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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 May 2008 (29.05.2008)

PCT

(10) International Publication Number
WO 2008/061537 A2

(51) International Patent Classification:
C12N 15/11 (2006.01) *C12Q 1/68* (2006.01)
A61K 31/713 (2006.01)

(21) International Application Number:
PCT/DK2007/050174

(22) International Filing Date:
23 November 2007 (23.11.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2006 01543	23 November 2006 (23.11.2006)	DK
PA 2006 01544	23 November 2006 (23.11.2006)	DK
60/888,094	4 February 2007 (04.02.2007)	US
60/888,095	4 February 2007 (04.02.2007)	US
PA 2007 01081	24 July 2007 (24.07.2007)	DK

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2008/061537 A2

(54) Title: OLIGONUCLEOTIDES FOR MODULATING TARGET RNA ACTIVITY

(57) Abstract: The present invention describes oligonucleotides that bind to microRNA target sites in target RNAs, such as mRNAs. The oligonucleotides of the invention may mediate RNase H degradation of the target RNA, mediate RNAi of the target RNA or prevent microRNA regulation of the target RNA. The oligonucleotides of the invention are useful e.g. as research tools for studying microRNA:mRNA interactions and for therapeutic development. The present invention also describes methods of identifying microRNA target sites, methods of validating microRNA target sites, methods of identifying oligonucleotides of the invention and methods of modulating the activity of a target RNA using the oligonucleotides of the invention.

Oligonucleotides for modulating target RNA activity

Background of the invention

The present invention relates to oligonucleotides that can be used to affect the activity of target RNAs.

The first generation of such oligonucleotides were antisense oligonucleotides that were intended to affect the activity of target mRNAs. One reason for interest in such oligonucleotides is the potential for exquisite and predictable specificity that can be achieved because of specific base pairing. In other words, it is in theory very simple to design an oligonucleotide that is highly specific for a given nucleic acid, such as an mRNA.

However, it has turned out that not all sequences are available for antisense targeting and accessibility may vary e.g. because of secondary structure or protein binding.

Moreover, it has turned out simple base pairing is not enough to achieve regulation of a given target mRNA, i.e. an oligonucleotide complementary to a given target mRNA does not necessarily affect the activity of the target mRNA. If the oligonucleotide targets the open reading frame of an mRNA, it may e.g. be that the translational apparatus simply displaces the oligonucleotide during translation. Therefore, means where developed that would improve the regulatory activity of the oligonucleotide.

E.g. oligonucleotides that can activate RNase H cleavage of the target mRNA were developed. One potential disadvantage of such oligonucleotides is that they may mediate cleavage of other RNAs than the intended target mRNA, i.e. giving rise to off-target effects. Still, oligonucleotides acting through RNase H cleavage are in clinical trials for treatment of various diseases.

Recently, research has shown that eukaryotic cells, including mammalian cells, comprise a complex gene regulatory system (herein also termed RNAi machinery)

that uses RNA as specificity determinants. This system can be triggered by so called siRNAs that may be introduced into a cell of interest to regulate the activity of a target mRNA. Currently, massive efforts go into triggering the RNAi machinery with siRNAs for specific regulation of target RNAs, in particular target mRNAs. This approach is widely regarded as having great promise for the development of new therapeutics. As will also be outlined below, a major advantage of this approach is that specificity of the siRNA lies in the degree of complementarity between the guide strand of the siRNA and the target RNA, i.e. target specificity can be controlled. However, it has turned out that siRNAs may be less specific than initially thought. Initially, it was believed that only target RNAs that harboured stretches of complete complementarity to the guide strand of the siRNA would be affected, i.e. targeted by the RNAi machinery. New research indicates that siRNAs indeed do result in significant off-target effects, i.e. regulation of non-intended targets. It is now believed that these off-targets stem from the siRNAs, or rather the guide strand of the siRNAs, acting as microRNAs.

MicroRNAs are a class of endogenous RNA molecules that has recently been discovered and that, as siRNA, function via the RNAi machinery. Currently, about 500 human microRNAs have been discovered and the number is rapidly increasing. It is now believed that more than one third of all human genes may be regulated by microRNAs. Therefore, microRNAs themselves may be used to regulate the activity of target RNAs, and consequently e.g. be used as therapeutics.

However, as also described below, microRNAs generally act at more than one target RNA, i.e. they are promiscuous. Thus, introduction of a microRNA into the cell or regulating the level of a microRNA will affect the activity of more than one target mRNA and consequently may give rise to undesired off-target effects.

A recent approach has been put forward, wherein the activity of a target RNA is regulated by inhibiting the activity of a microRNA. The microRNA can be inhibited using complementary oligonucleotides that have been termed antimirs and antagomirs. Since the microRNA is itself promiscuous, also an antimir or antagomir will be promiscuous and affect the activity of more than one target RNA.

Detailed description

In previous applications (PA 2006 01543 and PA 2006 01544 filed in Denmark, November 23, and US 60,888,094 and US60/888,095 filed 02/04/2007 in the US) the term Xmir was used, when referring to oligonucleotides of the invention. In this application, the term oligonucleotides of the invention are preferentially used over the term Xmir. However, when the term Xmir is used, reference is to oligonucleotides of the invention.

Thus, as used herein, the term Xmir refers to an oligonucleotide of the invention as specified further in the following embodiments and in the claims.

All references mentioned herein are hereby incorporated by reference.

It is to be understood that features described in one aspect of the invention equally applies for the other aspects.

Definitions

An "oligonucleotide capable of regulating the activity of a target RNA" refers to an oligonucleotide with a particular activity. Such oligonucleotides are also termed active oligonucleotides.

The terms "regulate" and "modulate" are used interchangeably herein.

An "oligonucleotide potentially capable of regulating the activity of a target mRNA" refers to an oligonucleotide which activity has not yet been experimentally confirmed. Such an oligonucleotide may also be termed a candidate regulator.

When reference is made to an oligonucleotide without further specification, the oligonucleotide may be an "oligonucleotide capable of regulating the activity of an mRNA" or an "oligonucleotide potentially capable of regulating the activity of a target mRNA" or both.

When referring to a "target RNA", what is meant is the target for an oligonucleotide of the invention. Typically, an oligonucleotide of the invention can interact with a target RNA by way of base pairing.

The target RNA may be any RNA. Preferably, the target RNA is a mRNA or a viral RNA, such as a genomic viral RNA.

When referring to the "activity of a target mRNA", what is typically meant is the expression of the target mRNA, i.e. translation into a protein or peptide. Thus, regulation of the activity of a target mRNA may include degradation of the mRNA and/or translational regulation. Regulation may also include affecting intracellular transport of the mRNA. In a preferred embodiment of the invention, the oligonucleotide is capable of regulating the expression of the target mRNA. In another preferred embodiment, the oligonucleotide may mediate degradation of the target mRNA (in turn also regulating expression of the target mRNA). The activity may also be replication.

When the target RNA is a viral RNA, the oligonucleotide of the invention may affect replication of the virus or otherwise interfere with the proliferation of the virus.

As used herein, regulation may be either positive or negative. I.e. a regulator (e.g. oligonucleotide or microRNA) may increase the activity of the target (e.g. target mRNA) or it may decrease the activity of the target.

When referring to the "target sequence of an RNA", what is meant is the region of the RNA involved in or necessary for microRNA regulation. The terms "target region" and "target sequence" are used interchangeably herein.

Not intended to be bound by theory, it is believed that this region comprise bases that interact directly with the microRNA during microRNA regulation of the target RNA. In a preferred embodiment, the target sequence is the region of the target RNA necessary for microRNA regulation. Such region may be defined using a reporter system, wherein systematic deletions of the target RNA are tested for

activity to define the target sequence. Assessing the effect of introducing point mutations in the target region is also valuable for defining the target region.

As will be clear from the specification, also oligonucleotides of the invention may be used to define the region of the target RNA necessary for microRNA regulation. Preferably, the target sequence comprises an antiseed sequence, which is complementary to the seed sequence of a microRNA and also complementary to a guide sequence of a oligonucleotide of the invention. Introduction of mutations in the antiseed sequence will typically affect microRNA regulation and hence may be used to verify that given positions are involved in microRNA regulation.

The term microRNA as used herein has the same meaning as typically in the art. I.e. the term microRNA refers to a small non-translated RNA of typically 18-22 nucleotides that is capable of regulating the activity of a target mRNA. A microRNA is typically processed from pri-microRNA to short stem-loop structures called pre-microRNA and finally to mature miRNA. Both strands of the stem of the pre-microRNA may be processed to a mature microRNA.

The miRBase (http://microrna.sanger.ac.uk/sequences/) is a compilation of known microRNAs. Also predicted and known targets of the microRNAs can be found on this site.

The term siRNA (short interfering RNA) as used herein has the same meaning as typically in the art. I.e. the term siRNA refers to double stranded RNA complex wherein the strands are typically 18-22 nucleotides in length. Very often, the complex has 3'-overhangs.

When referring to the RNAi machinery herein, what is meant are the cellular components necessary for the activity of siRNAs and microRNAs or for the RNAi pathway. A major player of the RNAi machinery is the RNA induced silencing complex (the RISC complex).

As referred to herein, a RNA unit is one of the monomers that make up an RNA polymer. Thus, an RNA unit is also referred to as an RNA monomer or a RNA nucleotide. Likewise, a DNA unit is one of the monomers that make up a DNA

polymer and a DNA unit may also be referred to as a DNA monomer or a DNA nucleotide.

When referring to a base, what is meant is the base of a nucleotide. The base may be part of DNA, RNA, INA, LNA or any other nucleic acid or nucleic acid capable of specific base pairing. The base may also be part of PNA (peptide nucleic acid). In some embodiments, the base may be an universal base.

When referring the length of a sequence, reference may be made to the number of units or to the number of bases.

When referring to a complementary sequence, G pairs to C, A pairs to T and U and vice versa. In a preferred embodiment, G also pairs to U and vice versa to form a so-called wobble base pair. In another preferred embodiment, the base inosine (I) may be comprised within either in a microRNA or oligonucleotide of the invention. I basepairs to A, C and U. In still another preferred embodiment, universal bases may be used. Universal bases can typically basepair to G, C, A, U and T. Often universal bases do not form hydrogen bonds with the opposing base on the other strand. In still another preferred embodiment, a complementary sequence refers to a contiguous sequence exclusively of Watson-Crick base pairs.

Summary of the invention

In a first aspect, the present invention provides oligonucleotides that are useful for modulating the activity of a target RNA. In a preferred embodiment, the oligonucleotides target a microRNA target region of the target RNA. Another aspect of the invention is a method for modulating the activity of a target RNA. Still other aspects relate to providing an oligonucleotide of the invention, identifying microRNA target regions of target RNAs, validating microRNA target regions of target RNAs and identifying microRNA regulators of a given target RNA.

Disclosure of the invention

The present invention provides oligonucleotides that target microRNA target regions of target RNAs. In one embodiment, the oligonucleotides draws use of the

accessibility of microRNA target regions of target RNAs. The oligonucleotides of the invention may recruit the RNAi machinery to the target RNA to mediate translational repression or cleavage of the target RNA. The oligonucleotides of the invention may also recruit RNase H to mediate cleavage of the target RNA. Moreover, the oligonucleotides of the invention may modulate the activity of the target RNA by preventing a microRNA from regulating the target RNA.
The invention also provides methods for providing microRNA targets of target RNAs, methods for validating microRNA target regions of target RNAs and methods of modulating the activity of target RNAs using oligonucleotides of the invention.

First aspect – bioactive oligonucleotides

In a first aspect, the present invention provides an oligonucleotide comprising an antisense sequence that comprises a guide sequence corresponding to the seed sequence of a microRNA, with the proviso that the oligonucleotide is not a microRNA or does not comprise a sequence corresponding the complete sequence of a microRNA.

Such an oligonucleotide is of interest because it can be used to target the target region of a target RNA, said target region being involved in microRNA regulation of the target RNA. Not intended to be bound by theory, it is believed that said target region will be more accessible for interaction (with microRNAs, oligonucleotides or other nucleic acids) than will other regions of the target RNA, because the target region is evolved for interaction with a microRNA or because endogenous microRNAs chooses target regions that are more accessible.

Support for the above view comes from work published after the priority date of this patent application. One publication investigated the effect of target secondary structure on the efficacy of repression by microRNAs (Long D, 2007). The results indicate a potent effect of target structure on target recognition by microRNAs, at least for microRNA regulation in Caenorhabditis elegans and Drosophila melanogaster. The authors suggest that target secondary structure probably contributes to accessibility in most miRNA-target interactions.

Another study systematically investigated the role of target-site accessibility in microRNA target recognition (Kertesz M, 2007). The authors demonstrated that mutations diminishing target accessibility substantially reduce microRNA mediated translational repression. Moreover, the authors performed a genome-wide analysis of target accessibility to all 3'UTRs of fly, worm, mouse and human. They found that microRNA seed sequences in all four organisms showed a notable preference for highly accessible regions and the authors suggest that target accessibility is a critical factor in microRNA function.

We suggest that target accessibility will most likely be determined by a combination of target secondary structure and occlusion by other factors such as RNA binding proteins.

Thus, in one embodiment of the present invention, the target region may be targeted by e.g. RNase H inducing oligonucleotides or siRNAs. The oligonucleotide may e.g. be a 10-mer that induces RNase H cleavage of the target RNA. The oligonucleotide may also prevent a microRNA from exerting its action on the target RNA. These various embodiments will be further outlined below.

As used in the context of the guide sequence (of an oligonucleotide of the invention) and the seed sequence (of a microRNA), the word "corresponding" refers to the ability of the seed sequence and the guide sequence of being capable of base pairing with the same sequence. I.e. the guide sequence and the seed sequence may not necessarily be identical, but they are capable of base pairing to the same sequence, e.g. the anti-seed sequence of a target RNA.

The phrase "a sequence corresponding to the complete sequence of a microRNA sequence" is intended to cover e.g. a precursor of the microRNA or a DNA molecule that encode the microRNA. The DNA molecule may e.g. be a PCR product intended for T7 RNA polymerase transcription of the microRNA. Such molecules are not included in the scope of the invention, as neither are naturally occurring microRNAs.

Origin

The target RNAs to be used in the methods of the present invention are preferably of animal or plant origin. More preferably, the target RNAs are of mammalian origin. Most preferably they are of human origin. The target RNAs may also be of viral origin, preferably from virus that infects humans. In a preferred embodiment, the term human target RNA also include viral target RNAs of virus that infects humans.

The microRNAs to be used in the methods of the present invention are also preferably of animal or plant origin. More preferably, they are of mammalian origin. Most preferably, they are of human origin. The microRNAs to be used in the methods of the present invention may also be of viral origin. If they are of viral origin, they are preferably from virus that infects humans, e.g. mir-LAT of HSV-1. In a preferred embodiment, the term human microRNAs also include viral microRNAs of virus that infect humans.

It is most preferred that the oligonucleotides of the invention comprise a guide sequence that corresponds to the seed sequence of a human microRNA or of a microRNA from a virus that infects humans.

In a preferred embodiment, the oligonucleotide of the invention comprise a sequence selected from the group consisting of sequences that are capable of base pairing to the complementary sequence of a sequence selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

In a preferred embodiment, the oligonucleotide of the invention comprise a sequence selected from the group consisting of sequences that are capable of base pairing to the complementary sequence of a sequence selected from the

group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723 and are not capable of forming a consecutive base pair with the neighbouring nucleotide of either side of the aforementioned positions.

The term complementary sequence has been defined above. The phrase "are capable of base pairing to" is related to the term complementary sequence. I.e. a first sequence is capable of base pairing to a second sequence, which is complementary to the first sequence.

In another preferred embodiment, the oligonucleotide of the invention consists of an antisense sequence selected from the group consisting of sequences that are capable of base pairing to the complementary sequence of a sequence selected from the group consisting selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

The oligonucleotides of the invention can also be defined by base pairing rules. Thus, in another preferred embodiment, the antisense sequence of the oligonucleotides of the invention comprises an sequence selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20,

position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723, wherein

a. A may be exchanged with only G, C, U, T or I
b. G may be exchanged with only A or I
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C, A, T or I

- and 3 additional positions may be exchanged with any base.

The exchange rules are based on the following considerations:
An A in the microRNA can base pair to U or I in the target RNA. U and I in the target RNA can base pair to A, G, I, C, U or T. Likewise for the other bases.

Moreover, editing of A to I in microRNAs has been shown to redirect silencing targets of microRNAs (Kawahara Y, 2007). Therefore, A in the microRNAs may be substituted for I some embodiments.

Also the target RNA may comprise I that have been edited from A.

Moreover, G:U base pairs may be accepted for microRNAs – target RNA interaction in some embodiments, but not all.

The rules are described in table 1:

Inosines in target RNA and miRNA + GU basepairs						
MicroRNA	U	G	C	A	I	A/I
target RNA	A, G, I	U, C	G, I	U, I	A, C, U	
Xmir	U, I , A, C, T	A, G, I	U, C, A, T	A, G, I, C, U, T	U, I, A, G, T	A, G, I, C, U, T

	Inosines in target RNA and miRNA + GU pairs, no T-I pairs					
MicroRNA	U	G	C	A	I	A/I
target RNA	A, G, I	U, C	G, I	U, I	A, C, U	
Xmir	U, I , A, C, T	A, G, I	U, C, A	A, G, I, C, U	U, I, A, G, T	A, G, I, C, U, T

	Inosines in target RNA and miRNA, no GU basepairs					
MicroRNA	U	G	C	A	I	A/I
target RNA	A, I	C	G, I	U, I	A, C, U	
Xmir	U, I, A, C, T	G, I	A, C, U, T	A, I, C, U, T	U, I, G, A, T	A, G, I, C, U, T

	Inosines in target RNA and miRNA, no GU pairs, no I-T pairs					
MicroRNA	U	G	C	A	I	A/I
target RNA	A, I	C	G, I	U, I	A, C, U	
Xmir	U, I, A, C, T	G, I	A, C, U	A, I, C, U	U, I, G, A, T	A, G, I, C, U, T

	No inosine in target RNA					
MicroRNA	U	G	C	A	I	A/I
target RNA	A, G	U, C	G, I	U	A, C, U	
Xmir	U, C, T	A, G, I	U, C, A, T	A, G, I	U, G, I, A, T	U, G, I, A, T

	No inosine in either target RNA or miRNA			
MicroRNA	U	G	C	A
target RNA	A, G	U, C	G	U
Xmir	U, C, T	A, G	U, C, T	A, G

No GU pairs and no inosine in either target RNA or miRNA				
MicroRNA	U	G	C	A
target RNA	A	C	G	U
Xmir	U, T	G	C	A

Additional positions that may be exchanged with any base are included to account for single nucleotide polymorphisms (SNPs) and other mutations. Furthermore, some target sequences interacting with microRNAs may not posses' perfect complementarity to the interacting microRNA. I.e. there may be a mismatch in the complex formed between the seed sequence of the microRNA and the antiseed sequence of the target RNA.

Thus, in another preferred embodiment,

a. A may be exchanged with only G, C, U, T or I
b. G may be exchanged with only A or I
c. C may be exchanged with only A or U
d. U may be exchanged with only C, A, T or I

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment,

a. A may be exchanged with only C, U, T or I
b. G may be exchanged with only I
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C, A, T or I

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment,

a. A may be exchanged with only C, U, or I
b. G may be exchanged with only I
c. C may be exchanged with only A or U
d. U may be exchanged with only C, A, T or I

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment,

a. A may be exchanged with only G or I
b. G may be exchanged with only I or A
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C or T

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment,

a. A may be exchanged with only G
b. G may be exchanged with only A or G
c. C may be exchanged with only T or U
d. U may be exchanged with only C or T

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment, U may be exchanged with only T

-and 3 additional positions may be exchanged with any base.

In yet another preferred embodiment, 2 additional positions may be exchanged with any base.

In yet another preferred embodiment, 1 additional position may be exchanged with any base.

In yet another preferred embodiment, no additional positions may be exchanged with any base.

In a preferred embodiment, the oligonucleotide may further comprise 1 or 2 additions or deletions. More preferred is 1 addition/substitution and most preferred is zero additions/deletions. Additions and deletions are relevant where the complex between the microRNA and target RNA comprise bulges. If a nucleotide on the microRNA is bulged, this accounts to a deletion of the oligonucleotide of the invention. If a nucleotide on the target RNA is bulged, this accounts for a addition of the oligonucleotide of the invention.

It is even more preferred that the oligonucleotide of the invention comprise an antisense sequence that comprises a guide sequence selected from the group consisting of: position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-10 and position 3-9 of any SEQ ID NOs:1-723 wherein it is to be understood that the exchange rules outlined above also apply for this group, i.e. in various embodiments.

It is most preferred that the oligonucleotide of the invention comprise an antisense sequence that comprises a guide sequence selected from the group consisting of: position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723 wherein it is to be understood that the exchange rules outlined above also apply for this group, i.e. in various embodiments.

In one embodiment, the oligonucleotide does not comprise the neighbouring nucleotide of either side of the aforementioned positions of any of SEQ ID NOs 1-723. I.e. the neighbouring positions of any of the aforementioned positions of any of SEQ ID NOs 1-723 are not the same as the corresponding neighbouring positions of the oligonucleotides of the invention.

It still another preferred embodiment, the oligonucleotide of the invention consists of an antisense sequence comprises a guide sequence selected from the group consisting of: position 1-8, position 1-7, position 2-8 and position 2-7 of any of SEQ ID NOs:1-723 wherein it is to be understood that the exchange rules outlined above also apply for this group, i.e. in various embodiments.

Second sequence

In another preferred embodiment, the antisense sequence of the oligonucleotide of the invention further comprises a second sequence selected from the group consisting of: position 12-17, position 12-16, position 13-17 and position 13-16 of any of SEQ ID NOs:1-723, wherein the guide sequence and the second sequence are derived from the same SEQ ID NO and wherein it is to be understood that the exchange rules outlined above also apply for this group, i.e. in various embodiments.

Contiguous stretch of bases

Preferably, the oligonucleotide of the invention comprises a antisense sequence that comprises a contiguous stretch of bases, complementary to the micro RNA target sequence of a target RNA selected from the group consisting of: less than 60 bases, less than 50 bases, less than 40 bases, less than 39 bases, less than 38 bases, less than 37 bases, less than 36 bases, less than 35, less than 34 bases, less than 33 bases, less than 32 bases, less than 31 bases, bases, less than 30

bases, less than 29 bases, less than 28 bases, less than 27 bases, less than 26 bases, less than 25 bases, less than 24 bases, less than 23 bases, less than 22 bases, less than 21 bases, less than 20 bases, less than 19 bases, less than 18 bases, less than 17 bases, less than 16 bases, less than 15 bases, less than 14 bases, less than 13 bases, less than 12 bases, less than 11 bases, less than 10 bases, less than 9 bases, less than 8 bases, less than 7 bases, more than 60 bases, more than 50 bases, more than 40 bases, more than 39 bases, more than 38 bases, more than 37 more, more than 36 bases, more than 35, more than 34 bases, more than 33 bases, more than 32 bases, more than 31, more than 30 bases, more than 29 bases, more than 28 bases, more than 27 bases, more than 26 bases, more than 25 bases, more than 24 bases, more than 23 bases, more than 22 bases, more than 21 bases, more than 20 bases, more than 19 bases, more than 18 bases, more than 17 bases, more than 16 bases, more than 15 bases, more than 14 bases, more than 13 bases, more than 12 bases, more than 11 bases, more than 10 bases, more than 9 bases, more than 8 bases, more than 7 bases, more than 6 bases and more than 5 bases.

A contiguous stretch of bases is intended to mean a non-interrupted sequence of bases that all fit into a duplex formed between the oligonucleotide of the invention and the target RNA. I.e. there are preferably no bulges in the duplex and it is preferred that the sequences are complementary (see the definition of complementary sequences above). Most preferred is perfect Watson-Crick duplex between the oligonucleotide of the invention and target region of the target RNA.

The terms contiguous and continuous are used interchangeably herein.

In another embodiment, the oligonucleotide of the invention comprise an antisense sequence that comprises a contiguous stretch of bases complementary to the micro RNA target sequence of a target RNA, said contiguous stretch of bases being selected from the group consisting of between 10 and 14 bases, between 12 and 16 bases, between 14 and 18 bases, between 16 and 20, between 10 and 25 bases, between 12 and 24 bases, between 14 and 22 bases, between 15 and and 22 bases and between 15 and 20 bases.

More preferred is a contiguous stretch of bases between 8 and 25 bases.

Most preferred is a contiguous stretch of bases between 10 and 20 bases.

Preferably, the oligonucleotide can interact with the same region of the target RNA as a microRNA. One advantage of such an oligonucleotide is that it targets an exposed region of the target RNA (see discussion above). Another advantage of such an oligonucleotide is that is can be used to mask the microRNA target such that the (endogenous) microRNA targeting the target RNA will be prevented from interacting with the target RNA, and thus exerts its effects on the target RNA.

The oligonucleotide of the invention may have a degree of identity to its corresponding microRNA selected from the group consisting of less than 99%, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30% and less than 25%. When referring to the degree of identity, the degree is counted over the length of the shortest molecule of the micro RNA and the oligonucleotide of the invention. The guide sequence of the oligonucleotide of the invention and the seed sequence of the microRNA is used for alignment. Hence, if the microRNAs is 20 bases and the oligonucleotide is 14 and the number of identical positions are 12, the degree of identity is 12/14 = 86%. If the microRNAs is 20, the oligonucleotide 20 and the number of positions is 10, then the degree of identity is 10/20 = 50%.

Preferably, the position of the guide sequence within the oligonucleotide of the invention is selected from the group consisting of: position 1, position 2, position 3, position 4, position 5, position 6, position 7, position 8, position 9, position 10, position 11, position 12, position 13, position 14, position 15, position 16, position 17, position 18 and position 19, wherein the position is counted in the 5'-3' direction from the first base of the guide sequence and the first base of the oligonucleotide.

More preferably the position is selected from the group consisting of: position 1, position 2, position 3, position 4 and position 5.

As mentioned earlier, the guide sequence corresponds to the seed sequence of a microRNA, which is defined elsewhere in the specification.

The length of the oligonucleotide of the invention may be adjusted for various purposes. A stronger interaction with the target RNA may be achieved by increasing the length of the oligonucleotide, as well as the stretch of bases complementary to the micro RNA target sequence of a target RNA. On the other hand, the length may be decreased for better delivery and bioavailability. A reduced length will give a decreased tm value (melting temperature) of the oligonucleotide. However, increasing the concentration of the oligonucleotide may be used to counteract this. Also affinity increasing nucleotides and affinity increasing modifications may be used.

In a preferred embodiment, the length of the oligonucleotide is selected from the group consisting of: less than 60 bases, less than 50 bases, less than 40 bases, less than 39 bases, less than 38 bases, less than 37 bases, less than 36 bases, less than 35, less than 34 bases, less than 33 bases, less than 32 bases, less than 31 bases, bases, less than 30 bases, less than 29 bases, less than 28 bases, less than 27 bases, less than 26 bases, less than 25 bases, less than 24 bases, less than 23 bases, less than 22 bases, less than 21 bases, less than 20 bases, less than 19 bases, less than 18 bases, less than 17 bases, less than 16 bases, less than 15 bases, less than 14 bases, less than 13 bases, less than 12 bases, less than 11 bases, less than 10 bases, less than 9 bases, less than 8 bases, less than 7 bases, more than 60 bases, more than 50 bases, more than 40 bases, more than 39 bases, more than 38 bases, more than 37 more, more than 36 bases, more than 35, more than 34 bases, more than 33 bases, more than 32 bases, more than 31, more than 30 bases, more than 29 bases, more than 28 bases, more than 27 bases, more than 26 bases, more than 25 bases, more than 24 bases, more than 23 bases, more than 22 bases, more than 21 bases, more than 20 bases, more than 19 bases, more than 18 bases, more than 17 bases, more than 16 bases, more than 15 bases, more than 14 bases, more than 13 bases, more than 12 bases, more than 11 bases, more than 10 bases, more than 9 bases, more than 8 bases, more than 7 bases, more than 6 bases and more than 5 bases.

In another preferred embodiment, the length of the oligonucleotide is selected from the group consisting of between 10 and 14 bases, between 12 and 16 bases, between 14 and 18 bases, between 16 and 20, between 10 and 25 bases, between 12 and 24 bases, between 14 and 22 bases, between 15 and and 22 bases and between 15 and 20 bases.

More preferred is a length between 8 and 25 bases.

Most preferred is a length between 10 and 20 bases.

In a preferred embodiment of the invention, the microRNA has a sequence selected from the group consisting of SEQ NO:1-723.

Preferred microRNAs are also listed in table 2. Note that the sequences of the sequence list of the priority applications mentioned earlier have been renumbered and additional sequences have been added.

Table 2. A list of human micro RNAs. Sequences are shown from the 5′ to 3′ direction.

MicroRNA	Sequence	SEQ ID NO
hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	1
hsa-let-7a*	CUAUACAAUCUACUGUCUUUC	2
hsa-let-7b	UGAGGUAGUAGGUUGUGUGGUU	3
hsa-let-7b*	CUAUACAACCUACUGCCUUCCC	4
hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU	5
hsa-let-7c*	UAGAGUUACACCCUGGGAGUUA	6
hsa-let-7d	AGAGGUAGUAGGUUGCAUAGUU	7
hsa-let-7d*	CUAUACGACCUGCUGCCUUUCU	8
hsa-let-7e	UGAGGUAGGAGGUUGUAUAGUU	9
hsa-let-7e*	CUAUACGGCCUCCUAGCUUUCC	10
hsa-let-7f	UGAGGUAGUAGAUUGUAUAGUU	11
hsa-let-7f-1*	CUAUACAAUCUAUUGCCUUCCC	12
hsa-let-7f-2*	CUAUACAGUCUACUGUCUUUCC	13
hsa-let-7g	UGAGGUAGUAGUUUGUACAGUU	14
hsa-let-7g*	CUGUACAGGCCACUGCCUUGC	15
hsa-let-7i	UGAGGUAGUAGUUUGUGCUGUU	16
hsa-let-7i*	CUGCGCAAGCUACUGCCUUGCU	17
hsa-miR-1	UGGAAUGUAAAGAAGUAUGUAU	18

hsa-miR-100	AACCCGUAGAUCCGAACUUGUG	19
hsa-miR-100*	CAAGCUUGUAUCUAUAGGUAUG	20
hsa-miR-101	UACAGUACUGUGAUAACUGAA	21
hsa-miR-101*	CAGUUAUCACAGUGCUGAUGCU	22
hsa-miR-103	AGCAGCAUUGUACAGGGCUAUGA	23
hsa-miR-105	UCAAAUGCUCAGACUCCUGUGGU	24
hsa-miR-105*	ACGGAUGUUUGAGCAUGUGCUA	25
hsa-miR-106a	AAAAGUGCUUACAGUGCAGGUAG	26
hsa-miR-106a*	CUGCAAUGUAAGCACUUCUUAC	27
hsa-miR-106b	UAAAGUGCUGACAGUGCAGAU	28
hsa-miR-106b*	CCGCACUGUGGGUACUUGCUGC	29
hsa-miR-107	AGCAGCAUUGUACAGGGCUAUCA	30
hsa-miR-10a	UACCCUGUAGAUCCGAAUUUGUG	31
hsa-miR-10a*	CAAAUUCGUAUCUAGGGGAAUA	32
hsa-miR-10b	UACCCUGUAGAACCGAAUUUGUG	33
hsa-miR-10b*	ACAGAUUCGAUUCUAGGGGAAU	34
hsa-miR-122	UGGAGUGUGACAAUGGUGUUUG	35
hsa-miR-122*	AACGCCAUUAUCACACUAAAUA	36
hsa-miR-124	UAAGGCACGCGGUGAAUGCC	37
hsa-miR-124*	CGUGUUCACAGCGGACCUUGAU	38
hsa-miR-125a-3p	ACAGGUGAGGUUCUUGGGAGCC	39
hsa-miR-125a-5p	UCCCUGAGACCCUUUAACCUGUGA	40
hsa-miR-125b	UCCCUGAGACCCUAACUUGUGA	41
hsa-miR-125b-1*	ACGGGUUAGGCUCUUGGGAGCU	42
hsa-miR-125b-2*	UCACAAGUCAGGCUCUUGGGAC	43
hsa-miR-126	UCGUACCGUGAGUAAUAAUGCG	44
hsa-miR-126*	CAUUAUUACUUUUGGUACGCG	45
hsa-miR-127-3p	UCGGAUCCGUCUGAGCUUGGCU	46
hsa-miR-127-5p	CUGAAGCUCAGAGGGCUCUGAU	47
hsa-miR-128a	UCACAGUGAACCGGUCUCUUU	48
hsa-miR-128b	UCACAGUGAACCGGUCUCUUU	49
hsa-miR-129*	AAGCCCUUACCCCAAAAAGUAU	50
hsa-miR-129-3p	AAGCCCUUACCCCAAAAAGCAU	51
hsa-miR-129-5p	CUUUUUGCGGUCUGGGCUUGC	52
hsa-miR-130a	CAGUGCAAUGUUAAAAGGGCAU	53
hsa-miR-130a*	UUCACAUUGUGCUACUGUCUGC	54
hsa-miR-130b	CAGUGCAAUGAUGAAAGGGCAU	55
hsa-miR-130b*	ACUCUUUCCCUGUUGCACUAC	56
hsa-miR-132	UAACAGUCUACAGCCAUGGUCG	57
hsa-miR-132*	ACCGUGGCUUUCGAUUGUUACU	58
hsa-miR-133a	UUUGGUCCCCUUCAACCAGCUG	59
hsa-miR-133b	UUUGGUCCCCUUCAACCAGCUA	60
hsa-miR-134	UGUGACUGGUUGACCAGAGGGG	61

hsa-miR-135a	UAUGGCUUUUUAUUCCUAUGUGA	62
hsa-miR-135a*	UAUAGGGAUUGGAGCCGUGGCG	63
hsa-miR-135b	UAUGGCUUUUCAUUCCUAUGUGA	64
hsa-miR-135b*	AUGUAGGGCUAAAAGCCAUGGG	65
hsa-miR-136	ACUCCAUUUGUUUUGAUGAUGGA	66
hsa-miR-136*	CAUCAUCGUCUCAAAUGAGUCU	67
hsa-miR-137	UUAUUGCUUAAGAAUACGCGUAG	68
hsa-miR-138	AGCUGGUGUUGUGAAUCAGGCCG	69
hsa-miR-138-1*	GCUACUUCACAACACCAGGGCC	70
hsa-miR-138-2*	GCUAUUUCACGACACCAGGGUU	71
hsa-miR-139-3p	GGAGACGCGGCCCUGUUGGAGU	72
hsa-miR-139-5p	UCUACAGUGCACGUGUCUCCAG	73
hsa-miR-140-3p	UACCACAGGGUAGAACCACGG	74
hsa-miR-140-5p	CAGUGGUUUUACCCUAUGGUAG	75
hsa-miR-141	UAACACUGUCUGGUAAAGAUGG	76
hsa-miR-141*	CAUCUUCCAGUACAGUGUUGGA	77
hsa-miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA	78
hsa-miR-142-5p	CAUAAAGUAGAAAGCACUACU	79
hsa-miR-143	UGAGAUGAAGCACUGUAGCUC	80
hsa-miR-143*	GGUGCAGUGCUGCAUCUCUGGU	81
hsa-miR-144	UACAGUAUAGAUGAUGUACU	82
hsa-miR-144*	GGAUAUCAUCAUAUACUGUAAG	83
hsa-miR-145	GUCCAGUUUUCCCAGGAAUCCCU	84
hsa-miR-145*	GGAUUCCUGGAAAUACUGUUCU	85
hsa-miR-146a	UGAGAACUGAAUUCCAUGGGUU	86
hsa-miR-146a*	CCUCUGAAAUUCAGUUCUUCAG	87
hsa-miR-146b-3p	UGCCCUGUGGACUCAGUUCUGG	88
hsa-miR-146b-5p	UGAGAACUGAAUUCCAUAGGCU	89
hsa-miR-147	GUGUGUGGAAAUGCUUCUGC	90
hsa-miR-147b	GUGUGCGGAAAUGCUUCUGCUA	91
hsa-miR-148a	UCAGUGCACUACAGAACUUUGU	92
hsa-miR-148a*	AAAGUUCUGAGACACUCCGACU	93
hsa-miR-148b	UCAGUGCAUCACAGAACUUUGU	94
hsa-miR-148b*	AAGUUCUGUUAUACACUCAGGC	95
hsa-miR-149	UCUGGCUCCGUGUCUUCACUCCC	96
hsa-miR-149*	AGGGAGGGACGGGGGCUGUGC	97
hsa-miR-150	UCUCCCAACCCUUGUACCAGUG	98
hsa-miR-150*	CUGGUACAGGCCUGGGGGACAG	99
hsa-miR-151-3p	CUAGACUGAAGCUCCUUGAGG	100
hsa-miR-151-5p	UCGAGGAGCUCACAGUCUAGU	101
hsa-miR-152	UCAGUGCAUGACAGAACUUGG	102
hsa-miR-153	UUGCAUAGUCACAAAAGUGAUC	103
hsa-miR-154	UAGGUUAUCCGUGUUGCCUUCG	104

hsa-miR-154*	AAUCAUACACGGUUGACCUAUU	105
hsa-miR-155	UUAAUGCUAAUCGUGAUAGGGGU	106
hsa-miR-155*	CUCCUACAUAUUAGCAUUAACA	107
hsa-miR-15a	UAGCAGCACAUAAUGGUUUGUG	108
hsa-miR-15a*	CAGGCCAUAUUGUGCUGCCUCA	109
hsa-miR-15b	UAGCAGCACAUCAUGGUUUACA	110
hsa-miR-15b*	CGAAUCAUUAUUUGCUGCUCUA	111
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG	112
hsa-miR-16-1*	CCAGUAUUAACUGUGCUGCUGA	113
hsa-miR-16-2*	CCAAUAUUACUGUGCUGCUUUA	114
hsa-miR-17	CAAAGUGCUUACAGUGCAGGUAG	115
hsa-miR-17*	ACUGCAGUGAAGGCACUUGUAG	116
hsa-miR-181a	AACAUUCAACGCUGUCGGUGAGU	117
hsa-miR-181a*	ACCAUCGACCGUUGAUUGUACC	118
hsa-miR-181a-2*	ACCACUGACCGUUGACUGUACC	119
hsa-miR-181b	AACAUUCAUUGCUGUCGGUGGGU	120
hsa-miR-181c	AACAUUCAACCUGUCGGUGAGU	121
hsa-miR-181c*	AACCAUCGACCGUUGAGUGGAC	122
hsa-miR-181d	AACAUUCAUUGUUGUCGGUGGGU	123
hsa-miR-182	UUUGGCAAUGGUAGAACUCACACU	124
hsa-miR-182*	UGGUUCUAGACUUGCCAACUA	125
hsa-miR-183	UAUGGCACUGGUAGAAUUCACU	126
hsa-miR-183*	GUGAAUUACCGAAGGGCCAUAA	127
hsa-miR-184	UGGACGGAGAACUGAUAAGGGU	128
hsa-miR-185	UGGAGAGAAAGGCAGUUCCUGA	129
hsa-miR-185*	AGGGGCUGGCUUUCCUCUGGUC	130
hsa-miR-186	CAAAGAAUUCUCCUUUUGGGCU	131
hsa-miR-186*	GCCCAAAGGUGAAUUUUUUGGG	132
hsa-miR-187	UCGUGUCUUGUGUUGCAGCCGG	133
hsa-miR-187*	GGCUACAACACAGGACCCGGGC	134
hsa-miR-188-3p	CUCCCACAUGCAGGGUUUGCA	135
hsa-miR-188-5p	CAUCCCUUGCAUGGUGGAGGG	136
hsa-miR-18a	UAAGGUGCAUCUAGUGCAGAUAG	137
hsa-miR-18a*	ACUGCCCUAAGUGCUCCUUCUGG	138
hsa-miR-18b	UAAGGUGCAUCUAGUGCAGUUAG	139
hsa-miR-18b*	UGCCCUAAAUGCCCCUUCUGGC	140
hsa-miR-190	UGAUAUGUUUGAUAUAUUAGGU	141
hsa-miR-190b	UGAUAUGUUUGAUAUUGGGUU	142
hsa-miR-191	CAACGGAAUCCCAAAAGCAGCUG	143
hsa-miR-191*	GCUGCGCUUGGAUUUCGUCCCC	144
hsa-miR-192	CUGACCUAUGAAUUGACAGCC	145
hsa-miR-192*	CUGCCAAUUCCAUAGGUCACAG	146
hsa-miR-193a-3p	AACUGGCCUACAAAGUCCCAGU	147

hsa-miR-193a-5p	UGGGUCUUUGCGGGCGAGAUGA	148
hsa-miR-193b	AACUGGCCCUCAAAGUCCCGCU	149
hsa-miR-193b*	CGGGGUUUUGAGGGCGAGAUGA	150
hsa-miR-194	UGUAACAGCAACUCCAUGUGGA	151
hsa-miR-194*	CCAGUGGGGCUGCUGUUAUCUG	152
hsa-miR-195	UAGCAGCACAGAAAUAUUGGC	153
hsa-miR-195*	CCAAUAUUGGCUGUGCUGCUCC	154
hsa-miR-196a	UAGGUAGUUUCAUGUUGUUGGG	155
hsa-miR-196a*	CGGCAACAAGAAACUGCCUGAG	156
hsa-miR-196b	UAGGUAGUUUCCUGUUGUUGGG	157
hsa-miR-197	UUCACCACCUUCUCCACCCAGC	158
hsa-miR-198	GGUCCAGAGGGGAGAUAGGUUC	159
hsa-miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA	160
hsa-miR-199a-5p	CCCAGUGUUCAGACUACCUGUUC	161
hsa-miR-199b-3p	ACAGUAGUCUGCACAUUGGUUA	162
hsa-miR-199b-5p	CCCAGUGUUUAGACUAUCUGUUC	163
hsa-miR-19a	UGUGCAAAUCUAUGCAAAACUGA	164
hsa-miR-19a*	AGUUUUGCAUAGUUGCACUACA	165
hsa-miR-19b	UGUGCAAAUCCAUGCAAAACUGA	166
hsa-miR-19b-1*	AGUUUUGCAGGUUUGCAUCCAGC	167
hsa-miR-19b-2*	AGUUUUGCAGGUUUGCAUUUCA	168
hsa-miR-200a	UAACACUGUCUGGUAACGAUGU	169
hsa-miR-200a*	CAUCUUACCGGACAGUGCUGGA	170
hsa-miR-200b	UAAUACUGCCUGGUAAUGAUGA	171
hsa-miR-200b*	CAUCUUACUGGGCAGCAUUGGA	172
hsa-miR-200c	UAAUACUGCCGGGUAAUGAUGGA	173
hsa-miR-200c*	CGUCUUACCCAGCAGUGUUUGG	174
hsa-miR-202	AGAGGUAUAGGGCAUGGGAA	175
hsa-miR-202*	UUCCUAUGCAUAUACUUCUUUG	176
hsa-miR-203	GUGAAAUGUUUAGGACCACUAG	177
hsa-miR-204	UUCCCUUUGUCAUCCUAUGCCU	178
hsa-miR-205	UCCUUCAUUCCACCGGAGUCUG	179
hsa-miR-206	UGGAAUGUAAGGAAGUGUGUGG	180
hsa-miR-208	AUAAGACGAGCAAAAAGCUUGU	181
hsa-miR-208b	AUAAGACGAACAAAAGGUUUGU	182
hsa-miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	183
hsa-miR-20a*	ACUGCAUUAUGAGCACUUAAAG	184
hsa-miR-20b	CAAAGUGCUCAUAGUGCAGGUAG	185
hsa-miR-20b*	ACUGUAGUAUGGGCACUUCCAG	186
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA	187
hsa-miR-21*	CAACACCAGUCGAUGGGCUGU	188
hsa-miR-210	CUGUGCGUGUGACAGCGGCUGA	189
hsa-miR-211	UUCCCUUUGUCAUCCUUCGCCU	190

hsa-miR-212	UAACAGUCUCCAGUCACGGCC	191
hsa-miR-214	ACAGCAGGCACAGACAGGCAGU	192
hsa-miR-214*	UGCCUGUCUACACUUGCUGUGC	193
hsa-miR-215	AUGACCUAUGAAUUGACAGAC	194
hsa-miR-216a	UAAUCUCAGCUGGCAACUGUGA	195
hsa-miR-216b	AAAUCUCUGCAGGCAAAUGUGA	196
hsa-miR-217	UACUGCAUCAGGAACUGAUUGGA	197
hsa-miR-218	UUGUGCUUGAUCUAACCAUGU	198
hsa-miR-218-1*	AUGGUUCCGUCAAGCACCAUGG	199
hsa-miR-218-2*	CAUGGUUCUGUCAAGCACCGCG	200
hsa-miR-219-1-3p	AGAGUUGAGUCUGGACGUCCCG	201
hsa-miR-219-2-3p	AGAAUUGUGGCUGGACAUCUGU	202
hsa-miR-219-5p	UGAUUGUCCAAACGCAAUUCU	203
hsa-miR-22	AAGCUGCCAGUUGAAGAACUGU	204
hsa-miR-22*	AGUUCUUCAGUGGCAAGCUUUA	205
hsa-miR-220	CCACACCGUAUCUGACACUUU	206
hsa-miR-220b	CCACCACCGUGUCUGACACUU	207
hsa-miR-220c	ACACAGGGCUGUUGUGAAGACU	208
hsa-miR-221	AGCUACAUUGUCUGCUGGGUUUC	209
hsa-miR-221*	ACCUGGCAUACAAUGUAGAUUU	210
hsa-miR-222	AGCUACAUCUGGCUACUGGGU	211
hsa-miR-222*	CUCAGUAGCCAGUGUAGAUCCU	212
hsa-miR-223	UGUCAGUUUGUCAAAUACCCCA	213
hsa-miR-223*	CGUGUAUUUGACAAGCUGAGUU	214
hsa-miR-224	CAAGUCACUAGUGGUUCCGUU	215
hsa-miR-23a	AUCACAUUGCCAGGGAUUUCC	216
hsa-miR-23a*	GGGGUUCCUGGGGAUGGGAUUU	217
hsa-miR-23b	AUCACAUUGCCAGGGAUUACC	218
hsa-miR-23b*	UGGGUUCCUGGCAUGCUGAUUU	219
hsa-miR-24	UGGCUCAGUUCAGCAGGAACAG	220
hsa-miR-24-1*	UGCCUACUGAGCUGAUAUCAGU	221
hsa-miR-24-2*	UGCCUACUGAGCUGAAACACAG	222
hsa-miR-25	CAUUGCACUUGUCUCGGUCUGA	223
hsa-miR-25*	AGGCGGAGACUUGGGCAAUUG	224
hsa-miR-26a	UUCAAGUAAUCCAGGAUAGGCU	225
hsa-miR-26a-1*	CCUAUUCUUGGUUACUUGCACG	226
hsa-miR-26a-2*	CCUAUUCUUGAUUACUUGUUUC	227
hsa-miR-26b	UUCAAGUAAUUCAGGAUAGGU	228
hsa-miR-26b*	CCUGUUCUCCAUUACUUGGCUC	229
hsa-miR-27a	UUCACAGUGGCUAAGUUCCGC	230
hsa-miR-27a*	AGGGCUUAGCUGCUUGUGAGCA	231
hsa-miR-27b	UUCACAGUGGCUAAGUUCUGC	232
hsa-miR-27b*	AGAGCUUAGCUGAUUGGUGAAC	233

hsa-miR-28-3p	CACUAGAUUGUGAGCUCCUGGA	234
hsa-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	235
hsa-miR-296-3p	GAGGGUUGGGUGGAGGCUCUCC	236
hsa-miR-296-5p	AGGGCCCCCCCUCAAUCCUGU	237
hsa-miR-297	AUGUAUGUGUGCAUGUGCAUG	238
hsa-miR-298	AGCAGAAGCAGGGAGGUUCUCCCA	239
hsa-miR-299-3p	UAUGUGGGAUGGUAAACCGCUU	240
hsa-miR-299-5p	UGGUUUACCGUCCCACAUACAU	241
hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA	242
hsa-miR-29a*	ACUGAUUUCUUUUGGUGUUCAG	243
hsa-miR-29b	UAGCACCAUUUGAAAUCAGUGUU	244
hsa-miR-29b-1*	GCUGGUUUCAUAUGGUGGUUUAGA	245
hsa-miR-29b-2*	CUGGUUUCACAUGGUGGCUUAG	246
hsa-miR-29c	UAGCACCAUUUGAAAUCGGUUA	247
hsa-miR-29c*	UGACCGAUUUCUCCUGGUGUUC	248
hsa-miR-300	UAUACAAGGGCAGACUCUCUCU	249
hsa-miR-301a	CAGUGCAAUAGUAUUGUCAAAGC	250
hsa-miR-301b	CAGUGCAAUGAUAUUGUCAAAGC	251
hsa-miR-302a	UAAGUGCUUCCAUGUUUUGGUGA	252
hsa-miR-302a*	ACUUAAACGUGGAUGUACUUGCU	253
hsa-miR-302b	UAAGUGCUUCCAUGUUUUAGUAG	254
hsa-miR-302b*	ACUUUAACAUGGAAGUGCUUUC	255
hsa-miR-302c	UAAGUGCUUCCAUGUUUCAGUGG	256
hsa-miR-302c*	UUUAACAUGGGGGUACCUGCUG	257
hsa-miR-302d	UAAGUGCUUCCAUGUUUGAGUGU	258
hsa-miR-302d*	ACUUUAACAUGGAGGCACUUGC	259
hsa-miR-30a	UGUAAACAUCCUCGACUGGAAG	260
hsa-miR-30a*	CUUUCAGUCGGAUGUUUGCAGC	261
hsa-miR-30b	UGUAAACAUCCUACACUCAGCU	262
hsa-miR-30b*	CUGGGAGGUGGAUGUUUACUUC	263
hsa-miR-30c	UGUAAACAUCCUACACUCUCAGC	264
hsa-miR-30c-1*	CUGGGAGAGGGUUGUUUACUCC	265
hsa-miR-30c-2*	CUGGGAGAAGGCUGUUUACUCU	266
hsa-miR-30d	UGUAAACAUCCCCGACUGGAAG	267
hsa-miR-30d*	CUUUCAGUCAGAUGUUUGCUGC	268
hsa-miR-30e	UGUAAACAUCCUUGACUGGAAG	269
hsa-miR-30e*	CUUUCAGUCGGAUGUUUACAGC	270
hsa-miR-31	AGGCAAGAUGCUGGCAUAGCU	271
hsa-miR-31*	UGCUAUGCCAACAUAUUGCCAU	272
hsa-miR-32	UAUUGCACAUUACUAAGUUGCA	273
hsa-miR-32*	CAAUUUAGUGUGUGUGAUAUUU	274
hsa-miR-320	AAAAGCUGGGUUGAGAGGGCGA	275
hsa-miR-323-3p	CACAUUACACGGUCGACCUCU	276

hsa-miR-323-5p	AGGUGGUCCGUGGCGCGUUCGC	277
hsa-miR-324-3p	ACUGCCCCAGGUGCUGCUGG	278
hsa-miR-324-5p	CGCAUCCCCUAGGGCAUUGGUGU	279
hsa-miR-325	CCUAGUAGGUGUCCAGUAAGUGU	280
hsa-miR-326	CCUCUGGGCCCUUCCUCCAG	281
hsa-miR-328	CUGGCCCUCUCUGCCCUUCCGU	282
hsa-miR-329	AACACACCUGGUUAACCUCUUU	283
hsa-miR-330-3p	GCAAAGCACACGGCCUGCAGAGA	284
hsa-miR-330-5p	UCUCUGGGCCUGUGUCUUAGGC	285
hsa-miR-331-3p	GCCCCUGGGCCUAUCCUAGAA	286
hsa-miR-331-5p	CUAGGUAUGGUCCCAGGGAUCC	287
hsa-miR-335	UCAAGAGCAAUAACGAAAAAUGU	288
hsa-miR-335*	UUUUUCAUUAUUGCUCCUGACC	289
hsa-miR-337-3p	CUCCUAUAUGAUGCCUUUCUUC	290
hsa-miR-337-5p	GAACGGCUUCAUACAGGAGUU	291
hsa-miR-338-3p	UCCAGCAUCAGUGAUUUUGUUG	292
hsa-miR-338-5p	AACAAUAUCCUGGUGCUGAGUG	293
hsa-miR-339-3p	UGAGCGCCUCGACGACAGAGCCG	294
hsa-miR-339-5p	UCCCUGUCCUCCAGGAGCUCACG	295
hsa-miR-33a	GUGCAUUGUAGUUGCAUUGCA	296
hsa-miR-33a*	CAAUGUUUCCACAGUGCAUCAC	297
hsa-miR-33b	GUGCAUUGCUGUUGCAUUGC	298
hsa-miR-33b*	CAGUGCCUCGGCAGUGCAGCCC	299
hsa-miR-340	UUAUAAAGCAAUGAGACUGAUU	300
hsa-miR-340*	UCCGUCUCAGUUACUUUAUAGC	301
hsa-miR-342-3p	UCUCACACAGAAAUCGCACCCGU	302
hsa-miR-342-5p	AGGGGUGCUAUCUGUGAUUGA	303
hsa-miR-345	GCUGACUCCUAGUCCAGGGCUC	304
hsa-miR-346	UGUCUGCCCGCAUGCCUGCCUCU	305
hsa-miR-34a	UGGCAGUGUCUUAGCUGGUUGU	306
hsa-miR-34a*	CAAUCAGCAAGUAUACUGCCCU	307
hsa-miR-34b	CAAUCACUAACUCCACUGCCAU	308
hsa-miR-34b*	UAGGCAGUGUCAUUAGCUGAUUG	309
hsa-miR-34c-3p	AAUCACUAACCACACGGCCAGG	310
hsa-miR-34c-5p	AGGCAGUGUAGUUAGCUGAUUGC	311
hsa-miR-361-3p	UCCCCCAGGUGUGAUUCUGAUUU	312
hsa-miR-361-5p	UUAUCAGAAUCUCCAGGGGUAC	313
hsa-miR-362-3p	AACACACCUAUUCAAGGAUUCA	314
hsa-miR-362-5p	AAUCCUUGGAACCUAGGUGUGAGU	315
hsa-miR-363	AAUUGCACGGUAUCCAUCUGUA	316
hsa-miR-363*	CGGGUGGAUCACGAUGCAAUUU	317
hsa-miR-365	UAAUGCCCCUAAAAAUCCUUAU	318
hsa-miR-367	AAUUGCACUUUAGCAAUGGUGA	319

hsa-miR-367*	ACUGUUGCUAAUAUGCAACUCU	320
hsa-miR-369-3p	AAUAAUACAUGGUUGAUCUUU	321
hsa-miR-369-5p	AGAUCGACCGUGUUAUAUUCGC	322
hsa-miR-370	GCCUGCUGGGGUGGAACCUGGU	323
hsa-miR-371-3p	AAGUGCCGCCAUCUUUUGAGUGU	324
hsa-miR-371-5p	ACUCAAACUGUGGGGGCACU	325
hsa-miR-372	AAAGUGCUGCGACAUUUGAGCGU	326
hsa-miR-373	GAAGUGCUUCGAUUUUGGGGUGU	327
hsa-miR-373*	ACUCAAAAUGGGGGCGCUUUCC	328
hsa-miR-374a	UUAUAAUACAACCUGAUAAGUG	329
hsa-miR-374a*	CUUAUCAGAUUGUAUUGUAAUU	330
hsa-miR-374b	AUAUAAUACAACCUGCUAAGUG	331
hsa-miR-374b*	CUUAGCAGGUUGUAUUAUCAUU	332
hsa-miR-375	UUUGUUCGUUCGGCUCGCGUGA	333
hsa-miR-376a	AUCAUAGAGGAAAAUCCACGU	334
hsa-miR-376a*	GUAGAUUCUCCUUCUAUGAGUA	335
hsa-miR-376b	AUCAUAGAGGAAAAUCCAUGUU	336
hsa-miR-376c	AACAUAGAGGAAAUUCCACGU	337
hsa-miR-377	AUCACACAAAGGCAACUUUUGU	338
hsa-miR-377*	AGAGGUUGCCCUUGGUGAAUUC	339
hsa-miR-378	ACUGGACUUGGAGUCAGAAGG	340
hsa-miR-378*	CUCCUGACUCCAGGUCCUGUGU	341
hsa-miR-379	UGGUAGACUAUGGAACGUAGG	342
hsa-miR-379*	UAUGUAACAUGGUCCACUAACU	343
hsa-miR-380	UAUGUAAUAUGGUCCACAUCUU	344
hsa-miR-380*	UGGUUGACCAUAGAACAUGCGC	345
hsa-miR-381	UAUACAAGGGCAAGCUCUCUGU	346
hsa-miR-382	GAAGUUGUUCGUGGUGGAUUCG	347
hsa-miR-383	AGAUCAGAAGGUGAUUGUGGCU	348
hsa-miR-384	AUUCCUAGAAAUUGUUCAUA	349
hsa-miR-409-3p	GAAUGUUGCUCGGUGAACCCCU	350
hsa-miR-409-5p	AGGUUACCCGAGCAACUUUGCAU	351
hsa-miR-410	AAUAUAACACAGAUGGCCUGU	352
hsa-miR-411	UAGUAGACCGUAUAGCGUACG	353
hsa-miR-411*	UAUGUAACACGGUCCACUAACC	354
hsa-miR-412	ACUUCACCUGGUCCACUAGCCGU	355
hsa-miR-421	AUCAACAGACAUUAAUUGGGCGC	356
hsa-miR-422a	ACUGGACUUAGGGUCAGAAGGC	357
hsa-miR-423-3p	AGCUCGGUCUGAGGCCCCUCAGU	358
hsa-miR-423-5p	UGAGGGGCAGAGAGCGAGACUUU	359
hsa-miR-424	CAGCAGCAAUUCAUGUUUUGAA	360
hsa-miR-424*	CAAAACGUGAGGCGCUGCUAU	361
hsa-miR-425	AAUGACACGAUCACUCCCGUUGA	362

hsa-miR-425*	AUCGGGAAUGUCGUGUCCGCCC	363
hsa-miR-429	UAAUACUGUCUGGUAAAACCGU	364
hsa-miR-431	UGUCUUGCAGGCCGUCAUGCA	365
hsa-miR-431*	CAGGUCGUCUUGCAGGGCUUCU	366
hsa-miR-432	UCUUGGAGUAGGUCAUUGGGUGG	367
hsa-miR-432*	CUGGAUGGCUCCUCCAUGUCU	368
hsa-miR-433	AUCAUGAUGGGCUCCUCGGUGU	369
hsa-miR-448	UUGCAUAUGUAGGAUGUCCCAU	370
hsa-miR-449a	UGGCAGUGUAUUGUUAGCUGGU	371
hsa-miR-449b	AGGCAGUGUAUUGUUAGCUGGC	372
hsa-miR-450a	UUUUGCGAUGUGUUCCUAAUAU	373
hsa-miR-450b-3p	UUGGGAUCAUUUUGCAUCCAUA	374
hsa-miR-450b-5p	UUUUGCAAUAUGUUCCUGAAUA	375
hsa-miR-451	AAACCGUUACCAUUACUGAGUU	376
hsa-miR-452	AACUGUUUGCAGAGGAAACUGA	377
hsa-miR-452*	CUCAUCUGCAAAGAAGUAAGUG	378
hsa-miR-453	AGGUUGUCCGUGGUGAGUUCGCA	379
hsa-miR-454	UAGUGCAAUAUUGCUUAUAGGGU	380
hsa-miR-454*	ACCCUAUCAAUAUUGUCUCUGC	381
hsa-miR-455-3p	GCAGUCCAUGGGCAUAUACAC	382
hsa-miR-455-5p	UAUGUGCCUUUGGACUACAUCG	383
hsa-miR-483-3p	UCACUCCUCUCCUCCCGUCUU	384
hsa-miR-483-5p	AAGACGGGAGGAAAGAAGGGAG	385
hsa-miR-484	UCAGGCUCAGUCCCCUCCCGAU	386
hsa-miR-485-3p	GUCAUACACGGCUCUCCUCUCU	387
hsa-miR-485-5p	AGAGGCUGGCCGUGAUGAAUUC	388
hsa-miR-486-3p	CGGGGCAGCUCAGUACAGGAU	389
hsa-miR-486-5p	UCCUGUACUGAGCUGCCCCGAG	390
hsa-miR-487a	AAUCAUACAGGGACAUCCAGUU	391
hsa-miR-487b	AAUCGUACAGGGUCAUCCACUU	392
hsa-miR-488	UUGAAAGGCUAUUUCUUGGUC	393
hsa-miR-488*	CCCAGAUAAUGGCACUCUCAA	394
hsa-miR-489	GUGACAUCACAUAUACGGCAGC	395
hsa-miR-490-3p	CAACCUGGAGGACUCCAUGCUG	396
hsa-miR-490-5p	CCAUGGAUCUCCAGGUGGGU	397
hsa-miR-491-3p	CUUAUGCAAGAUUCCCUUCUAC	398
hsa-miR-491-5p	AGUGGGGAACCCUUCCAUGAGG	399
hsa-miR-492	AGGACCUGCGGGACAAGAUUCUU	400
hsa-miR-493	UGAAGGUCUACUGUGUGCCAGG	401
hsa-miR-493*	UUGUACAUGGUAGGCUUUCAUU	402
hsa-miR-494	UGAAACAUACACGGGAAACCUC	403
hsa-miR-495	AAACAAACAUGGUGCACUUCUU	404
hsa-miR-496	UGAGUAUUACAUGGCCAAUCUC	405

hsa-miR-497	CAGCAGCACACUGUGGUUUGU	406
hsa-miR-497*	CAAACCACACUGUGGUGUUAGA	407
hsa-miR-498	UUUCAAGCCAGGGGGCGUUUUUC	408
hsa-miR-499-3p	AACAUCACAGCAAGUCUGUGCU	409
hsa-miR-499-5p	UUAAGACUUGCAGUGAUGUUU	410
hsa-miR-500	UAAUCCUUGCUACCUGGGUGAGA	411
hsa-miR-500*	AUGCACCUGGGCAAGGAUUCUG	412
hsa-miR-501-3p	AAUGCACCCGGGCAAGGAUUCU	413
hsa-miR-501-5p	AAUCCUUUGUCCCUGGGUGAGA	414
hsa-miR-502-3p	AAUGCACCUGGGCAAGGAUUCA	415
hsa-miR-502-5p	AUCCUUGCUAUCUGGGUGCUA	416
hsa-miR-503	UAGCAGCGGGAACAGUUCUGCAG	417
hsa-miR-504	AGACCCUGGUCUGCACUCUAUC	418
hsa-miR-505	CGUCAACACUUGCUGGUUUCCU	419
hsa-miR-505*	GGGAGCCAGGAAGUAUUGAUGU	420
hsa-miR-506	UAAGGCACCCUUCUGAGUAGA	421
hsa-miR-507	UUUUGCACCUUUUGGAGUGAA	422
hsa-miR-508-3p	UGAUUGUAGCCUUUUGGAGUAGA	423
hsa-miR-508-5p	UACUCCAGAGGGCGUCACUCAUG	424
hsa-miR-509-3-5p	UACUGCAGACGUGGCAAUCAUG	425
hsa-miR-509-3p	UGAUUGGUACGUCUGUGGGUAG	426
hsa-miR-509-5p	UACUGCAGACAGUGGCAAUCA	427
hsa-miR-510	UACUCAGGAGAGUGGCAAUCAC	428
hsa-miR-511	GUGUCUUUUGCUCUGCAGUCA	429
hsa-miR-512-3p	AAGUGCUGUCAUAGCUGAGGUC	430
hsa-miR-512-5p	CACUCAGCCUUGAGGGCACUUUC	431
hsa-miR-513-3p	UAAAUUUCACCUUUCUGAGAAGG	432
hsa-miR-513-5p	UUCACAGGGAGGUGUCAU	433
hsa-miR-514	AUUGACACUUCUGUGAGUAGA	434
hsa-miR-515-3p	GAGUGCCUUCUUUUGGAGCGUU	435
hsa-miR-515-5p	UUCUCCAAAAGAAAGCACUUUCUG	436
hsa-miR-516a-3p	UGCUUCCUUUCAGAGGGU	437
hsa-miR-516a-5p	UUCUCGAGGAAAGAAGCACUUUC	438
hsa-miR-516b	AUCUGGAGGUAAGAAGCACUUU	439
hsa-miR-516b*	UGCUUCCUUUCAGAGGGU	440
hsa-miR-517*	CCUCUAGAUGGAAGCACUGUCU	441
hsa-miR-517a	AUCGUGCAUCCCUUUAGAGUGU	442
hsa-miR-517b	UCGUGCAUCCCUUUAGAGUGUU	443
hsa-miR-517c	AUCGUGCAUCCUUUUAGAGUGU	444
hsa-miR-518a-3p	GAAAGCGCUUCCCUUUGCUGGA	445
hsa-miR-518a-5p	CUGCAAAGGGAAGCCCUUUC	446
hsa-miR-518b	CAAAGCGCUCCCCUUUAGAGGU	447
hsa-miR-518c	CAAAGCGCUUCUCUUUAGAGUGU	448

hsa-miR-518c*	UCUCUGGAGGGAAGCACUUUCUG	449
hsa-miR-518d-3p	CAAAGCGCUUCCCUUUGGAGC	450
hsa-miR-518d-5p	CUCUAGAGGGAAGCACUUUCUG	451
hsa-miR-518e	AAAGCGCUUCCCUUCAGAGUG	452
hsa-miR-518e*	CUCUAGAGGGAAGCGCUUUCUG	453
hsa-miR-518f	GAAAGCGCUUCUCUUUAGAGG	454
hsa-miR-518f*	CUCUAGAGGGAAGCACUUUCUC	455
hsa-miR-519a	AAAGUGCAUCCUUUUAGAGUGU	456
hsa-miR-519a*	CUCUAGAGGGAAGCGCUUUCUG	457
hsa-miR-519b-3p	AAAGUGCAUCCUUUUAGAGGUU	458
hsa-miR-519b-5p	CUCUAGAGGGAAGCGCUUUCUG	459
hsa-miR-519c-3p	AAAGUGCAUCUUUUUAGAGGAU	460
hsa-miR-519c-5p	CUCUAGAGGGAAGCGCUUUCUG	461
hsa-miR-519d	CAAAGUGCCUCCCUUUAGAGUG	462
hsa-miR-519e	AAGUGCCUCCUUUUAGAGUGUU	463
hsa-miR-519e*	UUCUCCAAAAGGGAGCACUUUC	464
hsa-miR-520a-3p	AAAGUGCUUCCCUUUGGACUGU	465
hsa-miR-520a-5p	CUCCAGAGGGAAGUACUUUCU	466
hsa-miR-520b	AAAGUGCUUCCUUUUAGAGGG	467
hsa-miR-520c-3p	AAAGUGCUUCCUUUUAGAGGGU	468
hsa-miR-520c-5p	CUCUAGAGGGAAGCACUUUCUG	469
hsa-miR-520d-3p	AAAGUGCUUCUCUUUGGUGGGU	470
hsa-miR-520d-5p	CUACAAAGGGAAGCCCUUUC	471
hsa-miR-520e	AAAGUGCUUCCUUUUUGAGGG	472
hsa-miR-520f	AAGUGCUUCCUUUUAGAGGGUU	473
hsa-miR-520g	ACAAAGUGCUUCCCUUUAGAGUGU	474
hsa-miR-520h	ACAAAGUGCUUCCCUUUAGAGU	475
hsa-miR-521	AACGCACUUCCCUUUAGAGUGU	476
hsa-miR-522	AAAAUGGUUCCCUUUAGAGUGU	477
hsa-miR-522*	CUCUAGAGGGAAGCGCUUUCUG	478
hsa-miR-523	GAACGCGCUUCCCUAUAGAGGGU	479
hsa-miR-523*	CUCUAGAGGGAAGCGCUUUCUG	480
hsa-miR-524-3p	GAAGGCGCUUCCCUUUGGAGU	481
hsa-miR-524-5p	CUACAAAGGGAAGCACUUUCUC	482
hsa-miR-525-3p	GAAGGCGCUUCCCUUUAGAGCG	483
hsa-miR-525-5p	CUCCAGAGGGAUGCACUUUCU	484
hsa-miR-526a	CUCUAGAGGGAAGCACUUUCUG	485
hsa-miR-526b	CUCUUGAGGGAAGCACUUUCUGU	486
hsa-miR-526b*	GAAAGUGCUUCCUUUUAGAGGC	487
hsa-miR-527	CUGCAAAGGGAAGCCCUUUC	488
hsa-miR-532-3p	CCUCCCACACCCAAGGCUUGCA	489
hsa-miR-532-5p	CAUGCCUUGAGUGUAGGACCGU	490
hsa-miR-539	GGAGAAAUUAUCCUUGGUGUGU	491

hsa-miR-541	UGGUGGGCACAGAAUCUGGACU	492
hsa-miR-541*	AAAGGAUUCUGCUGUCGGUCCCACU	493
hsa-miR-542-3p	UGUGACAGAUUGAUAACUGAAA	494
hsa-miR-542-5p	UCGGGGAUCAUCAUGUCACGAGA	495
hsa-miR-543	AAACAUUCGCGGUGCACUUCUU	496
hsa-miR-544	AUUCUGCAUUUUUAGCAAGUUC	497
hsa-miR-545	UCAGCAAACAUUUAUUGUGUGC	498
hsa-miR-545*	UCAGUAAAUGUUUAUUAGAUGA	499
hsa-miR-548a-3p	CAAAACUGGCAAUUACUUUUGC	500
hsa-miR-548a-5p	AAAAGUAAUUGCGAGUUUUACC	501
hsa-miR-548b-3p	CAAGAACCUCAGUUGCUUUUGU	502
hsa-miR-548b-5p	AAAAGUAAUUGUGGUUUUGGCC	503
hsa-miR-548c-3p	CAAAAAUCUCAAUUACUUUUGC	504
hsa-miR-548c-5p	AAAAGUAAUUGCGGUUUUUGCC	505
hsa-miR-548d-3p	CAAAAACCACAGUUUCUUUUGC	506
hsa-miR-548d-5p	AAAAGUAAUUGUGGUUUUUGCC	507
hsa-miR-549	UGACAACUAUGGAUGAGCUCU	508
hsa-miR-550	AGUGCCUGAGGGAGUAAGAGCCC	509
hsa-miR-550*	UGUCUUACUCCCUCAGGCACAU	510
hsa-miR-551a	GCGACCCACUCUUGGUUUCCA	511
hsa-miR-551b	GCGACCCAUACUUGGUUUCAG	512
hsa-miR-551b*	GAAAUCAAGCGUGGGUGAGACC	513
hsa-miR-552	AACAGGUGACUGGUUAGACAA	514
hsa-miR-553	AAAACGGUGAGAUUUUGUUUU	515
hsa-miR-554	GCUAGUCCUGACUCAGCCAGU	516
hsa-miR-555	AGGGUAAGCUGAACCUCUGAU	517
hsa-miR-556-3p	AUAUUACCAUUAGCUCAUCUUU	518
hsa-miR-556-5p	GAUGAGCUCAUUGUAAUAUGAG	519
hsa-miR-557	GUUUGCACGGGUGGGCCUUGUCU	520
hsa-miR-558	UGAGCUGCUGUACCAAAAU	521
hsa-miR-559	UAAAGUAAAUAUGCACCAAAA	522
hsa-miR-560	GCGUGCGCCGGCCGGCCGCC	523
hsa-miR-561	CAAAGUUUAAGAUCCUUGAAGU	524
hsa-miR-562	AAAGUAGCUGUACCAUUUGC	525
hsa-miR-563	AGGUUGACAUACGUUUCCC	526
hsa-miR-564	AGGCACGGUGUCAGCAGGC	527
hsa-miR-565	GGCUGGCUCGCGAUGUCUGUUU	528
hsa-miR-566	GGGCGCCUGUGAUCCCAAC	529
hsa-miR-567	AGUAUGUUCUUCCAGGACAGAAC	530
hsa-miR-568	AUGUAUAAAUGUAUACACAC	531
hsa-miR-569	AGUUAAUGAAUCCUGGAAAGU	532
hsa-miR-570	CGAAAACAGCAAUUACCUUUGC	533
hsa-miR-571	UGAGUUGGCCAUCUGAGUGAG	534

hsa-miR-572	GUCCGCUCGGCGGUGGCCCA	535
hsa-miR-573	CUGAAGUGAUGUGUAACUGAUCAG	536
hsa-miR-574-3p	CACGCUCAUGCACACACCCACA	537
hsa-miR-574-5p	UGAGUGUGUGUGUGUGAGUGUGU	538
hsa-miR-575	GAGCCAGUUGGACAGGAGC	539
hsa-miR-576-3p	AAGAUGUGGAAAAAUUGGAAUC	540
hsa-miR-576-5p	AUUCUAAUUUCUCCACGUCUUU	541
hsa-miR-577	UAGAUAAAAUAUUGGUACCUG	542
hsa-miR-578	CUUCUUGUGCUCUAGGAUUGU	543
hsa-miR-579	UUCAUUUGGUAUAAACCGCGAUU	544
hsa-miR-580	UUGAGAAUGAUGAAUCAUUAGG	545
hsa-miR-581	UCUUGUGUUCUCUAGAUCAGU	546
hsa-miR-582-3p	UAACUGGUUGAACAACUGAACC	547
hsa-miR-582-5p	UUACAGUUGUUCAACCAGUUACU	548
hsa-miR-583	CAAAGAGGAAGGUCCCAUUAC	549
hsa-miR-584	UUAUGGUUUGCCUGGGACUGAG	550
hsa-miR-585	UGGGCGUAUCUGUAUGCUA	551
hsa-miR-586	UAUGCAUUGUAUUUUUAGGUCC	552
hsa-miR-587	UUUCCAUAGGUGAUGAGUCAC	553
hsa-miR-588	UUGGCCACAAUGGGUUAGAAC	554
hsa-miR-589	UGAGAACCACGUCUGCUCUGAG	555
hsa-miR-589*	UCAGAACAAAUGCCGGUUCCCAGA	556
hsa-miR-590-3p	UAAUUUUAUGUAUAAGCUAGU	557
hsa-miR-590-5p	GAGCUUAUUCAUAAAAGUGCAG	558
hsa-miR-591	AGACCAUGGGUUCUCAUUGU	559
hsa-miR-592	UUGUGUCAAUAUGCGAUGAUGU	560
hsa-miR-593	UGUCUCUGCUGGGGUUUCU	561
hsa-miR-593*	AGGCACCAGCCAGGCAUUGCUCAGC	562
hsa-miR-595	GAAGUGUGCCGUGGUGUGUCU	563
hsa-miR-596	AAGCCUGCCCGGCUCCUCGGG	564
hsa-miR-597	UGUGUCACUCGAUGACCACUGU	565
hsa-miR-598	UACGUCAUCGUUGUCAUCGUCA	566
hsa-miR-599	GUUGUGUCAGUUUAUCAAAC	567
hsa-miR-600	ACUUACAGACAAGAGCCUUGCUC	568
hsa-miR-601	UGGUCUAGGAUUGUUGGAGGAG	569
hsa-miR-602	GACACGGGCGACAGCUGCGGCCC	570
hsa-miR-603	CACACACUGCAAUUACUUUUGC	571
hsa-miR-604	AGGCUGCGGAAUUCAGGAC	572
hsa-miR-605	UAAAUCCCAUGGUGCCUUCUCCU	573
hsa-miR-606	AAACUACUGAAAAUCAAAGAU	574
hsa-miR-607	GUUCAAAUCCAGAUCUAUAAC	575
hsa-miR-608	AGGGGUGGUGUUGGGACAGCUCCGU	576
hsa-miR-609	AGGGUGUUUCUCUCAUCUCU	577

hsa-miR-610	UGAGCUAAAUGUGUGCUGGGA	578
hsa-miR-611	GCGAGGACCCCUCGGGGUCUGAC	579
hsa-miR-612	GCUGGGCAGGGCUUCUGAGCUCCUU	580
hsa-miR-613	AGGAAUGUUCCUUCUUUGCC	581
hsa-miR-614	GAACGCCUGUUCUUGCCAGGUGG	582
hsa-miR-615-3p	UCCGAGCCUGGGUCUCCCUCUU	583
hsa-miR-615-5p	GGGGGUCCCCGGUGCUCGGAUC	584
hsa-miR-616	AGUCAUUGGAGGGUUUGAGCAG	585
hsa-miR-616*	ACUCAAAACCCUUCAGUGACUU	586
hsa-miR-617	AGACUUCCCAUUUGAAGGUGGC	587
hsa-miR-618	AAACUCUACUUGUCCUUCUGAGU	588
hsa-miR-619	GACCUGGACAUGUUUGUGCCCAGU	589
hsa-miR-620	AUGGAGAUAGAUAUAGAAAU	590
hsa-miR-621	GGCUAGCAACAGCGCUUACCU	591
hsa-miR-622	ACAGUCUGCUGAGGUUGGAGC	592
hsa-miR-623	AUCCCUUGCAGGGGCUGUUGGGU	593
hsa-miR-624	CACAAGGUAUUGGUAUUACCU	594
hsa-miR-624*	UAGUACCAGUACCUUGUGUUCA	595
hsa-miR-625	AGGGGGAAAGUUCUAUAGUCC	596
hsa-miR-625*	GACUAUAGAACUUUCCCCCUCA	597
hsa-miR-626	AGCUGUCUGAAAAUGUCUU	598
hsa-miR-627	GUGAGUCUCUAAGAAAAGAGGA	599
hsa-miR-628-3p	UCUAGUAAGAGUGGCAGUCGA	600
hsa-miR-628-5p	AUGCUGACAUAUUUACUAGAGG	601
hsa-miR-629	UGGGUUUACGUUGGGAGAACU	602
hsa-miR-629*	GUUCUCCCAACGUAAGCCCAGC	603
hsa-miR-630	AGUAUUCUGUACCAGGGAAGGU	604
hsa-miR-631	AGACCUGGCCCAGACCUCAGC	605
hsa-miR-632	GUGUCUGCUUCCUGUGGGA	606
hsa-miR-633	CUAAUAGUAUCUACCACAAUAAA	607
hsa-miR-634	AACCAGCACCCCAACUUUGGAC	608
hsa-miR-635	ACUUGGGCACUGAAACAAUGUCC	609
hsa-miR-636	UGUGCUUGCUCGUCCCGCCCGCA	610
hsa-miR-637	ACUGGGGGCUUUCGGGCUCUGCGU	611
hsa-miR-638	AGGGAUCGCGGGCGGGUGGCGGCCU	612
hsa-miR-639	AUCGCUGCGGUUGCGAGCGCUGU	613
hsa-miR-640	AUGAUCCAGGAACCUGCCUCU	614
hsa-miR-641	AAAGACAUAGGAUAGAGUCACCUC	615
hsa-miR-642	GUCCCUCUCCAAAUGUGUCUUG	616
hsa-miR-643	ACUUGUAUGCUAGCUCAGGUAG	617
hsa-miR-644	AGUGUGGCUUUCUUAGAGC	618
hsa-miR-645	UCUAGGCUGGUACUGCUGA	619
hsa-miR-646	AAGCAGCUGCCUCUGAGGC	620

hsa-miR-647	GUGGCUGCACUCACUUCCUUC	621
hsa-miR-648	AAGUGUGCAGGGCACUGGU	622
hsa-miR-649	AAACCUGUGUUGUUCAAGAGUC	623
hsa-miR-650	AGGAGGCAGCGCUCUCAGGAC	624
hsa-miR-651	UUUAGGAUAAGCUUGACUUUUG	625
hsa-miR-652	AAUGGCGCCACUAGGGUUGUG	626
hsa-miR-653	GUGUUGAAACAAUCUCUACUG	627
hsa-miR-654-3p	UAUGUCUGCUGACCAUCACCUU	628
hsa-miR-654-5p	UGGUGGGCCGCAGAACAUGUGC	629
hsa-miR-655	AUAAUACAUGGUUAACCUCUUU	630
hsa-miR-656	AAUAUUAUACAGUCAACCUCU	631
hsa-miR-657	GGCAGGUUCUCACCCUCUCUAGG	632
hsa-miR-658	GGCGGAGGGAAGUAGGUCCGUUGGU	633
hsa-miR-659	CUUGGUUCAGGGAGGGUCCCCA	634
hsa-miR-660	UACCCAUUGCAUAUCGGAGUUG	635
hsa-miR-661	UGCCUGGGUCUCUGGCCUGCGCGU	636
hsa-miR-662	UCCCACGUUGUGGCCCAGCAG	637
hsa-miR-663	AGGCGGGGCGCCGCGGGACCGC	638
hsa-miR-665	ACCAGGAGGCUGAGGCCCCU	639
hsa-miR-668	UGUCACUCGGCUCGGCCCACUAC	640
hsa-miR-671-3p	UCCGGUUCUCAGGGCUCCACC	641
hsa-miR-671-5p	AGGAAGCCCUGGAGGGGCUGGAG	642
hsa-miR-672	UGAGGUUGGUGUACUGUGUGUGA	643
hsa-miR-674	GCACUGAGAUGGGAGUGGUGUA	644
hsa-miR-675	UGGUGCGGAGAGGGCCCACAGUG	645
hsa-miR-7	UGGAAGACUAGUGAUUUUGUUGU	646
hsa-miR-708	AAGGAGCUUACAAUCUAGCUGGG	647
hsa-miR-708*	CAACUAGACUGUGAGCUUCUAG	648
hsa-miR-7-1*	CAACAAAUCACAGUCUGCCAUA	649
hsa-miR-7-2*	CAACAAAUCCCAGUCUACCUAA	650
hsa-miR-744	UGCGGGGCUAGGGCUAACAGCA	651
hsa-miR-744*	CUGUUGCCACUAACCUCAACCU	652
hsa-miR-758	UUUGUGACCUGGUCCACUAACC	653
hsa-miR-760	CGGCUCUGGGUCUGUGGGGA	654
hsa-miR-765	UGGAGGAGAAGGAAGGUGAUG	655
hsa-miR-766	ACUCCAGCCCCACAGCCUCAGC	656
hsa-miR-767-3p	UCUGCUCAUACCCCAUGGUUUCU	657
hsa-miR-767-5p	UGCACCAUGGUUGUCUGAGCAUG	658
hsa-miR-768-3p	UCACAAUGCUGACACUCAAACUGCUGAC	659
hsa-miR-768-5p	GUUGGAGGAUGAAAGUACGGAGUGAU	660
hsa-miR-769-3p	CUGGGAUCUCCGGGGUCUUGGUU	661
hsa-miR-769-5p	UGAGACCUCUGGGUUCUGAGCU	662
hsa-miR-770-5p	UCCAGUACCACGUGUCAGGGCCA	663

hsa-miR-801	GAUUGCUCUGCGUGCGGAAUCGAC	664
hsa-miR-802	CAGUAACAAAGAUUCAUCCUUGU	665
hsa-miR-871	UAUUCAGAUUAGUGCCAGUCAUG	666
hsa-miR-872	AAGGUUACUUGUUAGUUCAGG	667
hsa-miR-873	GCAGGAACUUGUGAGUCUCCU	668
hsa-miR-874	CUGCCCUGGCCCGAGGGACCGA	669
hsa-miR-875-3p	CCUGGAAACACUGAGGUUGUG	670
hsa-miR-875-5p	UAUACCUCAGUUUUAUCAGGUG	671
hsa-miR-876-3p	UGGUGGUUUACAAAGUAAUUCA	672
hsa-miR-876-5p	UGGAUUUCUUUGUGAAUCACCA	673
hsa-miR-877	GUAGAGGAGAUGGCGCAGGG	674
hsa-miR-877*	UCCUCUUCUCCCUCCUCCCAGG	675
hsa-miR-885-3p	AGGCAGCGGGGUGUAGUGGAUA	676
hsa-miR-885-5p	UCCAUUACACUACCCUGCCUCU	677
hsa-miR-886-3p	CGCGGGUGCUUACUGACCCUU	678
hsa-miR-886-5p	CGGGUCGGAGUUAGCUCAAGCGG	679
hsa-miR-887	GUGAACGGGCGCCAUCCCGAGG	680
hsa-miR-888	UACUCAAAAAGCUGUCAGUCA	681
hsa-miR-888*	GACUGACACCUCUUUGGGUGAA	682
hsa-miR-889	UUAAUAUCGGACAACCAUUGU	683
hsa-miR-890	UACUUGGAAAGGCAUCAGUUG	684
hsa-miR-891a	UGCAACGAACCUGAGCCACUGA	685
hsa-miR-891b	UGCAACUUACCUGAGUCAUUGA	686
hsa-miR-892a	CACUGUGUCCUUUCUGCGUAG	687
hsa-miR-892b	CACUGGCUCCUUUCUGGGUAGA	688
hsa-miR-9	UCUUUGGUUAUCUAGCUGUAUGA	689
hsa-miR-9*	AUAAAGCUAGAUAACCGAAAGU	690
hsa-miR-920	GGGGAGCUGUGGAAGCAGUA	691
hsa-miR-921	CUAGUGAGGGACAGAACCAGGAUUC	692
hsa-miR-922	GCAGCAGAGAAUAGGACUACGUC	693
hsa-miR-923	GUCAGCGGAGGAAAAGAAACU	694
hsa-miR-924	AGAGUCUUGUGAUGUCUUGC	695
hsa-miR-92a	UAUUGCACUUGUCCCGGCCUGU	696
hsa-miR-92a-1*	AGGUUGGGAUCGGUUGCAAUGCU	697
hsa-miR-92a-2*	GGGUGGGGAUUUGUUGCAUUAC	698
hsa-miR-92b	UAUUGCACUCGUCCCGGCCUCC	699
hsa-miR-92b*	AGGGACGGGACGCGGUGCAGUG	700
hsa-miR-93	CAAAGUGCUGUUCGUGCAGGUAG	701
hsa-miR-93*	ACUGCUGAGCUAGCACUUCCCG	702
hsa-miR-933	UGUGCGCAGGGAGACCUCUCCC	703
hsa-miR-934	UGUCUACUACUGGAGACACUGG	704
hsa-miR-935	CCAGUUACCGCUUCCGCUACCGC	705
hsa-miR-936	ACAGUAGAGGGAGGAAUCGCAG	706

hsa-miR-937	AUCCGCGCUCUGACUCUCUGCC	707
hsa-miR-938	UGCCCUUAAAGGUGAACCCAGU	708
hsa-miR-939	UGGGGAGCUGAGGCUCUGGGGGUG	709
hsa-miR-940	AAGGCAGGGCCCCCGCUCCCC	710
hsa-miR-941	CACCCGGCUGUGUGCACAUGUGC	711
hsa-miR-942	UCUUCUCUGUUUUGGCCAUGUG	712
hsa-miR-943	CUGACUGUUGCCGUCCUCCAG	713
hsa-miR-944	AAAUUAUUGUACAUCGGAUGAG	714
hsa-miR-95	UUCAACGGGUAUUUAUUGAGCA	715
hsa-miR-96	UUUGGCACUAGCACAUUUUUGCU	716
hsa-miR-96*	AAUCAUGUGCAGUGCCAAUAUG	717
hsa-miR-98	UGAGGUAGUAAGUUGUAUUGUU	718
hsa-miR-99a	AACCCGUAGAUCCGAUCUUGUG	719
hsa-miR-99a*	CAAGCUCGCUUCUAUGGGUCUG	720
hsa-miR-99b	CACCCGUAGAACCGACCUUGCG	721
hsa-miR-99b*	CAAGCUCGUGUCUGUGGGUCCG	722
hsv-1 miR-LAT	UGGCGGCCCGGCCCGGGGCC	723

In still another embodiment, the seed sequence of the micro RNA is selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

In a more preferred embodiment, the seed sequence of the micro RNA is selected from the group consisting of: position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

In a most preferred embodiment, the seed sequence of the micro RNA is selected from the group consisting of: position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723.

Activity of the oligonucleotide of the invention

As will be clear, the oligonucleotides of the invention have a variety of utilities and advantages.

RNase H cleavage

In one embodiment, the oligonucleotide draws use of the accessibility of a target region of a target RNA. In this embodiment, the oligonucleotide may activate RNase H cleavage of the target. Because of the improved target accessibility, the oligonucleotide will preferentially affect the activity of the target RNA, even if the oligonucleotide is short, e.g. about 10 bases or just the guide sequence. I.e. complementary regions elsewhere may not be targeted because they are less accessible. They may e.g. be buried in RNA secondary structure or may be inaccessible because they are engaged in protein binding.

RNase H will cleave the RNA part of a RNA-DNA duplex. The structural requirements for RNase H activation are well-known to the skilled man. This mechanism is very often used to achieve traditional antisense regulation e.g. by employing so-called gapmers. Gapmers are antisense oligonucleotides that comprise a central region with deoxy sugars (the gap) and modified flanks. Gapmers very often comprises phosphorothioate internucleotide linkages to improve biostability and the flanks comprise e.g. 2-O-modifications that also improve biostability, i.e. resistance against nucleolytic attack. The flanks may also comprise modifications that increase the melting temperature of the gapmer base paired to a complementary nucleic acid. Also headmer and endmer structures have been described in the literature.

In another preferred embodiment, the oligonucleotide is not capable of inducing RNase H cleavage of the target RNA. The skilled man is well aware of the requirements for RNase H cleavage and will be able to design oligonucleotides that do or do not activate RNase H.

Thus, in a preferred embodiment, the oligonucleotide does not comprise a stretch of unmodified DNA that exceeds a length selected from the group consisting of: 3 bases, 4 bases, 5 bases, 6 bases, 7 bases, 8 bases, 9 bases, 10 bases and 11 bases. Most preferably, the stretch of unmodified DNA does not exceed 3 bases.

In another preferred embodiment, the oligonucleotide does not comprise any DNA monomers.

Recruiting the RNAi machinery

The RNAi machinery is a sophisticated gene regulatory system that is guided by RNA. Thus, microRNAs guide the RNAi machinery to target mRNAs to affect the activity of the target mRNA. The RNAi machinery may affect translation of the mRNA directly or it may affect the stability of the target mRNA, i.e. mediate direct degradation of the target mRNA. Not intended to be bound by theory, it is believed that the degree of complementarity between microRNA and target mRNA is a key element as to whether the target mRNA is subjected to translational regulation or degradation.

Endogenous microRNAs are processed from precursor stem-loops and incorporated into a so called RNA induced silencing complex (RISC complex). The details of this process are still poorly understood.

The cellular RNAi machinery has been extensively used to affect the activity of cellular mRNAs by introducing synthetic double stranded RNA complexes termed siRNAs into the cell. As mentioned above, siRNAs are short double stranded RNA complexes comprising a passenger strand and a complementary guide strand. The guide strand of siRNA is incorporated into the RISC complex, where after the RISC complex can affect the activity of mRNA harbouring complementary sequences to the guide strand. Thus, siRNAs are a new class of compounds that is thought to be capable of efficiently and specifically targeting any mRNA and consequently, siRNAs are regarded potentially as a new class of therapeutics.

A common feature of siRNAs and microRNAs is that they recruit the cellular RNAi complex to affect the activity of target RNAs.

In one embodiment, the oligonucleotides of the invention are capable of recruiting the RNAi machinery and hence direct the RNAi machinery to the target RNA. This may result in cleavage of the target RNA or translational repression of the target RNA. In this embodiment, the oligonucleotide may be a siRNA. I.e. the oligonucleotide is hybridised to a complementary oligonucleotide, typically over a length of 20-22 bases and very often with 3'overhangs of 1-3 bases. As the name implies, a siRNA essentially consists of RNA monomers, although modifications, such as e.g. 2'-O-modifications are acceptable at certain positions.

The oligonucleotide may also act as a microRNA, without being identical to a naturally occurring microRNA. When the oligonucleotide acts as a microRNA, it consists essentially of RNA monomers, although modifications may be acceptable at certain positions. The oligonucleotide may have a structure analogously to a mature endogenous microRNA or to a pre-microRNA (stem-loop with bulges in stem) that has to be processed by dicer to a mature microRNA.

Where naturally occurring microRNAs typically regulate many target RNAs, a oligonucleotide of the invention acting as a microRNA may be designed to only regulate a few target RNAs or only one target RNA. Promiscuity of the oligonucleotide can be adjusted by designing the oligonucleotide to target only one or a few targets. By using universal bases, a large degree of promiscuity can also be designed into the oligonucleotide. Universal bases will be discussed more below. Promiscouity can also be introduced by reducing the length of the oligonucleotide.

Importantly, when the oligonucleotides of the invention are capable of recruiting the RNAi machinery, they may still draw use of the accessibility of the target region of the target RNA.

Blockmir

In another embodiment, the oligonucleotides cannot recruit the RNAi machinery. In this embodiment, it is preferred that the oligonucleotides of the invention are capable of blocking the activity of the RNAi machinery at a particular target RNA. As mentioned above, the oligonucleotides may do so by sequestering the target

sequence of the target RNA, such that the RNAi machinery will not recognize the target sequence, as it is base paired to the oligonucleotides. Oligonucleotides of the invention with this activity may also be referred to as blockmirs.

In a preferred embodiment, the oligonucleotide is capable of blocking the regulatory activity of a microRNA at a particular target RNA. Preferably, the microRNA is an endogenous microRNA.

After the priority date of this patent application, examples of oligonucleotides capable of blocking the regulatory activity of a microRNA at a given mRNA has been published by two groups.

In the first publication (Xiao J, 2007), oligonucleotides termed microRNA masking antisense ODN (oligodeoxynucleotides) was used to interfere with the regulatory activity of mir-1 on HCN2 and HCN4 and the regulatory activity of mir-133 on HCN2. It was observed that microRNA masking antisense increased the protein level of HCN2 and HCN4 in a gene specific manner, as determined by immunoblotting using cultured neonatal rat ventricular cells and luciferase assays using HEK293 human embryonic kidney cell line. I.e. the mechanism of action of blockmirs was validated. In other words, it was demonstrated that an oligonucleotide that binds to the target site of a microRNA in the 3'UTR of a mRNA, can prevent microRNA regulation of the mRNA in mammalian cells (rat and human).

However, the design of the blockmirs in the work of Xiao et al., 2007 left some questions open. The microRNA masking antisense ODN consisted of deoxynucleotides with 5 LNA monomers at both ends. Thus, the central part of the oligonucleotide apparently consisted of a stretch of 12 unmodified deoxynucleotides. Such structure is typically expected to activate RNase H and hence mediate degradation of target RNAs.

In the second publication (Choi WY, 2007) blockmirs (termed target protectors) was used to prevent microRNA regulation of specific mRNAs in zebrafish. More specifically, the authors used morpholino oligonucleotides of 25 units with perfect complementarity to zebrafish mir-430 target sites in *squint* and *lefty* mRNA to

prevent mir-430 regulation of the target mRNAs (*squint* and *lefty).* Thus, the authors validate the blockmir approach in a different organism than did Xiao et al., and they also validate that a different chemistry can be used.

We suggest that the essence of blockmir activity is binding to a microRNA target site, and that this can achieved using a variety of chemistries and also in a variety of organisms.

Another report published after the priority date of this patent application studied the molecular basis for target RNA recognition and cleavage by human RISC (Ameres SL, 2007). These authors found that target accessibility determines RISC mediated cleavage in vitro and in vivo. Among others, they blocked target accessibility using oligonucleotides complementary to a siRNA target site, i.e. the oligonucleotides may be seen as functional analogues of the blockmirs of the present invention, except that they target a siRNA target site that is regulated by a siRNA with perfect complementary. Interestingly, the authors found that blocking 3 or 6 nt of the 21 nt target sequence in the region annealing to the 3'part of the siRNA had no effect on regulation (as seen by cleavage rates using affinity purified human RISC). In contrast, blocking 5 nt of the target site in the region annealing to the 5'part of the siRNA severely impaired regulation (as seen by cleavage) and even blocking only 2 nt impaired regulation.

Returning to blockmirs of the invention, if the microRNA is a positive regulator of the target RNA, the oligonucleotide will be a negative regulator of the target RNA.

Most often, the microRNA is a negative regulator of the target RNA. Thus, in another embodiment, the oligonucleotide is a positive regulator of the target RNA. This is contrary to traditional antisense oligonucleotides, microRNAs and siRNAs that typically act as negative regulators.

In a preferred embodiment, the blockmirs of the invention are DNAs, as these will not be recognized by the RNAi machinery and consequently function as neither microRNA nor siRNA. Preferably, the DNA units are modified such as to prevent RNase H activation. Alternatively, less than 5 consecutive DNA units are present, such as less than 4 consecutive DNA and less than 3 consecutive DNA units.

In still another embodiment, the blockmir does not comprise any DNA units.

In yet another embodiment, the blockmir does not comprise any RNA units.

In another embodiment, the blockmir does not comprise a stretch of RNA units that exceeds a length selected from the group of consisting of: a length of 5 units, 6 units, 7 units, 8 units, 9 units, 10 units, 11 units, 12 units, 13 units, 14 units, 15 units, 16 units, 17 units, 18 units, 19 units, 20 units, 21 units and 22 units.

In one embodiment, the oligonucleotides have been chemically modified such as to not being capable of recruiting the RNAi machinery. Preferred modifications include 2'-O-modications such as 2'-O-methyl and 2'O-F. Also conjugated RNAs are preferred. E.g. RNAs conjugated to a cholesterol moiety, in which case the cholesterol may both prevent the oligonucleotide from recruiting the RNAi machinery and improve the bioavailability of the oligonucleotide. The cholesterol moiety may be conjugated to a monomer within the guide sequence of the oligonucleotide or at the 3'end or the 5'end of the oligonucleotide. More modifications are described below.

In yet another embodiment, the blockmir may comprise a mix of DNA units and RNA units such as to prevent the oligonucleotide from activating RNase H and to at the same time prevent the oligonucleotide from recruiting the RNAi machinery. E.g. a DNA unit may be followed by a RNA unit that is again followed by a DNA unit and so on. Further, in a preferred embodiment, phosphorothioate internucleotide linkages may connect the units to improve the biostability of the oligonucleotide. Both DNA units and RNA units may be modified. Preferably, RNA units are modified in the 2'-O-position (2'-O-methyl, LNA etc.).

In yet another embodiment, the oligonucleotide (blockmir) comprise a mix of DNA units and RNA units such as to prevent the oligonucleotide from activating RNase H and to at the same time prevent the oligonucleotide from recruiting the RNAi machinery, wherein the DNA units and RNA units come in blocks. The blocks may have a length of 2 units, 3 units, 4 units, 5 units or 6 units and units of different length may be comprised with the same oligonucleotide. Both DNA units and RNA

units may be modified. Preferably, RNA units are modified in the 2'-O-position (2'-O-methyl, LNA etc).

In a preferred embodiment, also units selected from the group of LNA units, INA units and morpholino units are comprised within the oligonucleotide. In another preferred embodiment, the oligonucleotide comprises a mix of LNA units and RNA units with a 2'-O-methyl. Such mixmers have been used as steric block inhibitors of Human Immunodeficiency Virus Type 1 Tat-Dependent Trans-Activation and HIV-1 Infectivity.

In still another embodiment, the blockmir are entirely composed of units selected from the group of 2'-O-methyl modified units, LNA units, PNA units, INA units and morpholino units. In one embodiment, the units are mixed, while in another embodiment, the blockmir is composed of only one of the units.

In still another embodiment, the blockmir has been designed such as to able to bind to more than one target RNA. Promiscuity may be designed into blockmirs using universal bases. Also reducing the length of the blockmir will increase promiscuity. Thus, in one embodiment, the blockmir may only consist of the guide sequence corresponding to a seed sequence of a microRNA. In this embodiment, it is preferred that affinity increasing modifications are are used and the oligonucleotide may be fully modified in the 2'O-position with e.g. 2'-O-methyl, 2'-O-'flouro, 2'-O-(2-methoxyethyl) or the nucleotides may be locked (LNA).

Off-target effects

In most embodiments, off-target binding of the blockmir will have very few or no effects. This is contrary to antimirs, RNAi mediated by siRNAs and microRNAs, and RNase H mediated antisense regulation, which may all give rise to off-targets effects. The blockmir only has an effect if it binds to a microRNA target region and thereby prevents microRNA regulation of the target RNA.

Thus, in a preferred embodiment, the blockmir will have reduced off-target effects, as compared to regulating the activity of the target mRNA using an antimir.

An antimir, as used in the present context, is an oligonucleotide that can base pair with a microRNA and thereby inhibit the activity of the microRNA. Since most microRNAs are promiscuous, i.e. they regulate more than one target, regulation of a particular microRNA will affect the activity of more than one target mRNA. Thus, when it is desired to only regulate the activity of one particular target mRNA, regulation of other target mRNAs may be referred to as off-target effects of the antimir.

Using a (exogenous) promiscuous microRNA to affect or regulate the activity of a target mRNA, instead of an antimir may obviously also have off-target effects.

Moreover, the target repertoire of a given microRNA may vary in different cells, wherefore an antimir may have different off target effects in different cells. Likewise for regulation using a promiscuous microRNA. A blockmir will only have an effect in the particular cells wherein the target RNA is regulated by a microRNA. Thus, a blockmir enables targeting of cell specific microRNA:mRNA interactions. If the blockmir enter a cell that does not have the particular microRNA:mRNA interaction, the blockmir will have little or no effect.

siRNAs are double stranded RNA complexes comprising a passenger strand and a guide strand that mediate degradation of target mRNAs that are complementary to the guide strand of the RNA complex. It has now been recognized that siRNAs often have off-target effects, because the strand acting as guide strand can also function as microRNA, i.e. siRNAs may mediate regulation of target mRNAs that are not fully complementary to the guide strand of the siRNA.

Thus, in one embodiment, an blockmir of the present invention will have reduced off-target effects as compared to a siRNA directed to the same target mRNA.

In another preferred embodiment, the blockmir will also have reduced off-target effects as compared to using a traditional antisense oligonucleotide for regulation of the target mRNA.

Traditional antisense oligonucleotides are often designed such as to mediate RNase H cleavage of their target RNA. RNase H cleaves a duplex of RNA and DNA.

Thus, if such an antisense oligonucleotide base pairs to a non-intended mRNA, this mRNA will be inactivated by RNase H cleavage, and hence giving rise to off-target effects.

In conclusion, blockmirs of the present invention are characteristic in that they affect the activity of an RNA by preventing microRNA regulation of the target RNA. Thus, blockmirs of the present invention will have reduced off target effects as compared to both traditional antisense oligonucleotides, antimirs, and RNAi mediated regulation using microRNAs and siRNAs.

A consideration when designing short blockmirs is obviously that the transcriptome may comprise more than one site with perfect complementary to the blockmir. However, as outlined above, the blockmirs will only affect the target RNA if the target sequence is also a target sequence for microRNA regulation. Therefore, even very short blockmirs may have very little of no off-target effects. Thus, the blockmirs may deliberately be designed to target many sites. The blockmirs will then preferentially bind to microRNA target sites since these are more accessible, and the blockmirs will only have effects if they prevent microRNA binding to a target site.

Chemistry

In a preferred embodiment of the oligonucleotides of the invention, the oligonucleotide comprises nucleotide monomers that increase its affinity for complementary sequences or affinity increasing modifications. This is particular relevant for short oligonucleotides and may allow for generation of very short active oligonucleotides, e.g. of a length between 10 and 15 bases or even less than 10 bases, such as e.g. only the guide sequence corresponding to the seed sequence of a microRNA.

Nucleotide units that increase the affinity for complementary sequences may e.g. be LNA (locked nucleic acid) units, PNA (peptide nucleic acid) units or INA (intercalating nucleic acid) units. Also RNA units modified in the 2-O-position (e.g. 2'-O-(2-methoxyethyl)-RNA , 2'O-methyl-RNA, 2'O-flouro-RNA) increase the affinity for complementary sequences. At the same time, such modifications often

also improve the biostability of the oligonucleotides, as they become a poorer substrate for cellular nucleases.

The oligonucleotide may also comprise modifications that increase its biostability and/or bioavailability, such as phosphorothioate linkages. The oligonucleotide may be fully phosphorothiolated or only partly phosphorothiolated.

In a preferred embodiment, the oligonucleotide comprises a repeating pattern of one or more LNA units and one or more units that are substituted in the 2'-position. OMe/LNA mixmers have been shown to be powerful reagents for use as steric block inhibitors of gene expression regulated by protein-RNA interactions. Thus, when the oligonucleotides of the invention are used to block the activity of a microRNA at a target RNA, a OMe/LNA mixmer architecture may be used. A gapmer structure structure may also be used, however preferably without being capable of inducing RNase H if the oligonucleotide is intended to act as a blockmir.

In one embodiment, the oligonucleotide of the invention does not comprise any RNA units. Few or no RNA units may be used to prevent the oligonucleotide from being capable of recruiting the RNAi machinery. Chemical modifications can do the same.

In another embodiment, the oligonucleotide of the invention does not comprise any DNA units.

In still another embodiment, the oligonucleotide of the invention does not comprise any morpholino units and/or LNA units.

In yet another embodiment, the oligonucleotide comprises modifications that increase its biostability. The modifications may be the nucleotide units mentioned above for increasing the affinity toward complementary sequences.

In a preferred embodiment, the oligonucleotides comprise a number of nucleotide units that increase the affinity for complementary sequences selected from the group of: 1 units, 2 units, 3 units, 4 units, 5 units, 6 units, 7 units, 8 units, 9

units, 10 units, 11 units, 12 units, 13 units, 14 units, 15 units, 16 units, 17 units, 18 units, 19 units, 20 units, 21 units, and 22 units.

In a preferred embodiment, nucleotide units that increase the affinity for complementary sequences are located at the flanks of the oligonucleotide. E.g. if the oligonucleotide comprise e.g. 10 LNA units, 5 may be located at the 5'end and the other 5 units may be located at the 3'end.

In still another embodiment, the oligonucleotides comprise modifications that increase its bioavailability. Modifications that improve cellular delivery are particular preferred.

Promiscuity and specificity

In yet another embodiment, the oligonucleotide of the present invention may comprise nucleotides that do not hybridise specifically. Such nucleotides comprise so called universal bases. These are characterised in that they fit into a Watson-crick helix opposite to any base. Thus, they may be used to impose a certain degree of promiscuity on the oligonucleotides of the invention. That may e.g. be employed if the oligonucleotide is intended to target two particular mRNAs.

In a preferred embodiment, it may be desired to target most or all targets of a particular microRNA. In such case, the oligonucleotide may comprise a guide sequence corresponding to the seed sequence of the microRNA and one or two blocks of natural bases. The size of the blocks of natural bases can be adjusted such as to achieve a reasonable affinity to target sequences.

In still another embodiment, the oligonucleotide of the invention comprises a universal base selected from the group consisting of 3-nitropyrrole, 5-nitroindole, 3-methyl isocarbostyril or 5-methyl isocarbostyril.

In one embodiment, the oligonucleotides of the invention may comprise a guide sequence which is flanked by universal bases on the 3'side, the 5'side or both. Such an oligonucleotide may be used to mimic the promiscuous specificity of a microRNA and hence, block the activity of the microRNA at multiple target RNAs or even all target RNAs of the microRNAs. A combination of universal bases and

e.g. inosine may also be used to design an oligonucleotide that only targets a subset of the target RNAs of a microRNA.

In one embodiment, the bases between the guide sequence and the second sequence are universal bases.

In another embodiment, any bases not part of the guide sequence and the second sequence are universal bases.

Universal bases tend to decrease the melting temperature of the oligonucleotide, wherefore it is preferred to counteract this decrease by incorporation of affinity increasing modifications or units, e.g. LNA units or 2'-O-methyl groups.

Single-stranded vs. double stranded

In some embodiments, the oligonucleotide of the invention is preferably not base paired with a complementary oligonucleotide or intended for use with a base paired with a complementary oligonucleotide. I.e. it should be single stranded to facilitate interaction with a target RNA and in certain embodiments, also to prevent recruitment of the RNAi machinery.

In another embodiment, the oligonucleotide is base paired to a complementary oligonucleotide. In some situations, it may be desirable that the oligonucleotide is base paired to a complementary oligonucleotide to facilitate transport into a cell and/or intracellular transport. Also transport within an organism may be facilitated. Further, biostability may be positively affected.
Base pairing to a complementary oligonucleotide will also be used when the oligonucleotide is acting as a siRNA. When the oligonucleotide is acting as a exogenous miRNA, it may be formed as a stem-loop structure.

In another embodiment, the oligonucleotide is base paired to a RNA molecule that is degraded by RNase H, when the oligonucleotide enters its target cell. In this way, the oligonucleotide is liberated on site. In a preferred embodiment, the complementary oligonucleotide is not of the same type as the oligonucleotide of the invention. E.g. if the oligonucleotide is RNA, the complementary oligonucleotide will not be RNA.

Delivery

Various methods for delivery of oligonucleotides are known to the skilled man. Thus, oligonucleotides may be formulated in microparticles and nanoparticles. Liposomes are frequently used as delivery vehicle and a variety of liposome delivery systems exist. They may e.g. comprise cationic lipids or neutral lipids. Their size may be varied for various purposes and other components may be included in the liposomes or on the surface of the liposomes. Chitosan nanoparticles have been used for delivery of plasmids and siRNAs to various cells, among them primary cells. Thus, chitosan nanoparticles may also be used for delivery of the oligonucleotides of the invention. Others polymers for delivery are polyethyleneimine (PEI), cyclodextrin, atelocollagen, polyamidoamine (PAMAM) and poly(lactic-co-glycolic acid) (PLGA). Further, oligonucleotides of the invention may be conjugated to cationic peptides that have been shown to facilitate transport into cells.

Second aspect - Method of modulating the activity of a target RNA

A second aspect of the invention is a method of modulating the activity of a target RNA comprising the steps

a. Providing a system comprising a target RNA
b. Providing an oligonucleotide that comprises an antisense sequence complementary to a target region of the target RNA
c. Introducing the oligonucleotide of step b to the system of step a
d. Thereby modulating the activity of the target RNA

Preferably, the oligonucleotide is an oligonucleotide of the invention, as described in the first aspect of the invention in various embodiments.

And preferably, the target RNA comprises an anti-seed sequence which is complementary to the guide sequence of the oligonucleotide.

In a preferred embodiment, the oligonucleotide prevents the activity of a microRNA at the target RNA and thereby modulates the activity of the target RNA. I.e. the oligonucleotide is a blockmir as described in the first aspect.

In another embodiment, the oligonucleotide induces RNase H cleavage of the target RNA and thereby regulates the activity of the target RNA.

In yet another embodiment, the oligonucleotide recruits the RNAi machinery to the target RNA. Recruitment of the RNAi machinery may lead to translational repression of the target RNA or degradation of the target RNA.

Preferably, the system is either a cell extract or a cell. The method may be performed in vivo, ex vivo or in vitro.

In one embodiment, the method is a method for validating the activity of the oligonucleotide, i.e. verifying whether the oligonucleotide can indeed modulate the activity of the target RNA and to what extent. Such method may be used when aiming to identify oligonucleotides with optimal activity e.g. for therapeutic development. In such testing, typically different lengths and chemistries of the oligonucleotide will be tested.

In another embodiment, the method is a method of identifying or validating a micro RNA target of a target RNA. Very often, it is hypothesized that a microRNA regulates a given target RNA and in this case, the method of the second aspect is a method of verifying whether the target RNA is indeed regulated by a microRNA. Thus, the method may further comprise identifying the microRNA that regulates the target RNA. This is possible because the target RNA should comprise an anti-seed sequence which is complementary the seed sequence of the microRNA.

Third aspect – providing a bioactive oligonucleotide

A third aspect of the invention is a method comprising the steps of:

a. Providing a (predetermined) target sequence of a target RNA regulated by a microRNA, said target sequence being the sequence of the target RNA involved in microRNA regulation.
b. Designing an oligonucleotide sequence that comprises a continuous stretch of bases (antisense sequence) of at least 6 bases that is complementary to the target sequence
c. Synthesizing the oligonucleotide sequence of step b, said oligonucleotide being a candidate regulator of the activity of a target RNA.

In a preferred embodiment, the method is a method of providing a bioactive oligonucleotide.

Preferably, the continuous stretch of bases comprises the guide sequence corresponding to the seed sequence of the micro RNA regulating the target RNA.

Preferably, the method further comprises the steps

a. Providing a reporter system for activity of the target RNA
b. Determining the activity of the target RNA in the presence of the candidate regulator
c. Determining the activity of the target RNA in the absence of the candidate regulator
d. Comparing the activity levels in b and c and thereby verifying whether the oligonucleotide is indeed a capable of regulating the activity of the RNA and/or whether the potential target sequence of the RNA is indeed a target sequence.

In yet another preferred embodiment, the method further comprises a step of determining the activity of the target RNA in the presence of a negative control, said negative control being an oligonucleotide that does not have complementarity to any region in the target RNA. In another related embodiment, the negative control is an oligonucleotide which is complementary to the oligonucleotide it serves as a control for. In still another embodiment, the negative control is complementary to a region which is not part of the target region of the target RNA. Preferably, the oligonucleotide and its negative control are of the same type,

i.e., RNA, mixed RNA and DNA, and comprise the same modifications and nucleotide analogs such as LNA or INA.

Preferably, the activity of the target RNA is expression and the target RNA is a mRNA.

Hence, oligonucleotides (candidate regulators) potentially capable of regulating the activity of a target RNA are first identified, where after the activity of these oligonucleotides are tested using a reporter system such as to verify whether the oligonucleotides do indeed have the desired activity, i.e. are capable of regulating the activity of the target RNA.

Preferably, the oligonucleotides provided in the third aspect of the invention are oligonucleotides of the invention.

The activity of the target RNA is preferably gene expression and the target RNA is preferably a mRNA. The target RNA may also be a viral genomic RNA and the activity e.g. replication.

The predetermined target sequence may be retrieved from a scientific publication or a database of validated microRNA targets.

Reporter system

The reporter system for expression may be any system that enables a read-out indicative of the activity of the target RNA. It may be e.g. be cells harbouring a genetic construct, wherein the target RNA has been fused to another reporter gene.

In a preferred embodiment, the target sequence of the target RNA resides within the 3'-untranslated region of an mRNA. In such cases, the 3'UTR may be fused to a reporter gene without necessarily including the rest of the target mRNA.

The reporter gene may be e.g. the luciferase gene or GFP gene. Such reporter systems are well-known to the skilled man.

The reporter system could also be cells harbouring the endogenous target mRNA. In such an embodiment, the activity (expression) of the target mRNA may be determined by immunoblotting using antibodies targeting the polypeptide or protein encoded by the mRNA. 2D-gel analysis or protein chips may also be used to determine the activity of the target mRNA.

Microarrays, Northern blots and real time PCR (also known as quantitative PCR) may be used to determine any effects on mRNA levels. Also such reporter systems are well known to the skilled man.

Using the seed sequence and anti-seed sequence

In a preferred embodiment, the method of the third aspect further comprises providing the sequence of the microRNA regulating the target RNA and using the seed sequence of the microRNA to determine the anti-seed sequence of the target sequence.

The sequence of the microRNA regulating the target mRNA may e.g. be retrieved from a scientific paper or from a database. One such database collecting microRNA sequences is the so called miRBase (http://microrna.sanger.ac.uk/sequences/). In a preferred embodiment, the sequence of the microRNA is retrieved from a scientific paper describing regulation of the target mRNA by the microRNA. In another embodiment, the identity of the microRNA regulating the target mRNA is retrieved from a scientific publication, where after the sequence of the microRNAs is retrieved from a database. Such information is often the starting point for the method of the third aspect.

The seed sequence of the microRNA typically resides in the 5'end of the microRNA. Seed sequences are interesting, because it is believed that these are important predictors of the target mRNAs that are regulated by a particular microRNA. I.e. it is believed that they base-pair to complementary regions on target mRNAs. Such complementary regions of target mRNAs are herein also referred to as anti-seed regions or anti-seed sequences. Unfortunately, the seed

sequences are often too short to allow prediction of target mRNAs, i.e. there are too many anti-seed sequences in the transcriptome of a cell. Thus, identification of target mRNAs regulated by a given microRNA still poses a significant challenge and so far hinge on experimental proof rather than theoretical prediction.

Nonetheless, progress is continually made with regards to determine which mRNAs are regulated by which microRNAs and it is an object of the present invention to use such knowledge to carry out the method of the third aspect and to design and provide oligonucleotides of the invention.

In a preferred embodiment, the target region of the target mRNA is comprised within the 3'UTR and comprise a sequence that is complementary to a sequence selected from the group consisting of: position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs 1-723.

In a more preferred embodiment, the target region of the target mRNA is comprised within the 3'UTR and comprise a sequence that is complementary to a sequence selected from the group consisting of: position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

In a most preferred embodiment, the target region of the target mRNA is comprised within the 3'UTR and comprise a sequence that is complementary to a sequence selected from the group consisting of: position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723.

Fourth aspect - Identifying target regions, microRNA regulators thereof and oligonucleotides of the invention

In a fourth aspect, the invention provides a method comprising the steps

a. Providing a reporter system for activity of a target RNA
b. Providing a oligonucleotide that is complementary to a part of the target RNA
c. Determining the activity of the target RNA in the presence of the oligonucleotide of step b
d. Determining the activity of the target RNA in the absence of the oligonucleotide of step b
e. Comparing the activity levels in c and d and thereby verifying whether the oligonucleotide affect the activity of the RNA
f. Thereby identifying active oligonucleotides capable of regulating the activity of the target RNA and/or identifying microRNA target sequences of a RNA

Reporter systems have been described in the previous aspect.

One object of the oligonucleotides of the present invention is that they should prevent access of a microRNA to at least one of the target mRNAs of the particular microRNA. Thus, depending on the strength of oligonucleotide interaction with the target mRNA, the oligonucleotide will prevent the microRNA in base pairing with the target sequence. In other words, the microRNA is no longer able to guide the RNAi machinery to the target mRNA and exert its effects on the target mRNA.

In a preferred embodiment, the target region of the target RNA is the 3'UTR (3'untranslated region) of an mRNA.

In another preferred embodiment, the target region of the target mRNA is comprised within the 3'UTR.

In another embodiment, the method is a method of identifying a micro RNA target sequence of the RNA. I.e. microRNA targets of a given mRNA may e.g. be identified using the method of the fourth aspect.

In still another embodiment, the method is a method of identifying an oligonucleotide capable of regulating the activity of the RNA.

The method may further comprise providing a series of oligonucleotides that each are complementary to a part of the target RNA and where the series of oligonucleotides has an overall coverage of more than 50% for a particular target region of the target RNA and wherein each oligonucleotide is tested for activity (with respect to regulating the activity of the target RNA).

Preferably, the sequence of active oligonucleotides is used to define oligonucleotide sensitive regions of the target region. Moreover, the sequences of oligonucleotide sensitive regions are preferably used to design one or more oligonucleotide with optimized sequences, i.e. optimized activity.

In another embodiment, the sequences of the active oligonucleotides are truncated and tested for activity again such as to define minimal lengths of the oligonucleotides that will function as regulators of the mRNA.

As referred to herein, an oligonucleotide sensitive region is a region of the RNA, which when base paired to an oligonucleotide, affects the activity of the RNA. Typically, an oligonucleotide base paired to the oligonucleotide sensitive region will prevent a microRNA from regulating the activity of the RNA.

In a preferred embodiment, the sequences of oligonucleotide sensitive regions are used to identify candidate microRNAs that potentially regulate the target RNA. Thus, the method is a method of verifying which microRNAs regulate a given target RNA.

Identification of microRNAs that regulate a particular mRNA is of interest for various reasons. First, it will provide insight into how the RNAi machinery is recruited to particular mRNA targets and this information may be used to direct the RNAi machinery one or more therapeutic targets, e.g. mRNAs that encode proteins involved in disease. Second, a particular mRNA may be targeted for regulation by an antimir oligonucleotide that inhibits the activity of the microRNA regulating the activity of the mRNA. Determining which mRNAs are regulated by a

particular microRNA or which microRNAs regulate a particular mRNA is currently one of, if not, the most important questions relating to RNAi, microRNAs and siRNAs.

It is an object of the present invention to provide such regulatory relationships between microRNAs and mRNAs.

Identification of candidate microRNAs preferably comprises the steps of:

a. Providing a sequence of an oligonucleotide sensitive region
b. Searching to sequence of the oligonucleotide sensitive region for complementarity to microRNAs to identify candidate microRNAs that potentially regulate the target RNA

When searching for complementarity, the seed sequence is particular important and the oligonucleotide sensitive region is preferably first searched for anti-seed sequences.

In a preferred embodiment, the activity of the identified candidate microRNAs that potentially regulate the target RNA is verified in a secondary test such as to identify microRNAs that do indeed regulate the activity of the target RNA

Preferably, the secondary test comprises the steps of:

a. Providing a reporter system for activity of the target RNA
b. Providing an antimir-oligonucleotide that comprises complementarity to the microRNA and is capable of inhibiting the activity of the candidate microRNA
c. Determining the activity of the target RNA in the presence of the antimir-oligonucleotide of step b
d. Determining the activity of the target RNA in the absence of the antimir-oligonucleotide of step b
e. Comparing the activity levels of step c and d and thereby verifying whether the identified candidate microRNA regulators are indeed active microRNA regulators of the target RNA

In a preferred embodiment, the secondary test further comprise a step of determining the expression of the target mRNA in the presence of the negative control, wherein said negative control is a oligonucleotide that do not comprise complementarity to the microRNAs.

Preferably, the method further comprises the steps of:

a. Determining the activity of the target RNA in the presence of an oligonucleotide directed to the target RNA
b. Determining the activity of the target RNA in the simultaneous presence of the oligonucleotide of step a in the presence of the antimir-oligonucleotide
c. Thereby verifying whether the oligonucleotide functions by blocking the activity of the micro RNA at the oligonucleotide sensitive region.

Thus, if the oligonucleotide has reduced or even no effect on the activity of the target RNA when the antimir is present, this indicates that the oligonucleotide functions by blocking the activity of the microRNA at the oligonucleotide sensitive region.

In a preferred embodiment, the coverage is selected from the group consisting of: more than 55 %, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% and 100%.

When referring to coverage, what is meant is the fraction of the target region that can be covered by the series of potential oligonucleotides. In other words, the fraction of target region that would be engaged in base pairing if the series of potential oligonucleotides where added to the target region under conditions of hybridisation.

In another preferred embodiment, the coverage is 100% and the oligonucleotides have an overlap in sequence.

In yet another preferred embodiment, a particular oligonucleotide has 50% overlap with the oligonucleotide to its 5'end and 50% overlap with the

oligonucleotide to its 3'end. Thus, any give sequence of the target region will be covered by at least two oligonucleotides. Such a setup will be beneficial in defining oligonucleotide sensitive regions.

Preferably, the target RNA is a mRNA or a viral RNA. When the target RNA is a mRNA, the activity of the target mRNA is preferably gene expression.

If the target RNA is a target mRNA, the target region preferably is in the 3'UTR of the target mRNA.

In a preferred embodiment of the fourth aspect, the oligonucleotide is an oligonucleotide as described in the first aspect of the invention.

Pharmaceutical composition and treatment

A fifth aspect of the present invention is a pharmaceutical composition comprising the oligonucleotide of the invention. As the skilled man will understand from the above description, the oligonucleotide may be used for therapy in the same manner as siRNAs, microRNAs and antisense oligonucleotides, because they can be used to specifically affect the expression of a particular gene.

A sixth aspect of the present invention is a method of treatment comprising administering an effective amount of the oligonucleotide of the invention or the pharmaceutical composition comprising the oligonucleotide of the invention to a person in need thereof.

A seventh aspect of the present invention is the oligonucleotide of the invention for use as medicine.

An eight aspect of the present invention is use of the oligonucleotide of the invention for the preparation of a medicament for treatment of cancer, viral infection, cardiovascular disease or immunogical disease.

The cancer may be glioblastoma, breast cancer, colorectal cancer and liver cancer.

The viral infection may be HIV infection, Hepatitis C infection, Hepatitis B infection, CMV infection and HSV infection.

The immunological disease may be psoriasis or eczema.

The cardiovascular disease may be treated by lowering high blood cholesterol.

A ninth aspect of the invention is use of the oligonucleotide of the invention for modulating the activity of a target RNA.

Method of transmission

A tenth aspect of the present invention is a method comprising transmission of information describing the oligonucleotide of the invention, oligonucleotide sensitive regions provided by the invention or information describing microRNA target regions of target RNAs provided by the invention. The information may describe either the oligonucleotide potentially capable of regulating the activity of a target mRNA or the oligonucleotide capable of regulating the activity of a target mRNA.

In a preferred embodiment of the tenth aspect, the transmission is electronic transmission.

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Examples

Example 1

A blockmir for treatment of diabetes

It has been demonstrated that mir-375 is a regulator of pancreatic island insulin secretion, and that Myotrophin (*Mtpn*) is a target of mir-375 regulation (Poy MN, 2004). Further, it has been shown that siRNA inhibition of Mtpn mimics the effects of miR-375 on glucose stimulated insulin secretion and exocytosis. Thus, it was concluded that by the authors that miR-375 is a regulator of insulin secretion

and may thereby constitute a novel pharmacological target for the treatment of diabetes.

Here we provide blockmirs that can regulate Mtpn expression by inhibiting mir-375 regulation of Mtpn. activity on the 3'UTR of the Mtpn mRNA.

The relevant portion of the target mRNA is:

5'GUGUUUUAAGUUUUGUGUUGCAA**GAACAA**AUGGAAUAAACUUGAAU

The anti-seed sequence is shown in bold. This target region of the target RNA can be identified e.g. by searching the target RNA for anti-seed sequences. Or the target region can be found using suitable databases available on the internet (www.pictar.com, target-scan). Obviously, the information may also be available from experiments or from a scientific publication (as e.g. Poy et al.)

The sequence of mir-375 is:

5'UUUGUUCGUUCGGCUCGCGUGA

Pairing the seed sequence to the anti-seed sequence gives e.g. the following interactions.

```
5' ...AGUUUUGUGUUGC---AAGAACAAAU...
              |:|  ||     |||||||
        3'AGUGCGC-UCGGCUUGCUUGUUU
```

It is seen that overall complementarity is scarce.

The blockmir:

A blockmir capable of regulating Mtpn expression by inhibition of mir-375 regulation will have to be able to sequester the anti-seed sequence of the target region, i.e. hide the anti-seed sequence in base pairing.

Thus, blockmirs (lower strand in 3'-5' direction) of Mtpn are exemplified here, base paired to the target sequence (upper strand in 5'-3' direction):

```
5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
          ||||||||||||||||||||||||||||||||
          GUUCAAAACACAACGUUCUUGUUUACCUUAUUUG

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
          ||||||||||||||||||||||||||||
          GUUCAAAACACAACGUUCUUGUUUACCUU

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
          ||||||||||||||||||||||||
          GUUCAAAACACAACGUUCUUGUUU

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
             ||||||||||||||||||||||||
             CAAAACACAACGUUCUUGUUUGCC

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
                ||||||||||||||||||
                CACAACGUUCUUGUUUACC

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
                   ||||||||||||||||
                   CAACGUUCUUGUUUACC

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
                      ||||||||||||
                      CGUUCUUGUUUACC

5'GUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAU
                        ||||||||||||
```

GUUCUUGUUUACC

5'GUGUUUUAAGUUUUGUGUUGCAA**GAACAA**AUGGAAUAAACUUGAAU

||||||||||||

GUUCUUGUUUAC

5'GUGUUUUAAGUUUUGUGUUGCAA**GAACAA**AUGGAAUAAACUUGAAU

||||||||||

GUUCUUGUUUA

5'GUGUUUUAAGUUUUGUGUUGCAA**GAACAA**AUGGAAUAAACUUGAAU

||||||||

CUUGUUUA

The blockmirs designed above may be synthesized using methods known in the art. As described in the specification, they may be synthesised as DNA, RNA, LNA, INA or with mixed monomers.

Obviously, U monomers may be exchanged with T monomers, while still allowing base pairing. Also G-C base pairs may be substituted with G-U wobble base pairs.

Methods for synthesizing various embodiments of the above designed blockmirs targeting the Mtpn mRNA are well known to the skilled man within the field of oligonucleotide synthesis. Particular preferred embodiments are described in the detailed description of the invention.

Conjugation of the blockmirs to e.g. cholesterol is also within the common knowledge of the skilled man.

Example 2

A blockmir for treatment of Herpes-simplex virus infection

Recently it was demonstrated that Herpes simplex virus-1 encoded a microRNA that enables the virus to establish a latent infection (Gupta A, 2006). The

microRNA that was termed mir-LAT was found to regulate TGF-beta and SMAD3, and thereby affect the ability of the cell to undergo apoptosis, the usual process by which an infected cell self-destructs in order to prevent production of viral progeny. Thus, it is of interest to be able to block the regulatory activity of mir-LAT on the expression of TGF-beta and SMAD3.

The sequence of the target region of the TGF-beta mRNA is:

5'AGGTCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC

The sequence of mir-LAT is:

5'**UGGCGG**CCCGGCCCGGGGCC

Thus, the following complex may be formed:

```
5'CGCCCCGGCAGGCCCG GCC-CCA
    |||||||    ||| |||| |||
3'CCGGGGCC---CGGCCCGGCGGU
```

A series of blockmirs can be designed as was also done in the previous example. The lower strand is the blockmir shown in the 3'-5' direction and the upper strand is the target region of TGF-beta mRNA:

```
5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCGGCCCCACC
  ||||||||||||||||||||||||||||||||||||||||||||
  UCCAGGGCGGGGCGGGGCGGGGCGGGGCCGUCCGGGCCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCGGCCCCACC
                ||||||||||||||||||||||||||||
                GGGGCGGGGCGGGGCCGUCCGGGCCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCGGCCCCACC
                          ||||||||||||||||||||||
                          GCGGGGCCGUCCGGGCCGGGGUGG
```

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC
||||||||||||||||||
CCGUCCGGGCCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC
||||||||||||
CGGGCCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC
||||||||||||
GGCCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC
|||||||||
CCGGGGUGG

5'AGGUCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG**GCCCCA**CC
||||||||
CCGGGGUG

Synthesis of various embodiments of such sequences is well within the ability of the skilled man. Particular preferred embodiments are described in the detailed description of the invention.

Example 3

Identification of an Oligonucleotide that regulate expression of Mtpn.

The following is a non-limiting example of how the method may be carried out. No wet experiments have actually been carried out.

The following sequence is a portion of the estimated target region of the Mtpn mRNA:

5′UUUGACGCAGUUGGGUUUCUCAUAAGUAUCCUAGUUCAUGUACAUCCGAAUGCUAAAU
AAUACUGUGUUUUAAGUUUUGUGUUGCAA**GAACAAA**GGAAUAAACUUGAAUUGUGCUAC

A series of potential blockmirs for this region with a 50% overlap is designed. Potential blockmirs are shown in italic and a reference sequence of the Mtpn mRNA and a complementary strand are shown for comparison:

```
5′UUUGACGCAGUUGGGUUUCUCAUAAGUAUCCUAGUUCAUGUACAUCCGAAUGCUAAAUAAU-
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3′AAACUGCGUCAACCCAAAGAGUAUUCAUAGGAUCAAGUACAUGUAGGCUUACGAUUUAUUA-

  AAACUGCGUCAACCCAAAGA
            AACCCAAAGAGUAUUCAUAG
                      GUAUUCAUAGGAUCAAGUAC
                                GAUCAAGUACAUGUAGGCUU
                                          AUGUAGGCUUACGAUUUAUUA
                                                    ACGAUUUAUUA-

5′-ACUGUGUUUUAAGUUUUGUGUUGCAAGAACAAAUGGAAUAAACUUGAAUUGUGCUAC
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3′-UGACACAAAAUUCAAAACACAACGUUCUUGUUUACCUUAUUUGAACUUAACACGAUG

  -UGACACAAAA
   UGACACAAAAUUCAAAACAC
             UUCAAAACACAACGUUCUUG
                       AACGUUCUUGUUUACCUUAU
                                 UUUACCUUAUUUGAACUUAA
                                           UUGAACUUAACACGAUG
```

Thus, 11 blockmirs have been designed. These are then synthesised, e.g. as 2-O-modified oligonucleotides with phosphorothioate linkages. Methods for synthesizing various embodiments of the above designed blockmirs targeting the Mtpn mRNA are well known to the skilled man within the field of oligonucleotide synthesis. Particular preferred embodiments are described in the detailed description of the invention.

Conjugation of the blockmirs to e.g. cholesterol is also within the common knowledge of the skilled man

The target sequence is then fused to a reporter gene, which expression can be detected. In this example, the reporter gene is eGFP. The reporter gene is then transfected to Hela cells, where after expression of eGFP is monitored after transfection of each of the 11 designed blockmirs.

Result:
Only blockmirs 7-9, counting from blockmirs complementary to the 5'end of the target region, affects the expression of eGFP. Blockmir 7 seven gives a slight increase in eGFP expression, whereas blockmir 8 and 9 has a more dramatic effect.

Thus, oligonucleotides that can affect the expression of the Mtpn mRNA have been identified.

The result indicates that the region covered by blockmirs 7-9 is a target for microRNA regulation. Furthermore, the result indicates that the region covered by blockmirs 8 and 9 is most important for microRNA regulation. The region covered by both oligonucleotide 8 and 9 may comprise the region that interact with the seed sequence of the microRNA or partly comprise the region that interact with the seed sequence of the microRNA.

The region covered by blockmirs 8 and 9 (AAGTTTTGTGTTGCAA**GAACAAA**TGGAATA) is then searched for complementarity to microRNAs.

More specifically, the region is searched for complementarity to seed sequences of microRNA. This search identifies human mir-375.

Whether this microRNA does indeed regulate the activity of the Mtpn mRNA may be verified by inhibiting the activity of mir-375 with an antimir.

The sequence of mir-375 is:

5'UUUGUUCGUUCGGCUCGCGUGA

Thus, an inhibitory antimir with the complementary sequence is synthesized.

Mir-375-antimir:

5'TCACGCGAGCCGAACGAACAAA

Truncated versions of the antimir is also produced:

5'ACGCGAGCCGAACGAACAAA

5'GCGAGCCGAACGAACAAA

5'GAGCCGAACGAACAAA

5'GCCGAACGAACAAA

5'CGAACGAACAAA

The antimirs are synthesised e.g. as 2-modified oligonucleotides with phosphorothioate linkages. Then it is tested whether these antimirs can prevent mir-375 from regulating the Mtpn mRNA using the reporter system.
The result shows that mir-375 does indeed regulate expression of the Mtpn mRNA.

Claims

1. An oligonucleotide comprising a antisense sequence that comprises a guide sequence corresponding to the seed sequence of a microRNA, with the proviso that the oligonucleotide is not a microRNA or does not comprise a sequence corresponding to the complete sequence of a microRNA.

2. The oligonucleotide of claim 1, wherein the microRNA is a human microRNA

3. The oligonucleotide of any of the preceding claims comprising a sequence selected from the group consisting of sequences that are capable of base pairing to the complementary sequence of a sequence selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723

4. The oligonucleotide of any of claims 1 or 2, wherein the antisense sequence comprises an sequence selected from the group consisting of position 1-20, position 1-19, position 1-18, position 1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723, wherein

a. A may be exchanged with only G, C, U, T or I
b. G may be exchanged with only A or I
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C, A, T or I

-and wherein 3 additional positions may be exchanged with any base.

5. The oligonucleotide of claim 3, wherein

a. A may be exchanged with only G, C, U, T or I
b. G may be exchanged with only A or I
c. C may be exchanged with only A or U
d. U may be exchanged with only C, A, T or I

-and wherein 3 additional positions may be exchanged with any base.

6. The oligonucleotide of claim 3, wherein
a. A may be exchanged with only C, U, T or I
b. G may be exchanged with only I
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C, A, T or I

-and wherein 3 additional positions may be exchanged with any base.

7. The oligonucleotide of claim 3, wherein
a. A may be exchanged with only C, U, or I
b. G may be exchanged with only I
c. C may be exchanged with only A or U
d. U may be exchanged with only C, A, T or I

-and wherein 3 additional positions may be exchanged with any base.

8. The oligonucleotide of claim 3, wherein
a. A may be exchanged with only G or I

b. G may be exchanged with only I or A
c. C may be exchanged with only A, U or T
d. U may be exchanged with only C or T

-and wherein 3 additional positions may be exchanged with any base.

9. The oligonucleotide of claim 3, wherein
a. A may be exchanged with only G
b. G may be exchanged with only A or G
c. C may be exchanged with only T or U
d. U may be exchanged with only C or T

-and wherein 3 additional positions may be exchanged with any base.

10. The oligonucleotide of claim 3, wherein U may be exchanged with only T

-and wherein 3 additional positions may be exchanged with any base.

11. The oligonucleotide of any of the preceding claims, wherein 2 additional positions may be exchanged with any base.

12. The oligonucleotide of any of the preceding claims, wherein 1 additional position may be exchanged with any base.

13. The oligonucleotide of any of the preceding claims, wherein no additional positions may be exchanged with any base.

14. The oligonucleotide of any of the preceding claims, wherein the antisense sequence comprises a sequence selected from the group consisting of: position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

15. The oligonucleotide of any of the preceding claims, wherein the antisense sequence comprises a sequence selected from the group consisting of:

position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723.

16. The oligonucleotide of any of the preceding claims, wherein the antisense sequence further comprises a second sequence selected from the group consisting of: position 12-17, position 12-16, position 13-17 and position 13-16 of any SEQ ID NOs:1-723, wherein the guide sequence and the second sequence are derived from the same SEQ ID NO.

17. The oligonucleotide of any of the preceding claims, wherein the antisense sequence comprises a contiguous stretch of bases complementary to the micro RNA target sequence of a target RNA selected from the group consisting of: less than 60 bases, less than 50 bases, less than 40 bases, less than 39 bases, less than 38 bases, less than 37 bases, less than 36 bases, less than 35, less than 34 bases, less than 33 bases, less than 32 bases, less than 31 bases, bases, less than 30 bases, less than 29 bases, less than 28 bases, less than 27 bases, less than 26 bases, less than 25 bases, less than 24 bases, less than 23 bases, less than 22 bases, less than 21 bases, less than 20 bases, less than 19 bases, less than 18 bases, less than 17 bases, less than 16 bases, less than 15 bases, less than 14 bases, less than 13 bases, less than 12 bases, less than 11 bases, less than 10 bases, less than 9 bases, less than 8 bases, less than 7 bases, more than 60 bases, more than 50 bases, more than 40 bases, more than 39 bases, more than 38 bases, more than 37 more, more than 36 bases, more than 35, more than 34 bases, more than 33 bases, more than 32 bases, more than 31, more than 30 bases, more than 29 bases, more than 28 bases, more than 27 bases, more than 26 bases, more than 25 bases, more than 24 bases, more than 23 bases, more than 22 bases, more than 21 bases, more than 20 bases, more than 19 bases, more than 18 bases, more than 17 bases, more than 16 bases, more than 15 bases, more than 14 bases, more than 13 bases, more than 12 bases, more than 11 bases, more than 10 bases, more than 9 bases, more than 8 bases, more than 7 bases, more than 6 bases and more than 5 bases.

18. The oligonucleotide of any of the preceding claims, wherein the antisense sequence comprises a contiguous stretch of bases complementary to the micro RNA target sequence of a target RNA, said continuous stretch of bases being selected from the group consisting of between 10 and 25 bases, between 12 and 24 bases, between 14 and 22 bases, between 15 and and 22 bases and between 15 and 20 bases.

19. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide has a percent identity to the corresponding the microRNA selected from the group of: less than 99%, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30% and less than 25%.

20. The oligonucleotide of any of the preceding claims, wherein the position of the guide sequence within the oligonucleotide is selected from the group consisting of: position 1, position 2, position 3, position 4, position 5, position 6, position 7, position 8, position 9, position 10, position 11, position 12, position 13, position 14, position 15, position 16, position 17, position 18 and position 19, wherein the position is counted in the 5'-3' direction from the first base of the guide sequence and the first base of the oligonucleotide.

21. The oligonucleotide of any of the preceding claims, wherein the length of the oligonucleotide is selected from the group consisting of: less than 60 bases, less than 50 bases, less than 40 bases, less than 39 bases, less than 38 bases, less than 37 bases, less than 36 bases, less than 35, less than 34 bases, less than 33 bases, less than 32 bases, less than 31 bases, bases, less than 30 bases, less than 29 bases, less than 28 bases, less than 27 bases, less than 26 bases, less than 25 bases, less than 24 bases, less than 23 bases, less than 22 bases, less than 21 bases, less than 20 bases, less than 19 bases, less than 18 bases, less than 17 bases, less than 16 bases, less than 15 bases, less than 14 bases, less than 13 bases, less than 12 bases, less than 11 bases, less than 10 bases, less than 9 bases, less than 8 bases, less than 7 bases, more than 60 bases, more than 50 bases,

more than 40 bases, more than 39 bases, more than 38 bases, more than 37 more, more than 36 bases, more than 35, more than 34 bases, more than 33 bases, more than 32 bases, more than 31, more than 30 bases, more than 29 bases, more than 28 bases, more than 27 bases, more than 26 bases, more than 25 bases, more than 24 bases, more than 23 bases, more than 22 bases, more than 21 bases, more than 20 bases, more than 19 bases, more than 18 bases, more than 17 bases, more than 16 bases, more than 15 bases, more than 14 bases, more than 13 bases, more than 12 bases, more than 11 bases, more than 10 bases, more than 9 bases, more than 8 bases, more than 7 bases, more than 6 bases and more than 5 bases.

22. The oligonucleotide of any of the preceding claims, wherein the length of the oligonucleotide is selected from the group consisting of between 10 and 25 bases, between 12 and 24 bases, between 14 and 22 bases, between 15 and and 22 bases and between 15 and 20 bases.

23. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is capable of inducing RNaseH cleavage of the target RNA.

24. The oligonucleotide of any of the preceding claims being an gapmer, endmer or headmer.

25. The oligonucleotide of any of claims 1-22, wherein the oligonucleotide is not capable of inducing RNase H cleavage of the target RNA.

26. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide does not comprise a stretch of unmodified DNA that exceeds a length selected from the group consisting of: 3 bases, 4 bases, 5 bases, 6 bases, 7 bases, 8 bases, 9 bases, 10 bases and 11 bases.

27. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is capable of recruiting the RNAi machinery.

28. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is not capable of recruiting the RNAi machinery.

29. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is capable of blocking the activity of the RNAi machinery at a particular target RNA.

30. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is capable of blocking the regulatory activity of a microRNA at a particular target RNA.

31. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is a positive regulator of the target RNA.

32. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is a negative regulator of the target RNA.

33. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide comprise nucleotide monomers that increase its affinity for complementary sequences or affinity increasing modifications.

34. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide comprise modifications that increase its biostability and/or bioavailability such as phosphorothioate linkages.

35. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide comprises INA units, PNA units, LNA units, units substituted in the 2'-position or any other nucleotide units that are capable of specific base pairing.

36. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide comprise nucleotide units that are substituted in the 2'-position and/or backbone modifications.

37. The oligonucleotide comprising a repeating pattern of one or more LNA units and one or more units that are substituted in the 2'-position.

38. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide do not comprise any RNA units.

39. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide do not comprise any DNA units.

40. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide do not comprise any morpholino units and/or LNA units.

41. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide comprise universal bases.

42. The oligonucleotide of any of the preceding claims, wherein the guide sequence is flanked by universal bases on the 3'side, the 5'side or both.

43. The oligonucleotide any of the preceding claims, wherein the bases between the guide sequence and the second sequence are universal bases.

44. The oligonucleotide of, any of the preceding claims, wherein any bases not part of the guide sequence and the second sequence are universal bases.

45. The oligonucleotide of any of the preceding claims wherein the oligonucleotide is not base paired with a complementary oligonucleotide or intended for use with a base paired with a complementary oligonucleotide.

46. The oligonucleotide of any of the preceding claims, wherein the oligonucleotide is base paired to a complementary oligonucleotide.

47. The oligonucleotide of any of the preceding claims, wherein the sequence of the microRNA is selected from the group consisting of: SEQ ID NOs: 1-723

48. The oligonucleotide of any of the preceding claims, wherein the target RNA is a target mRNA or target viral RNA

49. A method of modulating the activity of a target RNA comprising the steps of:

    a. Providing a system comprising a target RNA
    b. Introducing a oligonucleotide according to any of claims 1-xx of the target RNA to the system
    c. Thereby modulating the activity of the target RNA

50. The method of claim 49 wherein the oligonucleotide prevents the activity of a microRNA at the target RNA and thereby regulates the activity of the target RNA.

51. The method of any of claims 49-50 wherein the oligonucleotide induces RNase H cleavage of the target RNA and thereby regulates the activity of the target RNA.

52. The method of any of claims 49-51, wherein the system is either a cell extract or a cell.

53. The method of any of claims 49-52, wherein the method is performed in vivo, ex vivo or in vitro.

54. The method of any of claims 49-53, wherein the method is a method for validating a oligonucleotide regulator or identifying a oligonucleotide regulator.

55. The method of any of claims 49-54, wherein the method is a method for validating a micro RNA target of a target RNA.

56. The method of any of claims 49-54, wherein the target RNA is a mRNA or a viral RNA.

57. A method comprising the steps of:

a. Providing a target sequence of a target RNA regulated by a microRNA, said target sequence being the sequence of the target RNA involved in microRNA regulation.
b. Designing an oligonucleotide sequence that comprises a stretch of bases of at least 6 bases that is complementary to the target sequence
c. Synthesizing the oligonucleotide sequence of step b, thereby providing the oligonucleotide of step b, said oligonucleotide being a candidate regulator of the activity of a target RNA.

58. The method of claim 57 further comprising testing the steps of:

a. Providing a reporter system for activity of the target RNA
b. Determining the activity of the target RNA in the presence of the oligonucleotide of claim 57 step c
c. Determining the activity of the target RNA in the absence of the oligonucleotide of claim 57 step c
d. Comparing the activity levels in b and c and thereby verifying whether the oligonucleotide is indeed a capable of regulating the activity of the RNA and/or whether the potential target sequence of the RNA is indeed a target sequence.

59. The method of any of claims 57 and 58, wherein the activity is gene expression.

60. The method of any of claims 57-59, wherein the target sequence of the target RNA comprises a sequence complementary to the seed sequence of a microRNAs.

61. The method of any of claims 57-60, wherein the target sequence of the target RNA comprises a sequence selected from the group consisting of sequences that are capable of base pairing to the a sequence selected from the group consisting of position 1-20, position 1-19, position 1-18, position

1-17, position 1-16, position 1-15, position 1-14, position 1-13, position 1-12, position 1-11, position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-20, position 2-19, position 2-18, position 2-17, position 2-16, position 2-15, position 2-14, position 2-13, position 2-12, position 2-11, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-20, position 3-19, position 3-18, position 3-17, position 3-16, position 3-15, position 3-14, position 3-13, position 3-12, position 3-11, position 3-10 and position 3-9 of any SEQ ID NOs:1-723

62. The method of any of claims 57-61, wherein the target sequence of the RNA comprises a sequence selected from the group consisting sequences that are capable of base pairing to a sequence selected from the group consisting of: position 1-10, position 1-9, position 1-8, position 1-7, position 1-6, position 2-10, position 2-9, position 2-8, position 2-7, position 2-6, position 3-10 and position 3-9 of any SEQ ID NOs:1-723.

63. The method of any of claims 57-62, wherein the target sequence of the RNA comprises a sequence selected from the group consisting sequences that are capable of base pairing to a sequence selected from the group consisting of: position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723.

64. The method of any of claims 57-62, wherein the target sequence of the RNA further comprises a second sequence selected from the group consisting sequences that are capable of base pairing to a sequence selected from the group consisting of position 12-17, position 12-16, position 13-17 and position 13-16 of any SEQ ID NOs:1-723, wherein the guide sequence and the second sequence are derived from the same SEQ ID NO.

65. The method of any of claims 57-64, wherein the target sequence of the RNA is non-validated and the method is a method of validating the target sequence.

66. The method of any of claims 57-65, wherein the target sequence of the RNA is validated and the method is a means to provide a oligonucleotide capable of regulating the activity of the RNA.

67. The method of any of any of claims 57-66 further comprising providing the sequence of the microRNA regulating the RNA and using the seed sequence of the microRNA to determine the anti-seed sequence of the target sequence and the guide sequence of the oligonucleotide.

68. The method of any claims 57-67 wherein the seed sequence of the microRNA is selected from the group consisting of: position 1-10 of the microRNA, position 1-9 of the microRNA position 1-8 of the microRNA position 1-7 of the microRNA position 1-6 of the microRNA position 1-5 of the microRNA position 1-4 of the microRNA, position 2-10 of the microRNA, position 2-9 of the microRNA, position 2-8 of the microRNA, position 2-7 of the microRNA, position 2-6 of the microRNA and position 2-5 of any SEQ ID NOs:1-723 counted from the 5'end of the microRNAs.

69. The method of claims 57-68 wherein the seed sequence of the microRNA is selected from the group consisting of position 1-8, position 1-7, position 2-8 and position 2-7 of any SEQ ID NOs:1-723.

70. The method of any of claims 57-69 wherein the target RNA is a target mRNA or a target viral RNA.

71. The method of any of claims 57-70 wherein the oligonucleotide is an oligonucleotide of any of claims 1-48.

72. A method comprising the steps of

a. Providing a reporter system for expression of a target mRNA
b. Providing a oligonucleotide that is complementary to a part of the target mRNA
c. Determining the expression of the target mRNA in the presence of the oligonucleotide of step b

d. Determining the expression of the target mRNA in the absence of the oligonucleotide of step b
e. Comparing the expression levels in c and d and thereby verifying whether the oligonucleotide affect the expression of the mRNA.

73. The method of claim 72 wherein the method is a means of identifying a micro RNA target sequence of the mRNA.

74. The method of claim 72 wherein the method is a means of identifying a oligonucleotide capable of regulating the activity of the mRNA.

75. The method of any of claims 72-74 wherein a series of oligonucleotides are provided that each are complementary to a part of the target mRNA and where the series of oligonucleotides has an overall coverage of more than 50% for a particular target region of the target mRNA and wherein each oligonucleotide in the series are tested for activity.

76. The method of any of claims 72-75, wherein the target region of the target RNA is the 3'UTR of an mRNA.

77. The method of any of claims 72-76, wherein the sequences of active oligonucleotides are used to define oligonucleotide sensitive regions of the target region.

78. The method of any of claims 72-77, wherein the sequences of oligonucleotide sensitive regions are used to design one or more oligonucleotides with optimized sequences.

79. The method of any of claims 72-78, wherein the sequences of oligonucleotide sensitive regions are used to identify candidate microRNAs that potentially regulate the target RNA.

80. The method of any of claims 72-79 further comprising the steps

c. Providing a sequence of an oligonucleotide sensitive region

d. Searching to sequence of the oligonucleotide sensitive region for complementarity to microRNAs to identify candidate microRNAs that potentially regulate the target RNA

81. The method of claim 80, wherein the activity of the identified candidate microRNAs that potentially regulate the target mRNA is verified in a secondary test such as to identify microRNAs that do indeed regulate the activity of the target RNA

82. The method of claim 81, wherein the secondary test comprises the steps of

a. Providing a reporter system for activity of the target RNA
b. Providing an antimir-oligonucleotide that comprises complementarity to the microRNA and is capable of inhibiting the activity of the candidate microRNA
c. Determining the activity of the target RNA in the presence of the antimir-oligonucleotide of step b
d. Determining the activity of the target RNA in the absence of the antimir-oligonucleotide of step b
e. Comparing the activity levels of step c and d and thereby verifying whether the identified candidate microRNA regulators are indeed active microRNA regulators of the target RNA

83. The method of claim 82 further comprising the steps of

a. Determining the activity of the target RNA in the presence of an oligonucleotide directed to the target RNA
b. Determining the activity of the target RNA in the simultaneous presence of the oligonucleotide of step a in the presence of the antimir- oligonucleotide
c. Thereby verifying whether the oligonucleotide functions by blocking the activity of the micro RNA at the oligonucleotide sensitive region.

84. The method of any of claims 72-83, wherein the coverage is selected from the group consisting of: more than 55 %, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, more than 99% and 100%.

85. The method of any of claims 72-84, wherein the coverage is 100% and the oligonucleotides have an overlap in sequence.

86. The method of claim 85, wherein a particular oligonucleotide has 50% overlap with the oligonucleotide to its 5'end and 50% overlap with the oligonucleotide to its 3'end.

87. The method of any of claims 72-86, wherein the oligonucleotide is an oligonucleotide according the any of claims 1-48.

88. A pharmaceutical composition comprising the oligonucleotide of any of claims 1-48

89. A method of treatment comprising administering the oligonucleotide of any of claims 1-48 or the pharmaceutical composition of claim 88 to a person in need thereof.

90. The oligonucleotide of any of claims 1-48 for use as medicine

91. Use of the oligonucleotide of any of claims 1-48 for the preparation of a medicament for treatment of cancer, viral infection, immunological disease or cardiovascular disease.

92. A method comprising transmission of information describing the oligonucleotide of any of the preceding claims

93. The method of claim 92, wherein the transmission is electronic transmission