chain triglyceride (MCT), isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol. Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colouring agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

[0090] Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

[0091] Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

[0092] Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut

oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

[0093] Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, polyvinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

[0094] Emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

[0095] Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein. The composition may incorporate any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

[0096] Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as *Remington's Pharmaceutical Sciences*, 20th Edition, Williams & Wilkins, Pennsylvania, USA. The carrier will depend on the route of administration, and again the person skilled in the art will readily be able to determine the most suitable formulation for each particular case.

[0097] The compositions may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The compositions in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq., the contents of which is incorporated herein by reference.

Combination Regimens

[0098] Therapeutic advantages may be realised through combination regimens. In combination therapy the miRNA, antagonist thereof, or agent capable of stimulating or enhancing the expression or activity of the miRNA and at least an additional therapeutic agent may be coadministered. For example, in the context of cancer, one may seek to maintain ongoing anti-cancer therapies such as chemotherapy and/or radiotherapy, in order to manage the condition of the patient, to improve local tumour control and/or reduce the risk of metastasis, whilst employing agents in accordance with embodiments of the present invention. Accordingly, methods

of treatment according to the present invention may be applied in conjunction with conventional therapy, such as with tyrosine kinase inhibitors, radiotherapy, chemotherapy, surgery, or other forms of medical intervention. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time, difference of, for example, from seconds, minutes, hours, days, weeks or months between the administration of the two formulations or therapies. The formulations or therapies may be administered in any order.

[0099] The additional therapeutic agent(s) used will depend upon the condition to be treated or prevented. For example where the condition is a head and neck cancer, suitable therapeutic agents include erlotinib (Tarceva), gefitinib (Iressa or ZD1839) or cetuximab. Alternatively or in addition, the antagonist such as the miRNA may be administered simultaneously and/or consecutively in any order with an agent which counters the side effects of the miRNA.

[0100] Examples of chemotherapeutic agents include adriamycin, taxol, fluorouracil, melphalan, cisplatin, oxaliplatin, alpha interferon, vincristine, vinblastine, angiostatin, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide, SP-PG and the like. Other chemotherapeutic agents include alkylating agents such as nitrogen mustards including mechlorethamine, melphalan, chlorambucil, cyclophosphamide and ifosfamide, nitrosoureas including carmustine, lomustine, semustine and streptozocin; alkyl sulfonates including busulfan; triazines including dicarbazine; ethylenimines including thiotepa and hexamethylmelamine; folic acid analogues including methotrexate; pyrimidine analogues including 5-fluorouracil, cytosine arabinoside; purine analogues including 6-mercaptopurine and 6-thioguanine; antitumour antibiotics including actinomycin D; the anthracyclines including doxorubicin, bleomycin, mitomycin C and methramycin; hormones and hormone antagonists including tamoxifen and corticosteroids and miscellaneous agents including cisplatin and brequinar, and regimens such as COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), and PROMACE/MOPP (prednisone, methotrexate (w/leucovorin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine).

[0101] Agents and compositions disclosed herein may be administered therapeutically or preventively. In a therapeutic application, agents and compositions are administered to a patient already suffering from a condition, in an amount sufficient to cure or at least partially arrest the condition and its symptoms and/or complications. The agent or composition should provide a quantity of the active compound sufficient to effectively treat the patient.

Dosage

[0102] The effective dose level of the administered agent for any particular subject will depend upon a variety of factors including: the type of condition being treated and the stage of the condition; the activity and nature of the agent employed; the composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration; the route of administration; the rate of sequestration of compounds; the duration of the treatment; drugs used in combi-

nation or coincidental with the treatment, together with other related factors well known in medicine. One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic dosage which would be required to treat applicable conditions. These will most often be determined on a case-by-case basis.

[0103] Generally, an effective dosage is expected to be in the range of about 0.0001 mg to about 1000 mg per kg body weight per 24 hours; typically, about 0.001 mg to about 750 mg per kg body weight per 24 hours; about 0.01 mg to about 500 mg per kg body weight per 24 hours; about 0.1 mg to about 500 mg per kg body weight per 24 hours; about 0.1 mg to about 250 mg per kg body weight per 24 hours; or about 1.0 mg to about 250 mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range of about 10 mg to about 200 mg per kg body weight per 24 hours.

[0104] Alternatively, an effective dosage may be up to about 5000 mg/m². Generally, an effective dosage is expected to be in the range of about 10 to about 5000 mg/m², typically about 10 to about 2500 mg/m², about 25 to about 2000 mg/m², about 50 to about 1500 mg/m², about 50 to about 1000 mg/m², or about 75 to about 600 mg/m². Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques.

[0105] It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

[0106] In some embodiments effective dosages, optimal number of dosages, spacing of individual dosages and optimal courses of treatment may be determined by monitoring serum or plasma levels of an EGFR ligand. For example, a sample such as a blood serum or blood plasma, may be assayed by any method known in the art to determine the level of expression and/or activity of the EGFR ligand. After administration of the agent or at intervals during the course of treatment a further sample may be taken and assayed to determine the level of expression and/or activity of the EGFR ligands. In instances where the levels expression and/or activity of the EGFR ligand has not changed significantly the dose or frequency of doses may be increased to optimise the dosage or the treatment. In instances where the level of EGFR ligands have changed significantly the dose or frequency of doses may be decreased to optimise the dosage or treatment.

[0107] The efficacy of a treatment regime in a subject suffering from a disease or condition associated with dysregulated expression or activity of an EGFR ligand may be evaluated by monitoring the change in expression of an EGFR ligand in the subject. For example, a subject may be treated with a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA. After a first period of time a biological sample from the subject may be assayed by any method known in the art to determine the level of expression and/or activity of the EGFR ligand in the sample. After a further period of time an additional biological sample from the subject may be assayed by any method known in the art to determine the level of expression and/or

activity of the EGFR ligand in the additional sample. In some embodiments this process of sampling and determining EGFR ligand levels may be repeated at more than two intervals such that the level of EGFR ligand in response to the treatment regime can be measured over time. The efficacy of the regime can then be evaluated by determining whether the expression and/or activity of the EGFR ligand changes over the period of time. A change in the level of expression and/or activity of the EGFR ligand is indicative of the efficacy of the treatment regime.

[0108] The EGFR ligand may be TGF α , HB-EGF, amphiregulin, epiregulin, betacellulin, epigen NRG-1, NRG-2, NRG-3 or NRG-4. In particular embodiments the EGFR ligand may be TGF α .

[0109] The present invention will now be further described in greater detail by reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

EXAMPLES

Example 1

cDNA Microarray Expression Profiling and Data Analysis

[0110] Microarray analysis was used to identify novel genes down-regulated by miR-7. Specifically, cDNA microarray analysis of miR-7 transfected HN5 cells revealed new miR-7 targets with potential roles in HNC. HN5 cells were transfected for 24 h with a miR-NC precursor corresponding to human miR-7 (Pre-miR miRNA Precursor Product ID: PM10047) (Ambion; Victoria, Australia) or a negative control miRNA (miR-NC; Pre-miR miRNA Precursor Negative Control #1, Product ID: AM17110) (Ambion; Victoria, Australia). Total RNA was isolated for microarray analysis. The 24 h time point was selected on the basis of previous studies which identified a number of miRNA-regulated genes in a liver cancer and non small cell lung cancer cell line (Wang & Wang (2006), *Nucleic Acids Res* 34: 1646-1652; Webster et al., 2009, *J Biol Chem* 284:5731-5741).

[0111] Total RNA was isolated from HN5 cells 24 h after transfection (6 well plates seeded at a density of 5.0×10^5 cells per well) with miR-7 or miR-NC precursor molecules (30 nM) using TRIzol reagent (Invitrogen; Victoria, Australia). The quantity and integrity of extracted RNA was confirmed using a 2100 Bioanalyzer (Agilent Technologies; Victoria, Australia) before samples were judged suitable for array analysis. Gene expression profiling by microarray hybridisation was performed with two experimental replicates by the Australian Genome Research Facility (Victoria, Australia) using Human-6 v3 array chips (Illumina; Victoria, Australia). Raw data, consisting of genes significantly up or down-regulated ($p < 0.05$) in response to transfection with miR-7 precursor by at least 1.5-fold relative to miR-NC precursor, was generated using the Database for annotation, visualisation and integrated discovery (DAVID) (Dennis et al., (2003), *Genome Biol* 4:P3; Huang et al., (2009), *Nat Protoc* 4:44-57). This was followed by further DAVID analysis of the down-regulated gene list for identification of signalling pathways enriched for molecules down-regulated by miR-7. DIANA-miRExTra was used to confirm the over-representation of putative miR-7 target genes among the microarray list of genes down-regulated by miR-7. TargetScan (Lewis et al.,

(2005), Cell 120:15-20) was used for miR-7 target predictions within signalling pathways enriched for putative miR-7 target genes.

[0112] Total RNA was extracted from HN5 cells with TRIzol reagent (Invitrogen; Victoria, Australia) and treated with DNase I (Promega; Sydney, Australia) to eliminate contaminating genomic DNA. For qRT-PCR analysis of EGFR, RAF1, PAK1 and GAPDH mRNA expression, 0.5 µg of total RNA was reverse transcribed into cDNA with random hexamers using Thermoscript (Invitrogen; Victoria, Australia). Real-time PCR for EGFR, RAF1, PAK1 and GAPDH cDNA was performed on a Corbett 3000 RotorGene instrument (Corbett Research; Sydney, Australia) using a SensiMixPlus SYBR Kit (Quantace; New South Wales, Australia) and EGFR, RAF1, PAK1 and GAPDH primers from PrimerBank (Wang & Seed, 2003, Nucleic Acid Res., 31: e154): EGFR-F, 5'-GCG TTC GGC ACG GTG TAT AA-3' (SEQ ID NO:13); EGFR-R, 5'-GGC TTT CGG AGA TGT TGC TTC-3' (SEQ ID NO:14); RAF1-F, 5'-GCA CTG TAG CAC CAA AGT ACC-3' (SEQ ID NO:15); RAF1-R, 5'-CTG GGA CTC CAC TAT CAC CAA TA-3' (SEQ ID NO:16); PAK1-F, 5'-CAG CAC TAT GAT TGG AGT CGG-3' (SEQ ID NO:17); PAK1-R, 5'-TGG ATC GGT AAA ATC GGT CCT-3' (SEQ ID NO:18); GAPDH-F, 5'-ATG GGG MG G-TG MG GTC G-3' (SEQ ID NO:19); GAPDH-R, 5'-GGG GTC ATT GAT GGC AAC ATT A-3' (SEQ ID NO:20). Single peak melt curves and reaction efficiencies of >0.9 were required for further analysis of data. Expression of EGFR, RAF1 and PAK1 mRNA relative to GAPDH mRNA was determined using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen (2001), Methods 25:402-408).

[0113] All results are presented as means±standard deviation (S.D.). Statistical significance was calculated using Student's t test (two-tailed, unpaired) and the level of significance was set at $p < 0.05$. All samples for immunoblotting were loaded in duplicate to validate equal loading of protein. Statistical analysis of qRT-PCR data was performed using GenEx software (MultiD; California, USA). Normality of data was confirmed using the Kolmogorov-Smirnov test (KS test).

[0114] Two experimental replicates for each treatment, miR-7 precursor or miR-NC precursor, were analysed by microarray. The microarray analysis identified 189 genes that were significantly down-regulated ($p < 0.05$) by at least 1.5-fold in the miR-7-transfected cells relative to the miR-NC-transfected cells (data not shown). DAVID analysis of the down-regulated genes identified by microarray analysis revealed that miR-7 targets a variety of molecules belonging to the ErbB receptor signalling pathway, with this molecular pathway having the greatest fold-enrichment (7.2-fold) for miR-7 down-regulated genes ($p < 0.001$). The top six genes belonging to the ErbB receptor signalling pathway that were most down-regulated following miR-7 transfection are presented in Table 1 and confirmed that miR-7 was able to down-regulate multiple genes from the EGFR signalling pathway, genes previously unidentified as miR-7 targets in HNC. DIANA mirExTra was used to investigate whether there was enrichment for predicted miR-7 targets within the down-regulated genes. This analysis revealed that 135 of 189 down-regulated genes were putative miR-7 targets ($p < 0.001$), validating the microarray approach to identify genes down-regulated by miR-7, as it was hypothesised that a significant proportion of the genes down-regulated by miR-7 would contain miR-7 target sites.

TABLE 1

Gene Symbol (common name)	Fold Change	p-value	Number of putative miR-7 target sites
EGFR (EGFR)	↓3.27	1.89×10^{-3}	3
RAF1 (RAF1)	↓2.65	8.21×10^{-3}	2
TGFA (TGFα)	↓2.16	2.49×10^{-2}	5
PIK3CD (PI3K)	↓2.03	9.36×10^{-3}	4
ELK1 (ELK1)	↓1.86	4.78×10^{-3}	0
PAK1 (PAK1)	↓1.81	9.03×10^{-3}	1
HBEGF (HB-EGF)	↓1.50	2.75×10^{-2}	0

[0115] Of the genes down-regulated by miR-7 in the microarray, RAF1 and PAK1 (Table 1) were experimentally confirmed using qRT-PCR. These were chosen because of their known role in EGFR signaling (RAF1) and Akt activation (PAK1) in other cancers and normal tissues. qRT-PCR validation of RAF1 and PAK1 as miR-7 targets was performed using RNA from HN5 cells transfected with miR-7 precursor or miR-NC precursor and it was confirmed that RAF1, and PAK1 mRNA was significantly down-regulated relative to GAPDH mRNA in samples transfected with miR-7 (FIG. 1). RAF1 mRNA was down-regulated 2.49-fold ($p < 0.001$) and PAK1 mRNA was down-regulated 1.82-fold ($p < 0.01$) (FIG. 1), thus experimentally confirming that these genes are targets of miR-7 and suggesting that miR-7 promotes decay of RAF1 and PAK1 mRNA in HN5 cells.

[0116] As noted above, DAVID analysis of the down-regulated microarray genes identified the ErbB signalling pathway as that most enriched for genes down-regulated by miR-7. A schematic representation (FIG. 2) shows the possible interactions between these genes and miR-7. It is apparent that miR-7 has the capacity to regulate the EGFR signalling pathway miR-7 down-regulates multiple members of the EGFR signalling pathway in HN5 HNC cells. Genes significantly changing in expression by microarray analysis in response to miR-7 treatment vs. negative control are listed, and the fold changes, p-values and number of putative miR-7 target sites are indicated. The presence of putative miR-7 target sites within the 3'-UTR of a gene indicates that this gene is possibly a direct target of miR-7, whereas the lack of putative miR-7 target sites within the 3'-UTR of a gene indicates that this gene is possibly an indirect target of miR-7.

[0117] The observed down-regulation of EGFR following miR-7 transfection was in accordance with other experimental findings and served to validate the microarray analysis. Interestingly, two EGFR-activating ligands within the ErbB signalling pathway, TGFα and HB-EGF, were down-regulated in the microarray, both previously unidentified potential targets of miR-7. TGFα and HB-EGF are commonly over-expressed in cancers, including HNCs, and have been shown to contribute to increased proliferation of HNC cells (Grandis et al., (2008), J Cell Biochem 69:55-62). Thus down-regulation of these ligands indicates that miR-7 is able to disrupt EGFR signalling at the ligand and receptor levels as well as disrupting autocrine loops in order to down-regulate the EGFR signalling pathway and reduce tumour growth. It has also been shown that in colorectal, rectal and epidermoid carcinoma cell lines treated with increasing concentrations of cetuximab, a monoclonal antibody which blocks binding of ligands to EGFR, there is a dose dependent increase in concentration of TGFα in serum (Mutsaers et al., (2009), Clin Cancer Res 15:2397-2405). This suggests that blocking of TGFα binding results in upregulation of ligand production in these cancers. Furthermore, it has been found that TGFα and

EGFR mRNA expression is significantly increased in both HNC tumour tissue and surrounding histologically normal tissue, which could lead to malignant transformation of normal tissue (Grandis & Tweardy (1993), J Cell Biochem Suppl 17F:188-191). This reinforces the notion that down-regulation of EGFR ligands by miR-7 results in decreased EGFR pathway signalling and tumour growth and aids in preventing HNC recurrence in patients.

Example 2

miR-7 Modulates TGF α

[0118] The inventors then investigated the ability of miR-7 to directly modulate the level of expression of TGF α in HNC cell lines. The following DNA plasmids were used: pRL-CMV *Renilla luciferase* reporter (Promega) and pGL3-consensus miR-7 target site (SEQ ID NO:6) firefly luciferase plasmid (Webster et al., 2009). pGL3-TGF α miR-7 target site number 5 (SEQ ID NO:11) was generated by ligating annealed DNA oligonucleotides corresponding to nt 3699-3751 (SEQ ID NO:21) of the TGF α mRNA 3'-UTR (GenBank accession number NM_003236.2) into unique SpeI and ApaI sites that were inserted 3' of the luciferase open reading frame of pGL3-control (Promega) firefly luciferase reporter vector. The sequence of all plasmids was confirmed by sequencing.

[0119] The HNC cell line FaDu was obtained from the American Type Culture Collection (ATCC) and HNC cell line HN5 was kindly provided by A/Prof. Terrance Johns (Monash Institute of Medical Research). FaDu and HN5 cell lines were cultured at 37° C. in 5% CO₂ in low glucose DMEM (Invitrogen) supplemented with 10% foetal bovine serum (FBS). Cell lines were used within 20 passages of initial stock for all experiments. For analysis of basal EGFR pathway expression and signaling, cells were seeded in 6 well plates at a density ranging from 2.8-4.0×10⁵ cells per well, and 24 h after plating were serum starved for 24 h in DMEM supplemented with 0.5% FBS prior to protein extraction.

[0120] Cells were seeded at a density of 4.5×10⁵ (FaDu) or 5.0×10⁵ (HN5) cells in 6 well plates and transfected using Lipofectamine 2000 (Invitrogen) with miR-7 or miR-NC precursor molecules at final concentrations ranging from 1-30 nM. Cells were harvested at 24 h for RNA extraction or 3 d for protein extraction.

[0121] For quantitative reverse transcription PCR analysis total RNA was extracted from HN5 cells with TRIzol reagent (Invitrogen) and treated with DNase I (Promega) to eliminate contaminating genomic DNA. For qRT-PCR analysis of

TGF α and GAPDH mRNA expression, 0.5 μ g of total RNA was reverse transcribed into cDNA with random hexamers using Thermoscript (Invitrogen). Real-time PCR for TGF α and GAPDH cDNA was performed on a Corbett 3000 Rotor-Gene instrument (Corbett Research) using a SensiMixPlus SYBR Kit (Quantace) and TGF α and GAPDH primers from PrimerBank (Wang and Seed, 2003): TGF α -F, 5'-TGT MT CAC CTG TGC AGC CTT T-3' (SEQ ID NO:22); TGF α -R, 5'-GTG GTC CGC TGA TTT CTT CTC T-3' (SEQ ID NO:23); GAPDH-F, 5'-ATG GGG MG GTG MG GTC G-3' (SEQ ID NO:19); GAPDH-R, 5'-GGG GTC ATT GAT GGC MC ATT A-3' (SEQ ID NO:20). Single peak melt curves and reaction efficiencies of >0.9 were required for further analysis of data. Expression of TGF α mRNA relative to GAPDH mRNA was determined using the 2^{- $\Delta\Delta C_T$} method (Livak and Schmittgen, 2001).

[0122] For luciferase reporter assays, cells were seeded at a density of 2.0×10⁵ cells per well in 24 well plates and co-transfected using Lipofectamine 2000 (Invitrogen) with miR-7 or miR-NC precursor molecules (0.5-1 nM), and 100 ng per well of firefly luciferase reporter DNA and 5 ng per well of pRL-CMV *Renilla luciferase* reporter as a transfection control. Lysates were collected 24 h after transfection using 1× Passive Lysis Buffer (Promega), frozen at -80° C. overnight, thawed and centrifuged at 13,000×g for 5 min. Each supernatant was assayed for firefly and *Renilla luciferase* activity using a Dual-Luciferase Reporter Assay System (Promega) and a FLUOstar OPTIMA luminometer (BMG Labtech). Relative luciferase expression was determined by normalising firefly luciferase values to *Renilla luciferase* values.

[0123] All results are presented as means±standard deviation (S.D.). Statistical significance was calculated using Student's t test (two-tailed, unpaired) and the level of significance was set at p<0.05. All samples for immunoblotting were loaded in duplicate to validate equal loading of protein. Statistical analysis of qRT-PCR data was performed using GenEx software (MultiD). Normality of data was confirmed using the Kolmogorov-Smirnov test (KS test).

[0124] As shown in FIG. 3, expression of TGF α was significantly reduced both in HN5 and FaDu cells in the presence of miR-7, a reduction of 3.3 fold (p-value=6.61×10⁻⁴) relative to miR-NC in HN5 cells and a reduction of 1.41 fold (p-value=7.43×10⁻⁴) relative to miR-NC in FaDu cells. The results of the luciferase assays (FIG. 4) illustrate that in HN5 cells miR-7 binds to both a consensus miR-7 binding motif and the predicted miR-7 binding motif 5 provided in SEQ ID NO:11.

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1. A method for modulating the expression and/or activity of an epidermal growth factor receptor (EGFR) ligand in a cell or tissue, the method comprising contacting the cell or tissue with a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA.

2. (canceled)

3. The method of claim 1 wherein the miR-7 miRNA comprises the nucleotide sequence set forth in SEQ ID NO:1.

4. (canceled)

5. The method of claim 1 wherein the miR-7 miRNA precursor comprises a sequence as set forth in any one of SEQ ID Nos:2 to 4.

6. The method of claim 1 wherein contacting the cell or tissue with the miRNA reduces or inhibits the expression and/or activity of the EGFR ligand.

7. The method of claim 1 wherein contacting the cell or tissue with an antagonist of the miRNA increases the expression and/or activity of the EGFR ligand.

8. The method of claim 1 wherein the 3' untranslated region of the mRNA encoding the EGFR ligand comprises one or more miRNA binding sites.

9. The method of claim 8 wherein the miRNA binding sites comprise sequences as set forth in any of SEQ ID Nos:6 to 11.

10. The method of claim 1 wherein the EGFR ligand is TGF α or HB-EGF.

11. (canceled)

12. The method of claim **10** wherein the mRNA encoding TGF α comprises a 3' untranslated region comprising the sequence set forth in SEQ ID NO:12, or a variant thereof.

13-14. (canceled)

15. The method of claim **1** wherein a subject containing the cell or tissue, or from which the cell or tissue is derived, suffers from, is predisposed to, or is otherwise at risk of developing a disease or condition associated with dysregulated expression or activity of the EGFR ligand.

16. The method of claim **15** wherein the disease or condition is a cancer.

17. A method for treating a disease or condition associated with dysregulated expression or activity of an EGFR ligand in a subject, comprising administering to the subject an effective amount of a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, or an antagonist of any such miRNA, whereby the miRNA modulates the expression and/or activity of the EGFR ligand.

18. The method of claim **17** wherein the disease or condition is associated with upregulated or elevated expression or activity of the EGFR ligand and the subject is administered an effective amount of a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA.

19. (canceled)

20. The method of claim **17** wherein the miR-7 miRNA comprises the nucleotide sequence set forth in SEQ ID NO:1.

21. (canceled)

22. The method of claim **17** wherein the miR-7 miRNA precursor comprises a sequence as set forth in any one of SEQ ID Nos:2 to 4.

23. The method of claim **17** wherein the 3' untranslated region of the mRNA encoding the EGFR ligand comprises one or more miRNA binding sites.

24. The method of claim **23** wherein the miRNA binding sites comprise sequences as set forth in any of SEQ ID Nos:6 to 11.

25. The method of claim **17** wherein the EGFR ligand is TGF α or HB-EGF.

26. (canceled)

27. The method of claim **25** wherein the mRNA encoding TGF α comprises a 3' untranslated region comprising the sequence set forth in SEQ ID NO:12, or a variant thereof.

28. The method of claim **17** wherein the disease or condition is a cancer.

29. The method of claim **28** wherein the cancer is selected from the group consisting of head and neck cancer, glioblastoma, pancreatic cancer, colon cancer, lung cancer, prostate cancer, breast cancer, liver cancer, neuroblastoma and melanoma.

30-32. (canceled)

33. A method for preventing or reducing tumour growth, cancer metastasis or reoccurrence in a subject, wherein the tumour or cancer is associated with upregulated or elevated expression or activity of an EGFR ligand, the method comprising administering to the subject an effective amount of a miR-7 miRNA, a precursor or variant thereof, a miRNA comprising a seed region comprising the sequence GGAAGA, whereby the miRNA modulates the expression and/or activity of the EGFR ligand.

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