DESCRIPTION

Title of the Invention: ARTIFICIAL MATCH-TYPE miRNA FOR CONTROLLING GENE EXPRESSION AND USE THEREFOR

Technical Field

[0001]

The present invention relates to an artificial match-type miRNA that suppresses gene expression, and use thereof.

Background Art

[0002]

MicroRNA (miRNA) is known as a nucleic acid molecule that suppresses gene expression and has been reported to suppress transcription of a protein encoded by a gene via, for example, the following production process. That is, an miRNA transcription product (Pri-miRNA) having a cap structure on the 5'-terminal and poly(A) on the 3'-terminal is produced in the nucleus. The aforementioned Pri-miRNA is cleaved by RNase (Drosha) to produce a miRNA precursor (Pre-miRNA). The aforementioned Pre-miRNA forms a hairpin structure having a loop region and a stem region. The Pre-miRNA moves out from the nucleus and is degraded by RNase (Dicer) in the cytoplasm, and a double stranded miRNA (mature miRNA) having 1 - 4 bases of overhang on the 3'-terminal is cleaved out. One of the strands of the double stranded miRNA is called a guide strand and the other strand is called a passenger strand, and the aforementioned guide strand is bonded to a complex similar to RNA-induced Silencing Complex (RISC). This miRNA/RISC complex binds to the 3' untranslated region (3'UTR) of particular mRNA to suppress translation of protein from the aforementioned mRNA.

[0003]

It has been clarified that miRNA is deeply involved in life phenomena such as differentiation, cell proliferation, apoptosis and the like and many diseases such as viral infections, cancer and the like (patent document 1, non-patent document 1, non-patent document 2). Therefrom its application in, particularly, the medical field has been expected.

[Document List]

[Patent Document]

[0004]

patent document 1: WO 2010/056737 A2

[non-patent documents]

[0005]

non-patent document 1: Deiters, 2009, The AAPS Journal, 12, 51-60

non-patent document 2: Takeshita etal., 2010, Mol. Ther., 18, 181-187

SUMMARY OF THE INVENTION

Problems to be Solved by the Invention

[0006]

For application of the aforementioned miRNA, for example, a method including use of a double stranded mature miRNA and the like are available. However, this method requires, before application, annealing of two single strand nucleic acid molecules, which produces a possibility of developing autoimmunity by TLR3 and the like that recognize the double strand.

[0007]

Therefore, an object of the present invention is to provide a new artificial match-type miRNA utilizing miRNA.

Means of Solving the Problems

[0008]

To achieve the aforementioned object, the artificial match-type miRNA of the present invention is a single strand nucleic acid comprising X region and Y region, characterized in that

the 3'-terminal of the aforementioned X region and the 5'-terminal of the aforementioned Y region are linked via a linker region of a non-nucleotide structure,

the aforementioned X region comprises a guide strand sequence of a mature miRNA, and

the aforementioned Y region comprises a sequence completely

complementary to the aforementioned X region.

[0009]

The composition of the present invention is a composition for inhibiting the expression of a gene, and characteristically contains the above-mentioned artificial match-type miRNA of the present invention.

[0010]

The composition of the present invention is a pharmaceutical composition which characteristically contains the above-mentioned artificial match-type miRNA of the present invention.

[0011]

The expression inhibiting method of the present invention is a method of inhibiting the expression of a target gene, which characteristically uses the above-mentioned artificial match-type miRNA of the present invention.

[0012]

The method of treating a disease of the present invention includes a step of administering the above-mentioned artificial match-type miRNA of the present invention to a patient, wherein the aforementioned guide strand sequence in the above-mentioned artificial match-type miRNA is a guide strand sequence of a mature miRNA that suppresses expression of genes involved in the aforementioned diseases.

Effect of the Invention

[0013]

The artificial match-type miRNA of the present invention can be synthesized easily at a low cost, and can suppress translation of protein encoded by the aforementioned genes.

Brief Description of the Drawings

[0014]

Fig. 1 is a schematic showing of one embodiment of the artificial match-type miRNA of the present invention.

Fig. 2 is a graph showing the number of cells per well in Example 1 of the present invention.

Fig. 3 is a graph showing the relative values of cell proliferation in Example 1 of the present invention.

Fig. 4 is a graph showing the proportion of apoptosis in Example 1 of the present invention.

Fig. 5 is a graph showing the relative values of AXL mRNA amount and MET mRNA amount in Example 1 of the present invention.

Fig. 6 is a graph showing the relative values of AXL mRNA amount in Example 2 of the present invention.

Fig. 7 is a graph showing the relative values of MET mRNA amount in Example 2 of the present invention.

Fig. 8 is a graph showing the relative values of AXL mRNA amount in Example 3 of the present invention.

Fig. 9 is a graph showing the relative values of MET mRNA amount in Example 3 of the present invention.

Fig. 10 is a graph showing the relative values of HMGA2 mRNA amount in Example 4 of the present invention.

Fig. 11 is a graph showing the relative values of COLA1 mRNA amount in Example 5 of the present invention.

Description of Embodiments

[0015]

Unless otherwise specified, the terms used in the present specification mean what is generally meant by them in the art.

[0016]

(1) Artificial match-type miRNA

The artificial match-type miRNA of the present invention is, as mentioned above, a single strand nucleic acid comprising X region and Y region, characterized in that

the 3′-terminal of the aforementioned X region and the 5′-terminal of the aforementioned Y region are linked via a linker region of a non-nucleotide structure,

the aforementioned X region comprises a guide strand sequence of a mature miRNA, and

the aforementioned Y region comprises a sequence completely complementary to the aforementioned X region.

[0017]

The artificial match-type miRNA of the present invention can suppress, for example, expression of the target gene. Suppression of expression means, for example, suppression of the translation of the aforementioned target gene, that is, suppression of the translation of a protein encoded by the aforementioned target gene, more particularly, suppression of the translation of the aforementioned protein from mRNA of the aforementioned target gene. The aforementioned inhibition of the expression of the target gene can be verified by, for example, a decrease in the amount of a transcription product derived from the target gene; a decrease in the activity of the aforementioned transcription product; a decrease in the amount of a translation product generated from the aforementioned target gene; a decrease in the activity of the aforementioned translation product; or the like. The aforementioned proteins may be, for example, mature proteins, precursor proteins before being subjected to processing or post-translational modification.

[0018]

Since the artificial match-type miRNA of the present invention is a single strand nucleic acid molecule, annealing of two single strands is not necessary unlike mature miRNA, and can be produced at a low cost. Furthermore, since the artificial match-type miRNA of the present invention is a single strand nucleic acid molecule, for example, it can avoid recognition by TLR3, RIG-I, MDA5 and the like involved in autoimmunity.

[0019]

An outline of the configurational relationship between the aforementioned X region and the aforementioned Y region in the artificial match-type miRNA of the present invention is shown in Fig. 1. Fig. 1 shows an outline and, for example, the length, shape and the like of each region are not limited. The artificial match-type miRNA of the present invention has, as

shown in Fig. 1, the aforementioned X region on the 5'-side and the aforementioned Y region on the 3'-side, and the 3'-terminal of the aforementioned X region and the 5'-terminal of the aforementioned Y region are linked via linker region (shown with "P" in the Figure) of a non-nucleotide structure.

[0020]

In the artificial match-type miRNA of the present invention, since the aforementioned Y region contains a sequence completely complementary to the aforementioned X region, the aforementioned X region and the aforementioned Y region are, for example, intramolecularly annealed. Intramolecular annealing is also referred to as, for example, self-annealing. The artificial match-type miRNA of the present invention is also said to form a double strand in the aforementioned intramolecularly-annealed region.

[0021]

The artificial match-type miRNA of the present invention can also be referred to as a linear single strand nucleic acid molecule, wherein the 5'-terminal thereof and the 3'-terminal thereof are unlinked. To maintain the unbinding of the both termini, the 5'-terminal of the artificial match-type miRNA of the present invention is preferably, for example, a non-phosphoric acid group.

[0022]

In the artificial match-type miRNA of the present invention, the aforementioned X region contains, as mentioned above, a guide strand sequence of a mature miRNA. The guide strand sequence of a mature miRNA is, for example, registered in various databases (e.g., http://www.mirbase.org/ etc.). Therefore, the aforementioned X region can be set based on, for example, the information of known mature miRNAs. The guide strand of the aforementioned mature miRNA is a strand, which is taken into an Argonaute (Ago) protein of RNA-induced silencing complex (RISC) and binds to mRNA of the target.

[0023]

The aforementioned X region may consist solely of, for example, the aforementioned guide strand sequence, or may further have an additional sequence. In the latter case, the aforementioned X region consists of, for example, the aforementioned guide strand sequence and the aforementioned additional sequence, and the aforementioned additional sequence is linked to, for example, the 3'-terminal of the aforementioned guide strand sequence.

[0024]

In the artificial match-type miRNA of the present invention, when the aforementioned X region and the aforementioned Y region are aligned, the aforementioned Y region has a sequence completely complementary to the aforementioned X region. The aforementioned Y region may consist only of, for example, a sequence completely complementary to the aforementioned X region, or further have an overhang in addition to the aforementioned complementary sequence. That is, in the artificial match-type miRNA of the present invention, when, for example, the aforementioned Y region and the aforementioned X region are aligned, the aforementioned Y region may have an overhang on the 3'-terminal. As use herein, the aforementioned overhang in the Y region is, for example, a terminal base that the aforementioned Y region has in excess than the aforementioned X region when the aforementioned Y region and the aforementioned X region are aligned. The length (O) of the overhang is, for example, as shown in the following formula.

length (O) of overhang = [full-length base number (Y) of Y region]-[full-length base number (X) of X region]

[0025]

In the artificial match-type miRNA of the present invention, the length of each region is not particularly limited. While examples of the conditions are shown below, the artificial match-type miRNA of the present invention is not limited by such description. In the present invention, the

numerical range of the base number discloses all positive integers that fall within the range and, for example, "1 - 4 bases" means all of "1, 2, 3, 4 bases" (hereinafter the same).

[0026]

In the aforementioned X region, the length of the aforementioned guide strand sequence is not particularly limited and may be, for example, the length of a guide strand sequence of a reported mature miRNA. Specific examples thereof include a lower limit of 19 base length, 20 base length, and an upper limit of 25 base length, 24 base length, and ranges of 19 - 25 base length, 20 - 24 base length.

[0027]

The length of the aforementioned additional sequence of the aforementioned X region is not particularly limited, and the lower limit is, for example, 0 base length, 1 base length, 2 base length, and the upper limit is, for example, 5 base length, 4 base length, 3 base length, and the range is, for example, 0 - 5 base length, 1 - 5 base length, 1 - 4 base length, 2 - 3 base length, 3 - 5 base length.

[0028]

The length of the aforementioned X region is not particularly limited, the lower limit is, for example, 19 base length, 21 base length, 23 base length, the upper limit is, for example, 30 base length, 28 base length, 26 base length, and the range is, for example, 19 - 30 base length, 21 - 28 base length, 23 - 26 base length.

[0029]

The length of the aforementioned overhang in the aforementioned Y region is not particularly limited, and the lower limit is, for example, 0 base length, 1 base length, and the upper limit is, for example, 4 base length, 3 base length, and the range is, for example, 0 - 4 base length, 1 - 3 base length, 2 base length.

[0030]

The sequence of the aforementioned overhang is not

particularly limited and is, for example, UU, CU, GC, UA, AA, CC, UG, CG, AU, TT and the like from the 3'-side. The aforementioned overhang can be imparted with resistance to ribonuclease by being, for example, TT.

[0031]

The length of the aforementioned Y region is not particularly limited, and the lower limit is, for example, 19 base length, 21 base length, 23 base length, and the upper limit is, for example, 32 base length, 30 base length, 28 base length, and the range is, for example, 19 - 32 base length, 21 - 30 base length, 23 - 28 base length.

[0032]

The full-length (T) of the artificial match-type miRNA of the present invention is not particularly limited, and the lower limit is, for example, 38 base length, 42 base length, 46 base length, the upper limit is, for example, 62 base length, 58 base length, 54 base length, and the range is, for example, 38 - 62 base length, 42 - 58 base length, 46 - 54 base length.

[0033]

In the artificial match-type miRNA of the present invention, the kind of the aforementioned mature miRNA is not particularly limited, and can be appropriately selected according to the kind of the target gene.

[0034]

Examples of the aforementioned mature miRNA include mature miRNAs such as hsa-miR-34a (SEQ ID NO: 1), hsa-let-7a (SEQ ID NO: 2), hsa-let-7f (SEQ ID NO: 3), hsa-miR-150 (SEQ ID NO: 4), hsa-miR-29b (SEQ ID NO: 5) and the like.

hsa-miR-34a (SEQ ID NO: 1)

UGGCAGUGUCUUAGCUGGUUGU

hsa-let-7a (SEQ ID NO: 2)

UGAGGUAGUAGGUUGUAUAGUU

hsa-let-7f (SEQ ID NO: 3)

UGAGGUAGUAGAUUGUAUAGUU

hsa-miR-150 (SEQ ID NO: 4)

UCUCCCAACCCUUGUACCAGUG

hsa-miR-29b (SEQ ID NO: 5)

UAGCACCAUUUGAAAUCAGUGUU

The nucleotide sequence shown in each SEQ ID NO is a guide strand sequence.

[0035]

The guide strand of miR-34a targets, for example, AXL, MET, CDK4, CDK6, SIRT1, CCND1, SIRT1, BCL-2 and the like, and the suppression of the expression of these target genes can prevent or treat diseases such as lung cancer, colorectal cancer, stomach cancer, liver cancer, breast cancer and the like.

The guide strand of let-7a targets, for example, HMGA2 (high mobility group AT-hook 2), KRAS, NRAS, HRAS, MYC, TLR4 and the like, and the suppression of the expression of these target genes can prevent or treat diseases such as lung cancer, colorectal cancer, stomach cancer, liver cancer, breast cancer and the like.

The guide strand of let-7f targets, for example, HMGA2 (high mobility group AT-hook 2), KRAS, NRAS, HRAS, MYC, TLR4 and the like, and the suppression of the expression of these target genes can prevent or treat diseases such as lung cancer, colorectal cancer, stomach cancer, liver cancer, breast cancer and the like.

The guide strand of miR-150 targets, for example, COL1A1, COL4A4, SMAD2, SP1 and the like, and the suppression of the expression of these target genes can prevent or treat diseases such as lung fibrosis, hepatic fibrosis and the like.

The guide strand of miR-29b targets, for example, COL1A1, MCL1, DNMT3A, DNMT3B, TCL1A, TGFb3 and the like, and the suppression of the expression of these target genes can prevent or treat diseases such as lung cancer, colorectal cancer, stomach cancer, liver cancer, breast cancer, lung fibrosis, hepatic fibrosis and the like.

[0036]

The constitution units of the artificial match-type miRNA of the present invention are not particularly limited. Examples thereof include nucleotide residues. Examples of the aforementioned nucleotide residues include a ribonucleotide residue and a deoxyribonucleotide residue. In the artificial match-type miRNA of the present invention, the aforementioned nucleotide residue is preferably, for example, a ribonucleotide residue. The aforementioned nucleotide residue may be, for example, the one that is not modified (unmodified nucleotide residue) or the one that has been modified (modified nucleotide residue). By configuring the artificial match-type miRNA of the present invention to include the aforementioned modified nucleotide residue, for example, the resistance of the artificial match-type miRNA to nuclease can be improved, thereby allowing the stability of the artificial match-type miRNA to be improved. Furthermore, the artificial match-type miRNA of the present invention further may include, for example, a non-nucleotide residue in addition to the aforementioned nucleotide residue.

[0037]

When the artificial match-type miRNA includes, for example, the aforementioned modified ribonucleotide residue(s) in addition to the aforementioned unmodified ribonucleotide residues, the number of the aforementioned modified ribonucleotide residue(s) is not particularly limited, and is, for example, "one to several", specifically, for example, 1 to 5, preferably 1 to 4, more preferably 1 to 3, and most preferably 1 or 2. The aforementioned modified ribonucleotide residue as contrasted to the aforementioned unmodified ribonucleotide residue may be, for example, the aforementioned deoxyribonucleotide residue obtained by substituting a ribose residue with a deoxyribose residue. When the artificial match-type miRNA of the present invention includes, for example, the aforementioned deoxyribonucleotide residue(s) in addition to the aforementioned unmodified ribonucleotide residue(s), the

number of the aforementioned deoxyribonucleotide residue(s) is not particularly limited, and is, for example, "one to several", specifically, for example, 1 to 5, preferably 1 to 4, more preferably 1 to 3, and most preferably 1 or 2.

[0038]

The aforementioned nucleotide residue includes, for example, a sugar, a base, and a phosphate as its components. The aforementioned ribonucleotide residue has, for example, a ribose residue as the sugar; and adenine (A), guanine (G), cytosine (C), or uracil (U) as the base. The aforementioned deoxyribose residue has, for example, a deoxyribose residue as the sugar; and adenine (A), guanine (G), cytosine (C), or thymine (T) as the base.

[0039]

The aforementioned components of the aforementioned unmodified nucleotide residue are the same or substantially the same as, for example, the components of a naturally-occurring nucleotide residue. Specifically, for example, the components are the same or substantially the same as the components of a nucleotide residue occurring naturally in a human body.

[0040]

For example, the aforementioned modified nucleotide residue may be such that any of the components of the aforementioned unmodified nucleotide residue is modified. Examples of the aforementioned modified nucleotide residue include naturally-occurring nucleotide residues and artificially-modified nucleotide residues.

[0041]

The aforementioned modified nucleotide residue may be, for example, a residue of an alternative of the aforementioned nucleotide. Examples of the aforementioned alternative include artificial nucleic acid monomer residues. Specific examples thereof include PNA (Peptide Nucleic Acid), LNA (Locked Nucleic Acid), and ENA (2'-O,4'-C-Ethylenebridged Nucleic Acids).

[0042]

In the aforementioned nucleotide residue, the aforementioned base is not particularly limited. The aforementioned base may be, for example, a natural base or a non-natural base. The aforementioned base may be, for example, a naturally-derived base or a synthetic base. As the aforementioned base, for example, a common base, a modified analog thereof, and the like can be used.

[0043]

In the artificial match-type miRNA of the present invention, the linker region of the aforementioned non-nucleotide structure preferably contains at least one selected from the group consisting of an amino acid residue, a polyamine residue and a polycarboxylic acid residue. The aforementioned linker region may or may not contain a residue other than the amino acid residue, polyamine residue and polycarboxylic acid residue. For example, the aforementioned linker region may contain any of a polycarboxylic acid residue, a terephthalic acid residue and an amino acid residue.

[0044]

In the present invention, the "polyamine" means any compound containing a plurality of (two, three or more) amino groups. The aforementioned "amino group" is not limited to an $-NH_2$ group and also includes an imino group (-NH-). In the present invention, the aforementioned polyamine is not particularly limited, and examples thereof include 1,4-diaminobenzene, 1,3-diaminobenzene, 1,2-diaminobenzene and the like. In the present invention, moreover, the "polycarboxylic acid" means any compound containing a plurality of (two, three or more) carboxy groups. In the present invention, the aforementioned polycarboxylic acid is not particularly limited, and examples thereof include 1,4-dicarboxybenzene (terephthalic acid), 1,3-dicarboxybenzene (isophthalic acid), 1,2-dicarboxybenzene (phthalic acid) and the like. In the present invention, moreover, the "amino acid" means any organic compound containing one or more amino groups and one or more

carboxy groups in a molecule, as mentioned below. The aforementioned "amino group" is not limited to an $-NH_2$ group and also includes an imino group (-NH-).

[0045]

In the artificial match-type miRNA of the present invention, the aforementioned amino acid residue may be composed of a plurality of interlinked amino acid residues. In the present invention, the amino acid residue that is a plurality of interlinked amino acid residues is, for example, a residue containing a peptide structure. More specifically, the aforementioned amino acid residue that is a plurality of interlinked amino acid residues is, for example, an amino acid residue of the below-mentioned chemical formula (I) wherein the below-mentioned chemical formula (Ia) is a peptide (e.g., glycine dimer or glycine trimer etc.).

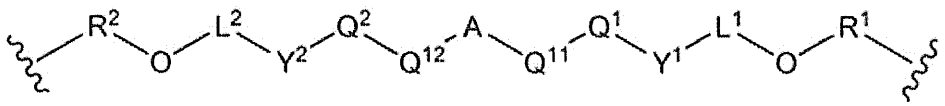
[0046]

In the artificial match-type miRNA of the present invention, the aforementioned amino acid residue may be a glycine residue, a terephthalic acid amide residue, a proline residue or a lysin residue. The aforementioned amino acid residue may be a modified amino acid residue or an amino acid derivative.

[0047]

In the artificial match-type miRNA of the present invention, the aforementioned linker region is represented by, for example, the following chemical formula (I-0).

[0048]



(I-0)

[0049]

in the aforementioned chemical formula (I-0),

Q11 and Q12 are each independently a single bond, CH_2 (a methylene group), NH (an imino group), C=O (a carbonyl group),

C=S (a thiocarbonyl group), C=NH (an iminomethylene group), O, or S,

Q1 and Q2 are each independently a single bond, CH_2 (a methylene group), NH (an imino group), C=O (a carbonyl group), C=S (a thiocarbonyl group), C=NH (an iminomethylene group), O, or S,

Y^1 and Y^2 are each independently a single bond, CH_2, NH, O, or S;

L^1 is an alkylene chain having n carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a, or,

L1 is a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom,

provided that: when Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other;

L^2 is an alkylene chain having m carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c, or

L^2 is a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom,

provided that: when Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other;

R^a, R^b, R^c, and R^d are each independently a substituent or a protecting group;

m is an integer in the range from 0 to 30;

n is an integer in the range from 0 to 30;

the aforementioned regions X and Y are each linked to the aforementioned linker residue via $-OR^1-$ or $-OR^2-$,

wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or the aforementioned structure (I-0); and

A is any atomic group.

[0050]

The combination of the aforementioned regions (X) and (Y) with $-OR^1-$ and $-OR^2-$ is not particularly limited, and may be, for example, any of the following conditions.

Condition (1):

the aforementioned regions (X) and (Y) are linked to the structure of the aforementioned formula (I) via $-OR^2-$ and $-OR^1-$, respectively.

Condition (2):

the aforementioned regions (X) and (Y) are linked to the structure of the aforementioned formula (I) via $-OR^1-$ and $-OR^2-$, respectively.

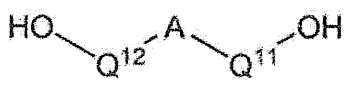
[0051]

In the aforementioned chemical formula (I-0), for example, Q^{11} may be C=O (a carbonyl group), and Q^1 may be NH (an imino group). In addition, for example, Q^{11} may be NH (an imino group), and Q^1 may be C=O (a carbonyl group). Furthermore, for example, Q^{12} may be C=O (a carbonyl group), and Q^2 may be NH (an imino group). Moreover, for example, Q^{12} may be NH (an imino group), and Q^2 may be C=O (a carbonyl group).

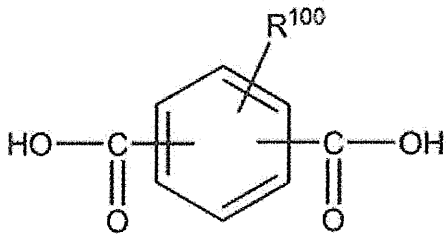
[0052]

In the aforementioned chemical formula (I-0), each of Q^{11} and Q^{12} may be, for example, a carbonyl group. In this case, each of Q^1 and Q^2 is preferably an imino group. In addition, in this case, the structure of the following chemical formula (Iα) is more preferably represented by the following chemical formula (Iα2).

[0053]



(Iα)

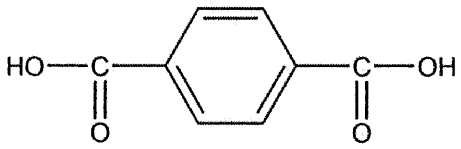


(Iα2)

[0054]

In the aforementioned chemical formula (Iα2), R^{100} is any substituent, which may or may not be present. When it is present, it may be present singly or in plurality. When it is present in plurality, they may be the same or different from each other. Examples of the aforementioned any substituent for R^{100} include the below-mentioned substituents exemplified as the aforementioned R^a, R^b, R^c and R^d. More specific examples thereof include halogen, hydroxy, alkoxy, amino, carboxy, sulfo, nitro, carbamoyl, sulfamoyl, alkyl, alkenyl, alkynyl, haloalkyl, aryl, arylalkyl, alkylaryl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cyclylalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, silyl, silyloxyalkyl, pyrrolyl, imidazolyl and the like. The structure of the aforementioned chemical formula (Iα2) is more preferably represented by the following chemical formula (Iα3).

[0055]



(Iα3)

[0056]

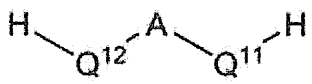
When Q^{11} and Q^{12} are carbonyl groups, and Q^1 and Q^2 are imino groups, the linker residue of the aforementioned chemical

formula (I-0) can be a carboxylic acid amide residue or a carboxylic acid residue. For example, the "TPA" structure in the below-mentioned Example can be a terephthalamide residue or a terephthalic acid residue represented by the aforementioned chemical formula (Iα3).

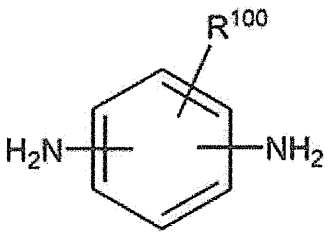
[0057]

In the aforementioned chemical formula (I-0), each of Q^{11} and Q^{12} may be an imino group. In this case, each of Q^{1} and Q^{2} is preferably a carbonyl group. In this case, the structure of the following chemical formula (Iβ) is more preferably represented by the following chemical formula (Iβ2).

[0058]



(Iβ)



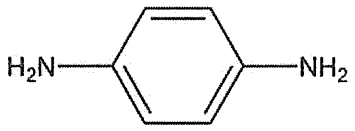
(Iβ2)

[0059]

In the aforementioned chemical formula (Iβ2),

R^{100} is any substituent, which may or may not be present. When it is present, it may be present singly or in plurality. When it is present in plurality, they may be the same or different from each other. Specifically, for example, it is the same as R^{100} in the aforementioned chemical formula (Iα2). In addition, the structure of the aforementioned chemical formula (Iβ2) is more preferably represented by the following chemical formula (Iβ3).

[0060]

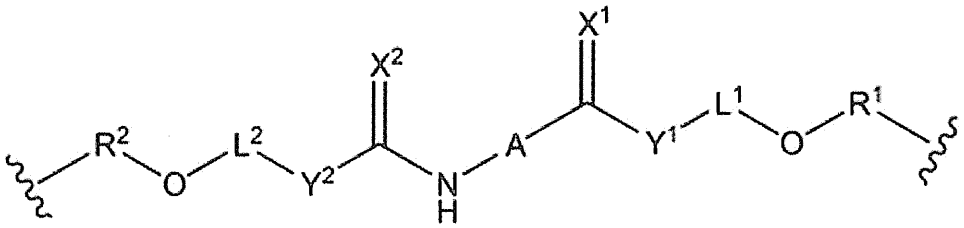


(Iβ3)

[0061]

In the artificial match-type miRNA of the present invention, when the aforementioned linker residue is an amino acid residue, the aforementioned amino acid residue is represented by, for example, the following chemical formula (I). The structure of the following chemical formula (I) is one example of the structure represented by the aforementioned chemical formula (I-0).

[0062]



(I)

[0063]

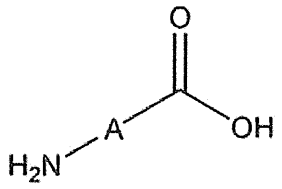
In the aforementioned formula (I), for example, X^1, X^2, Y^1, Y^2, L^1 and L^2 are as defined above.

The sequence complementary to the sequence of the aforementioned microRNA is each bound to the aforementioned amino acid residue via $-OR^1-$ or $-OR^2-$,

wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or the aforementioned structure (I); and

A is any atomic group, provided that the following chemical formula (Ia) is an amino acid or peptide.

[0064]



(I a)

[0065]

The atomic group A in the aforementioned chemical formula (I), (Iα) or (Ia) may or may not contain, for example, at least one selected from the group consisting of chain atomic group, alicyclic atomic group, aromatic atomic group, heteroaromatic atomic group, and heteroalicyclic atomic group. While the aforementioned chain atomic group is not particularly limited, for example, alkyl, alkenyl, alkynyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, silyl, silyloxyalkyl and the like can be mentioned. While the aforementioned alicyclic atomic group is not particularly limited, for example, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cyclylalkyl and the like can be mentioned. While the aforementioned aromatic atomic group is not particularly limited, for example, aryl, arylalkyl, alkylaryl, condensed-ring aryl, condensed-ring arylalkyl, condensed-ring alkylaryl and the like can be mentioned. The aforementioned heteroaromatic atomic group is not particularly limited, and examples thereof include heteroaryl, heteroarylalkyl, alkylheteroaryl, condensed-ring heteroaryl, condensed-ring heteroarylalkyl, condensed-ring alkylheteroaryl and the like. In the atomic group A in the aforementioned chemical formula (I), (Iα) or (Ia), each of the aforementioned atomic groups may or may not further have a substituent or a protecting group. When the aforementioned substituent or protecting group is in plurality, they may be the same or different. The aforementioned substituents are, for example, those exemplified for the aforementioned R^a, R^b, R^c and R^d, more specifically, for example, halogen, hydroxy, alkoxy, amino, carboxy, sulfo, nitro, carbamoyl, sulfamoyl, alkyl, alkenyl, alkynyl, haloalkyl, aryl, arylalkyl, alkylaryl, cycloalkyl,

cycloalkenyl, cycloalkylalkyl, cyclylalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, silyl, silyloxyalkyl, pyrrolyl, imidazolyl, and the like. The aforementioned protecting groups are, for example, the same as those exemplified for the aforementioned R^a, R^b, R^c and R^d.

[0066]

In the present invention, the "amino acid" refers to, as mentioned above, any organic compound containing at least one amino group and at least one carboxy group in a molecule. The aforementioned "amino group" is not limited to $-NH_2$ group, and also includes imino group (-NH-). For example, proline, hydroxyproline and the like not containing $-NH_2$ group in a molecule but containing imino group (-NH-) is included in the definition of the "amino acid" in the present invention. In the present invention, the aforementioned "amino acid" may be, as mentioned below, a natural amino acid or an artificial amino acid. For example, since a compound represented by the below-mentioned chemical formula (Ia2) or (Ia3) contains an amino group and a carboxy group in a molecule, it is encompassed in the definition of the "amino acid" in the present invention. Therefore, for example, the aforementioned chemical formula (I) wherein the atomic group A is a structure shown by the below-mentioned chemical formula (A2) or chemical formula (A2a) is included in the definition of "amino acid residue" in the present invention. For example, the "TPA" structure in the below-mentioned Example is also included in the definition of the "amino acid residue" in the present invention. The "peptide" in the present invention refers to an organic compound having a structure wherein not less than 2 molecules of amino acid are bonded via a peptide bond. The aforementioned peptide bond may be an acid amide structure or an acid imide structure. When plural amino groups are present in the amino acid or peptide molecule represented by the aforementioned chemical formula (Ia), the amino group clearly shown in the aforementioned chemical formula (Ia) may be any

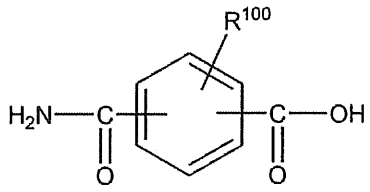
amino group. In addition, when plural carboxy groups are present in the amino acid or peptide molecule represented by the aforementioned chemical formula (Ia), the carboxy group clearly shown in the aforementioned chemical formula (Ia) may be any carboxy group.

[0067]

In the aforementioned amino acid residue of the artificial match-type miRNA of the present invention, the aforementioned amino acid may be, as mentioned above, natural amino acid or artificial amino acid. In the present invention, the "natural amino acid" refers to an amino acid having a naturally-occurring structure or an optical isomer thereof. The production method of the aforementioned natural amino acid is not particularly limited and, for example, it may be extracted from the nature, or may be synthesized. In the present invention, moreover, the "artificial amino acid" refers to an amino acid having a structure not occurring naturally. That is, the aforementioned artificial amino acid is an amino acid, i.e., a carboxylic acid derivative containing an amino group (organic compound containing at least one amino group and at least one carboxy group in a molecule) and having a structure not occurring naturally. The aforementioned artificial amino acid preferably does not contain, for example, a hetero ring. The aforementioned amino acid may be an amino acid constituting, for example, a protein. The aforementioned amino acid may be, for example, at least one kind selected from the group consisting of glycine, α-alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine, hydroxylysine, methionine, phenylalanine, serine, threonine, tyrosine, valine, proline, 4-hydroxyproline, tryptophan, β-alanine, 1-amino-2-carboxycyclopentane, aminobenzoic acid, aminopyridinecarboxylic acid and amino acid represented by the following chemical formula (Ia2), and may or may not further have a substituent or a protecting group. Examples of the

aforementioned substituent include the substituents exemplified for the aforementioned Ra, Rb, Rc and Rd. More specifically, for example, halogen, hydroxy, alkoxy, amino, carboxy, sulfo, nitro, carbamoyl, sulfamoyl, alkyl, alkenyl, alkynyl, haloalkyl, aryl, arylalkyl, alkylaryl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cyclylalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, silyl, silyloxyalkyl, pyrrolyl, imidazolyl, and the like can be mentioned. The aforementioned protecting group is the same as, for example, the protecting groups exemplified for the aforementioned Ra, Rb, Rc and Rd. When the amino acid of the aforementioned chemical formula (Ia), which is not peptide, contains isomers such as optical isomer, geometric isomer, stereoisomer and the like, any isomer can be used.

[0068]

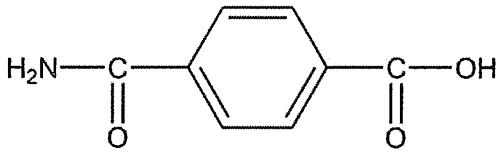


(Ia2)

[0069]

In the aforementioned chemical formula (Ia2), R100 is an optional substituent and may or may not be present. When it is present, the number thereof may be one or more and, when it is present in plurality, they may be the same or different. Examples of the aforementioned optional substituent for R100 include the substituents exemplified for the aforementioned Ra, Rb, Rc and Rd, more specifically, for example, halogen, hydroxy, alkoxy, amino, carboxy, sulfo, nitro, carbamoyl, sulfamoyl, alkyl, alkenyl, alkynyl, haloalkyl, aryl, arylalkyl, alkylaryl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cyclylalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, silyl, silyloxyalkyl, pyrrolyl, imidazolyl, and the like. The structure of the aforementioned chemical formula (Ia2) may be, for example, the following chemical formula (Ia3).

[0070]

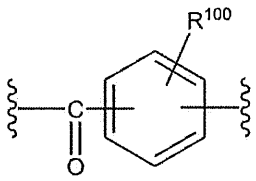


(Ia3)

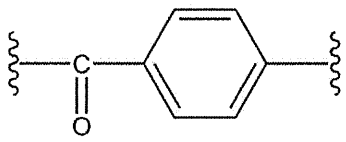
[0071]

When the structure of the aforementioned chemical formula (Ia) is the aforementioned chemical formula (Ia2), the structure of the atomic group A in the aforementioned chemical formula (I) is represented by the following chemical formula (A2). R100 in the following chemical formula (A2) is the same as that in the aforementioned chemical formula (Ia2). When the structure of the aforementioned chemical formula (Ia) is the aforementioned chemical formula (Ia3), the structure of the atomic group A in the aforementioned chemical formula (I) is represented by the following chemical formula (A2a).

[0072]



(A2)

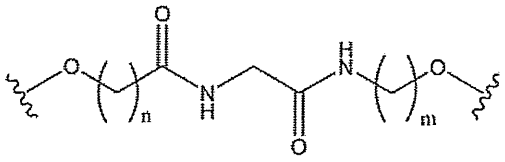


(A2a)

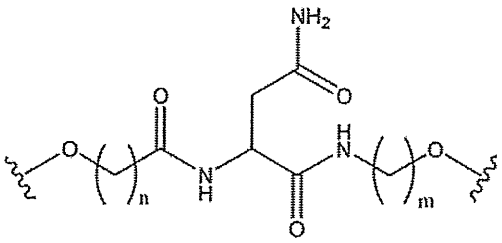
[0073]

The structure of the aforementioned chemical formula (I) is, for example, the following chemical formulae (I-1) - (I-7), wherein n and m are the same as those in the aforementioned chemical formula (I).

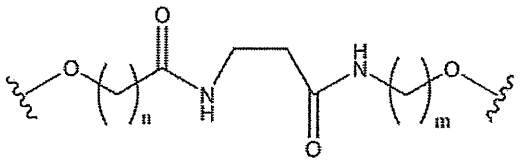
[0074]



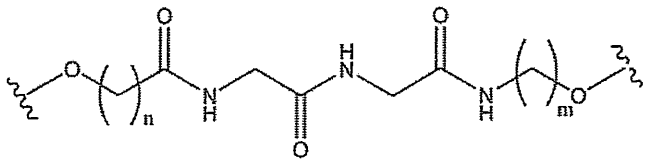
(I－1)



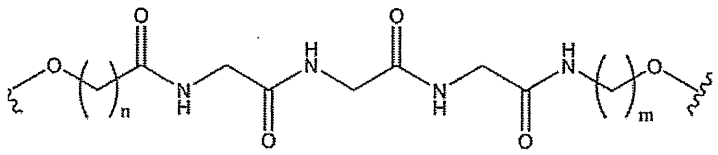
(I－2)



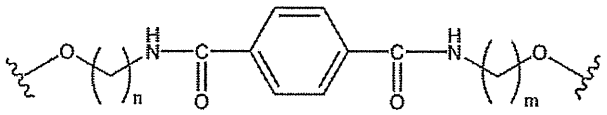
(I－3)



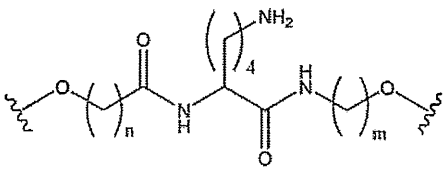
(I－4)



(I－5)



(I－6)



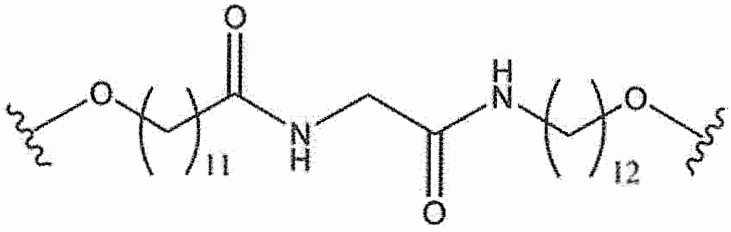
(I－7)

[0075]

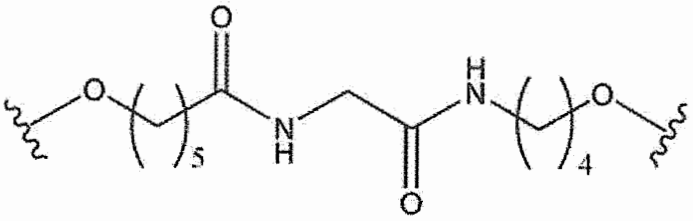
In the aforementioned chemical formulae (I-1) - (I-7), n

and m are not particularly limited, and as described above. Specific examples thereof include n=11 and m=12 or n=5 and m=4 in the aforementioned chemical formula (I-1), n=5 and m=4 in the aforementioned chemical formula (I-4), n=4 and m=4 in the aforementioned chemical formula (I-6), and n=5 and m=4 in the aforementioned chemical formula (1-7). The structures thereof are shown in the following chemical formulae (I-1a), (I-1b)(I-4a), (I-6a) and (I-7a).

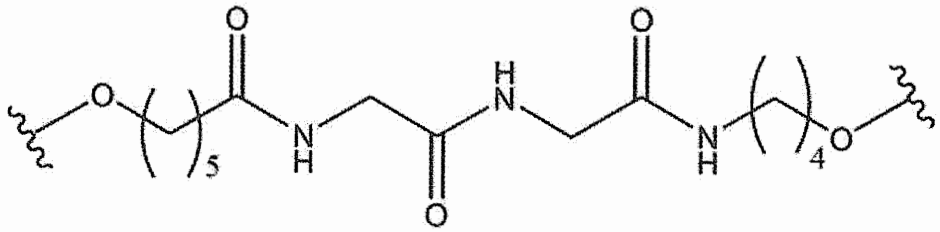
[0076]



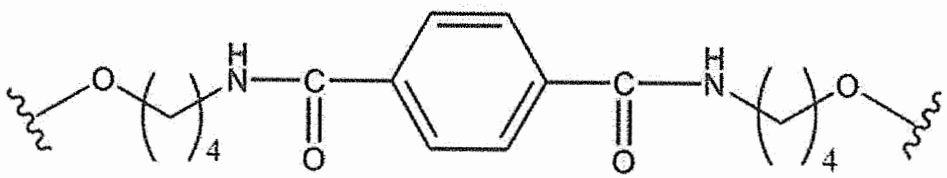
（Ｉ－１ａ）



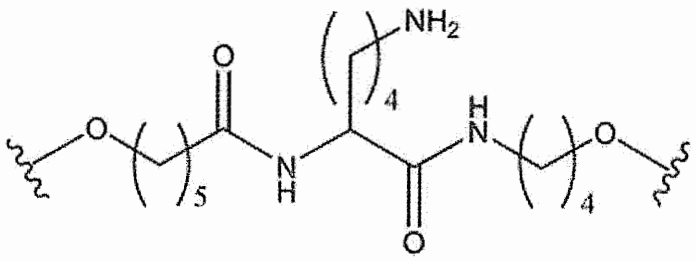
（Ｉ－１ｂ）



（Ｉ－４ａ）



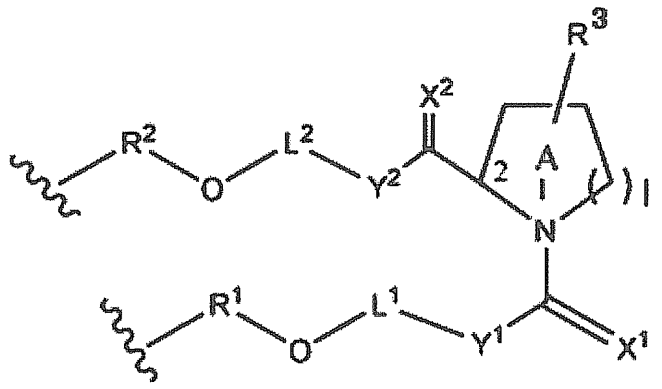
（Ｉ－６ａ）



（Ｉ－７ａ）

[0077]

In the artificial match-type miRNA of the present invention, the aforementioned linker region is represented, for example, by the following formula (II):



... (II)

[0078]

In the aforementioned formula (II), for example,

X^1 and X^2 are each independently H_2, O, S, or NH;

Y^1 and Y^2 are each independently a single bond, CH_2, NH, O, or S;

R^3 is a hydrogen atom or a substituent which is bonded to C-3, C-4, C-5 or C-6 on ring A,

L^1 is an alkylene chain having n atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a, or,

L^1 is a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom,

provided that: when Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other;

L^2 is an alkylene chain having m atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c, or

L^2 is a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom,

provided that: when Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other;

R^a, R^b, R^c, and R^d are each independently a substituent or a protecting group;

l is 1 or 2;

m is an integer in the range from 0 to 30;

n is an integer in the range from 0 to 30; and

in ring A, one carbon atom other than the aforementioned C-2 on the ring A may be substituted by nitrogen, oxygen or sulfur, and may contain, in the aforementioned ring A, a carbon-carbon double bond or a carbon-nitrogen double bond,

the aforementioned regions (X) and (Y) are each linked to the aforementioned non-nucleotide structure via $-OR^1-$ or $-OR^2-$, wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or the aforementioned structure (II).

[0079]

In the aforementioned formula (II), for example, X^1 and X^2 are each independently H_2, O, S, or NH. In the aforementioned formula (II), "X^1 is H_2" means that X^1 forms CH_2 (a methylene group) together with a carbon atom to which X^1 binds. The same applies to X^2.

[0080]

In the aforementioned formula (II), Y^1 and Y^2 are each independently a single bond, CH_2, NH, O, or S.

[0081]

In the aforementioned formula (II), l in ring A is 1 or 2. When l = 1, ring A is a 5-membered ring, for example, the aforementioned pyrrolidine skeleton. The aforementioned pyrrolidine skeleton is, for example, proline skeleton, prolinol skeleton or the like, and exemplified by the divalent structures thereof. When l = 2, ring A is a 6-membered ring, for example, the aforementioned piperidine skeleton. In ring A, one carbon atom other than C-2 on ring A may be substituted by

nitrogen, oxygen or sulfur. Ring A may contain, in ring A, a carbon-carbon double bond or a carbon-nitrogen double bond. Ring A is, for example, L type or D type.

[0082]

In the aforementioned formula (II), R^3 is a hydrogen atom or substituent bonded to C-3, C-4, C-5 or C-6 on ring A. When R^3 is the aforementioned substituent, substituent R^3 may be one or more, or may be absent. When R^3 is present in plurality, they may be the same or different.

[0083]

The substituent R^3 is, for example, halogen, OH, OR^4, NH_2, NHR^4, NR^4R^5, SH, SR^4, oxo group (=O) and the like.

[0084]

R^4 and R^5 are, for example, each independently a substituent or a protecting group, and may be the same or different. Examples of the aforementioned substituent include halogen, alkyl, alkenyl, alkynyl, haloalkyl, aryl, heteroaryl, arylalkyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cyclylalkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, heterocyclylalkenyl, heterocyclylalkyl, heteroarylalkyl, silyl, silyloxyalkyl and the like. The same applies hereinafter. The substituent R^3 may be selected from the substituents recited above.

[0085]

The aforementioned protecting group is a functional group that inactivates, for example, a highly-reactive functional group. Examples of the protecting group include known protecting groups and the like. Regarding the aforementioned protecting group, for example, the description in the literature (J. F. W. McOmie, "Protecting Groups in Organic Chemistry", Prenum Press, London and New York, 1973) can be incorporated herein. The aforementioned protecting group is not particularly limited, and examples thereof include a tert-butyldimethylsilyl group (TBDMS), a bis(2-acetoxyethyloxy)methyl group (ACE), a

triisopropylsilyloxymethyl group (TOM), a 1-(2-cyanoethoxy)ethyl group (CEE), a 2-cyanoethoxymethyl group (CEM), a tolylsulfonylethoxymethyl group (TEM), a dimethoxytrityl group (DMTr) and the like. When R^3 is OR^4, the aforementioned protecting group is not particularly limited, and examples thereof include a TBDMS group, an ACE group, a TOM group, a CEE group, a CEM group, a TEM group and the like. Other examples of the protecting group include silyl-containing groups. The same applies hereinafter.

[0086]

In the aforementioned formula (II), L^1 is an alkylene chain having n atoms. A hydrogen atom(s) on the aforementioned alkylene carbon atom(s) may or may not be substituted with, for example, OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a. Alternatively, L^1 may be a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom. The aforementioned polyether chain is, for example, polyethylene glycol. When Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other. That is, for example, when Y^1 is O, this oxygen atom and the oxygen atom in L^1 are not adjacent to each other, and the oxygen atom in OR^1 and the oxygen atom in L^1 are not adjacent to each other.

[0087]

In the aforementioned formula (II), L^2 is an alkylene chain having m atoms. A hydrogen atom(s) on the aforementioned alkylene carbon atom(s) may or may not be substituted with, for example, OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c. Alternatively, L^2 may be a polyether chain obtained by substituting at least one carbon atom on the aforementioned alkylene chain with an oxygen atom. When Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other. That is, for example, when Y^2 is O, this oxygen atom and the oxygen atom in L^2 are not adjacent to each other, and the oxygen atom in OR^2 and the

oxygen atom in L^2 are not adjacent to each other.

[0088]

n of L^1 and m of L^2 are not particularly limited, and the lower limit of each of them may be 0, for example, and the upper limit of the same is not particularly limited. For example, n and m can be set as appropriate depending on a desired length of the aforementioned non-nucleotide structure. For example, from the view point of manufacturing cost, yield, and the like, n and m are each preferably 0 to 30, more preferably 0 to 20, and still more preferably 0 to 15. n and m may be the same (n = m) or different. n + m is, for example, 0 to 30, preferably 0 to 20, and more preferably 0 to 15.

[0089]

For example, R^a, R^b, R^c and R^d are each independently a substituent or a protecting group. Examples of the aforementioned substituent and the aforementioned protecting group are the same as above.

[0090]

In the aforementioned formula (II), hydrogen atoms each independently may be substituted with, for example, a halogen such as Cl, Br, F, I and the like.

[0091]

The aforementioned X region and the aforementioned Y region are each bound to the aforementioned non-nucleotide structure via, for example, $-OR^1-$ or $-OR^2-$. Here, R^1 and R^2 may or may not be present. When R^1 and R^2 are present, R^1 and R^2 are each independently a nucleotide residue or the structure represented by the aforementioned formula (II). When R^1 and/or R^2 are/is the aforementioned nucleotide residue, the aforementioned non-nucleotide structure is formed by, for example, the aforementioned non-nucleotide residue having the structure of the aforementioned formula (II) excluding the nucleotide residue R^1 and/or R^2, and the aforementioned nucleotide residue(s). When R^1 and/or R^2 are/is the structure represented by the aforementioned formula (II), the structure

of the aforementioned non-nucleotide structure is such that, for example, two or more of the aforementioned non-nucleotide residues having the structure of the aforementioned formula (II) are linked to each other. The number of the structures of the aforementioned formula (II) may be, for example, 1, 2, 3, or 4. When the aforementioned structure includes a plurality of the aforementioned structures, the structures of the aforementioned (II) may be linked, for example, either directly or via the aforementioned nucleotide residue(s). On the other hand, when R^1 and R^2 are not present, the aforementioned non-nucleotide structure is formed by, for example, the aforementioned non-nucleotide residue having the structure of the aforementioned formula (II) alone.

[0092]

The combination of the aforementioned regions X and Y with $-OR^1-$ and $-OR^2-$ is not particularly limited, and may be, for example, any of the following conditions:

conditions (1)

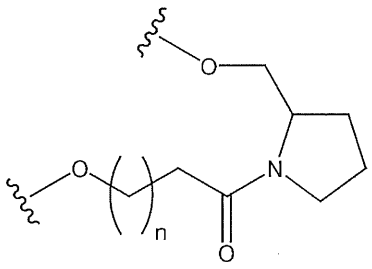
the aforementioned regions X and Y are linked to the structure of the aforementioned formula (II) via $-OR^2-$ and $-OR^1-$, respectively;

conditions (2)

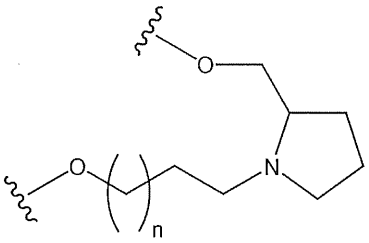
the aforementioned regions X and Y are linked to the structure of the aforementioned formula (II) via $-OR^1-$ and $-OR^2-$, respectively;

[0093]

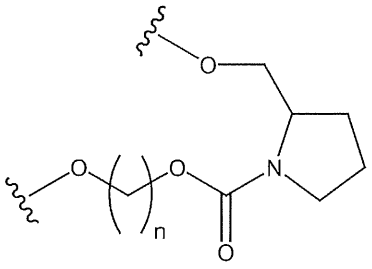
Examples of the structure of the aforementioned formula (II) include the structures of the following formulae (II-1) to (II-9). In the following formulae, n and m are the same as in the aforementioned formula (II). In the following formulae, q is an integer of 0 - 10.



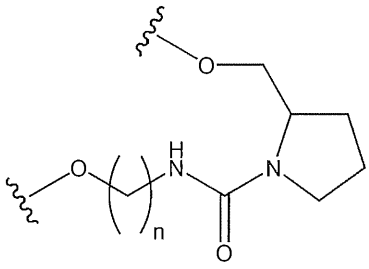
・・・(II－1)



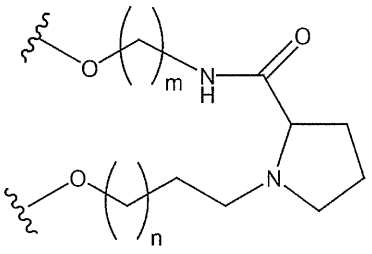
・・・(II－2)



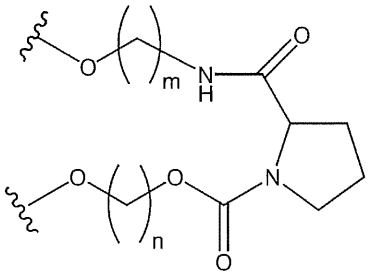
・・・(II－3)



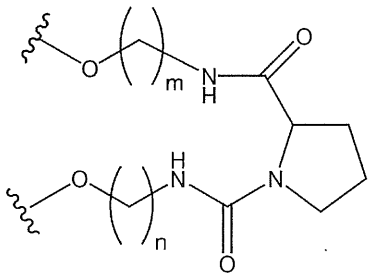
・・・(II－4)



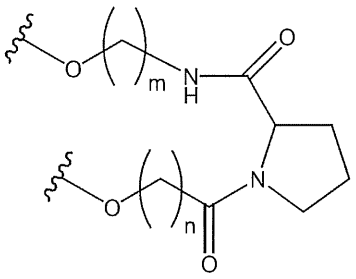
・・・(II－5)



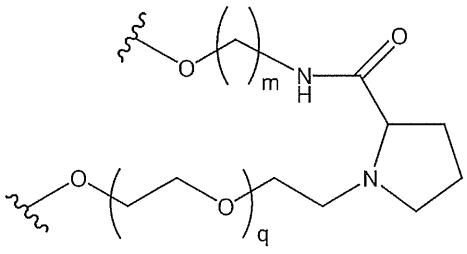
・・・(II－6)



・・・(II－7)



・・・(II－8)

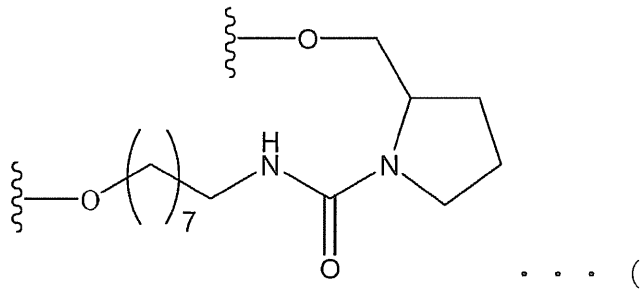


・・・(II－9)

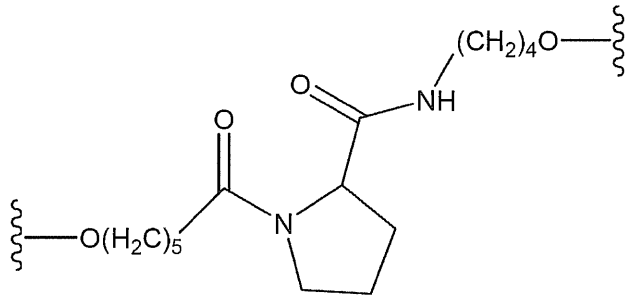
[0094]

In the aforementioned formulae (II-1) to (II-9), n, m and q are not particularly limited, and are as described above. Specific example thereof is the aforementioned formula (II-1)

wherein n=8, the aforementioned (II-2) wherein n=3, the aforementioned formula (II-3) wherein n=4 or 8, the aforementioned (II-4) wherein n=7 or 8, the aforementioned formula (II-5) wherein n=3 and m=4, the aforementioned (II-6) wherein n=8 and m=4, the aforementioned formula (II-7) wherein n=8 and m=4, the aforementioned (II-8) wherein n=5 and m=4, and the aforementioned formula (II-9) wherein q=1 and m=4. One embodiment (n=8) of the aforementioned formula (II-4) is shown in the following formula (II-4a), and one embodiment(n=5, m=4) of the aforementioned formula (II-8) is shown in the following formula (II-8a).



・・・（II－4a）



・・・（II－8a）

[0095]

In the present invention, the term "alkyl" encompasses, for example, straight-chain and branched alkyl groups. The number of carbon atoms in the aforementioned alkyl is not particularly limited, and is, for example, 1 to 30, preferably 1 to 6, more preferably 1 to 4. Examples of the aforementioned alkyl group include: methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl and the like. Among them, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl,

n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, and the like are preferable.

[0096]

In the present invention, the term "alkenyl" encompasses, for example, straight-chain and branched alkenyls. Examples of the aforementioned alkenyl include the aforementioned alkyls having one or more double bonds and the like. The number of carbon atoms in the aforementioned alkenyl is not particularly limited, and is, for example, the same as that in the aforementioned alkyl, preferably 2 to 8. Examples of the aforementioned alkenyl include vinyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,3-butadienyl, 3-methyl-2-butenyl and the like.

[0097]

In the present invention, the term "alkynyl" encompasses, for example, straight-chain and branched alkynyls. Examples of the aforementioned alkynyl include the aforementioned alkyls having one or more triple bonds and the like. The number of carbon atoms in the aforementioned alkynyl is not particularly limited, and is, for example, the same as that in the aforementioned alkyl, preferably 2 to 8. Examples of the aforementioned alkynyl include ethynyl, propynyl, butynyl and the like. The aforementioned alkynyl may further include, for example, one or more double bonds.

[0098]

In the present invention, the term "aryl" encompasses, for example, monocyclic aromatic hydrocarbon groups and polycyclic aromatic hydrocarbon groups. Examples of the aforementioned monocyclic aromatic hydrocarbon group include phenyl and the like. Examples of the aforementioned polycyclic aromatic hydrocarbon group include 1-naphthyl, 2-naphthyl, 1-anthryl, 2-anthryl, 9-anthryl, 1-phenanthryl, 2-phenanthryl, 3-phenanthryl, 4-phenanthryl, 9-phenanthryl and the like. Among them, for example, phenyl, naphthyls such as 1-naphthyl and 2-naphthyl, and the like are preferable.

[0099]

In the present invention, the term "heteroaryl" encompasses, for example, monocyclic aromatic heterocyclic groups and condensed aromatic heterocyclic groups. Examples of the aforementioned heteroaryl include furyls (e.g., 2-furyl, 3-furyl), thienyls (e.g., 2-thienyl, 3-thienyl), pyrrolyls (e.g., 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl), imidazolyls (e.g., 1-imidazolyl, 2-imidazolyl, 4-imidazolyl), pyrazolyls (e.g., 1-pyrazolyl, 3-pyrazolyl, 4-pyrazolyl), triazolyls (e.g., 1,2,4-triazol-1-yl, 1,2,4-triazol-3-yl, 1,2,4-triazol-4-yl), tetrazolyls (e.g., 1-tetrazolyl, 2-tetrazolyl, 5-tetrazolyl), oxazolyls (e.g., 2-oxazolyl, 4-oxazolyl, 5-oxazolyl), isoxazolyls (e.g., 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl), thiazolyls (e.g., 2-thiazolyl, 4-thiazolyl, 5-thiazolyl), thiadiazolyls, isothiazolyls (e.g., 3-isothiazolyl, 4-isothiazolyl, 5-isothiazolyl), pyridyls (e.g., 2-pyridyl, 3-pyridyl, 4-pyridyl), pyridazinyls (e.g., 3-pyridazinyl, 4-pyridazinyl), pyrimidinyls (e.g., 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl), furazanyls (e.g., 3-furazanyl), pyrazinyls (e.g., 2-pyrazinyl), oxadiazolyls (e.g., 1,3,4-oxadiazol-2-yl), benzofuryls (e.g., 2-benzo[b]furyl, 3-benzo[b]furyl, 4-benzo[b]furyl, 5-benzo[b]furyl, 6-benzo[b]furyl, 7-benzo[b]furyl), benzothienyls (e.g., 2-benzo[b]thienyl, 3-benzo[b]thienyl, 4-benzo[b]thienyl, 5-benzo[b]thienyl, 6-benzo[b]thienyl, 7-benzo[b]thienyl), benzimidazolyls (e.g., 1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl), dibenzofuryls, benzoxazolyls, benzothiazolyls, quinoxalinyls (e.g., 2-quinoxalinyl, 5-quinoxalinyl, 6-quinoxalinyl), cinnolinyls (e.g., 3-cinnolinyl, 4-cinnolinyl, 5-cinnolinyl, 6-cinnolinyl, 7-cinnolinyl, 8-cinnolinyl), quinazolinyls (e.g., 2-quinazolinyl, 4-quinazolinyl, 5-quinazolinyl, 6-quinazolinyl, 7-quinazolinyl, 8-quinazolinyl), quinolyls (e.g., 2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), phthalazinyls (e.g., 1-phthalazinyl, 5-phthalazinyl, 6-phthalazinyl), isoquinolyls

(e.g., 1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), puryls, pteridinyls (e.g., 2-pteridinyl, 4-pteridinyl, 6-pteridinyl, 7-pteridinyl), carbazolyls, phenanthridinyls, acridinyls (e.g., 1-acridinyl, 2-acridinyl, 3-acridinyl, 4-acridinyl, 9-acridinyl), indolyls (e.g., 1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), isoindolyls, phenazinyls (e.g., 1-phenazinyl, 2-phenazinyl), and phenothiazinyls (e.g., 1-phenothiazinyl, 2-phenothiazinyl, 3-phenothiazinyl, 4-phenothiazinyl) and the like.

[0100]

In the present invention, for example, the term "cycloalkyl" refers to cyclic saturated hydrocarbon groups and the number of carbon atoms in the cycloalkyl is, for example, 3 to 15. Examples of the aforementioned cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, bridged cyclic hydrocarbon groups, spiro hydrocarbon groups and the like. Among them, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, bridged cyclic hydrocarbon groups, and the like are preferable.

[0101]

In the present invention, examples of the "bridged cyclic hydrocarbon groups" include bicyclo[2.1.0]pentyl, bicyclo[2.2.1]heptyl, bicyclo[2.2.2]octyl, and bicyclo[3.2.1]octyl, tricyclo[2.2.1.0]heptyl, bicyclo[3.3.1]nonane, 1-adamantyl, 2-adamantyl and the like.

[0102]

In the present invention, examples of the "spiro hydrocarbon groups" include spiro[3.4]octyl and the like.

[0103]

In the present invention, the term "cycloalkenyl" encompasses, for example, unsaturated cyclic aliphatic hydrocarbon groups and the number of carbon atoms in the cycloalkenyl is, for example, 3 to 7. Examples of the aforementioned cycloalkenyl include cyclopropenyl, cyclobutenyl,

cyclopentenyl, cyclohexenyl, cycloheptenyl and the like. Among them, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, and the like are preferable. The aforementioned term "cycloalkenyl" also encompasses, for example, bridged cyclic hydrocarbon groups and spiro hydrocarbon groups having an unsaturated bond in their rings.

[0104]

In the present invention, examples of the "arylalkyl" include benzyl, 2-phenethyl, naphthalenylmethyl and the like. Examples of the "cycloalkylalkyl" and "cyclylalkyl" include cyclohexylmethyl adamantylmethyl and the like. Examples of the "hydroxyalkyl" include hydroxymethyl 2-hydroxyethyl and the like.

[0105]

In the present invention, the "alkoxy" encompasses, for example, groups composed of any of the aforementioned alkyls and oxygen (alkyl-O-groups) and examples thereof include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy and the like. Examples of the "alkoxyalkyl" include methoxymethyl and the like. Examples of the "aminoalkyl" include 2-aminoethyl and the like.

[0106]

In the present invention, examples of the "heterocyclyl" include 1-pyrrolinyl, 2-pyrrolinyl, 3-pyrrolinyl, 1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl, pyrrolidinone, 1-imidazolinyl, 2-imidazolinyl, 4-imidazolinyl, 1-imidazolidinyl, 2-imidazolidinyl, 4-imidazolidinyl, imidazolidinone, 1-pyrazolinyl, 3-pyrazolinyl, 4-pyrazolinyl, 1-pyrazolidinyl, 3-pyrazolidinyl, 4-pyrazolidinyl, piperidinone, piperidino, 2-piperidinyl, 3-piperidinyl, 4-piperidinyl, 1-piperazinyl, 2-piperazinyl, piperazinone, 2-morpholinyl, 3-morpholinyl, morpholino, tetrahydropyranyl, tetrahydrofuranyl and the like.

[0107]

In the present invention, examples of the "heterocyclylalkyl" include piperidinylmethyl,

piperazinylmethyl and the like. Examples of the "heterocyclylalkenyl" include 2-piperidinylethenyl and the like. Examples of the "heteroarylalkyl" include pyridylmethyl, quinolin-3-ylmethyl and the like.

[0108]

In the present invention, the term "silyl" encompasses groups represented by the chemical formula R_3Si-, where R independently can be selected from the aforementioned alkyls, aryls, and cycloalkyls. Examples of the silyl include a trimethylsilyl group, a tert-butyldimethylsilyl group and the like. Examples of the "silyloxy" include a trimethylsilyloxy group and the like. Examples of the "silyloxyalkyl" include trimethylsilyloxymethyl and the like.

[0109]

In the present invention, examples of the "alkylene" include methylene, ethylene, propylene and the like.

[0110]

In the present invention, the above-described various groups may be substituted. Examples of the aforementioned substituent include hydroxy, carboxy, sulfo, halogen, alkyl halide (haloalkyl, e.g., CF_3, CH_2CF_3, CH_2CCl_3), nitro, nitroso, cyano, alkyl (e.g., methyl, ethyl, isopropyl, tert-butyl), alkenyl (e.g., vinyl), alkynyl (e.g., ethynyl), cycloalkyl (e.g., cyclopropyl, adamantyl), cycloalkylalkyl (e.g., cyclohexylmethyl, adamantylmethyl), cycloalkenyl (e.g., cyclopropenyl), cyclylalkyl, hydroxyalkyl (e.g., hydroxymethyl, hydroxyethyl), alkoxyalkyl (e.g., methoxymethyl, ethoxymethyl, ethoxyethyl), aryl (e.g., phenyl, naphthyl), arylalkyl (e.g., benzyl, phenethyl), alkylaryl (e.g., p-methylphenyl), heteroaryl (e.g., pyridyl, furyl), heteroarylalkyl (e.g., pyridylmethyl), heterocyclyl (e.g., piperidyl), heterocyclylalkenyl, heterocyclylalkyl (e.g., morpholylmethyl), alkoxy (e.g., methoxy, ethoxy, propoxy, butoxy), halogenated alkoxy (e.g., OCF_3), alkenyloxy (e.g., vinyloxy, allyloxy), aryloxy (e.g., phenyloxy), alkyloxycarbonyl (e.g.,

methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl), arylalkyloxy (e.g., benzyloxy), amino [alkylamino (e.g., methylamino, ethylamino, dimethylamino), acylamino (e.g., acetylamino, benzoylamino), arylalkylamino (e.g., benzylamino, tritylamino), hydroxyamino], aminoalkyl (e.g., aminomethyl), alkylaminoalkyl (e.g., diethylaminomethyl), carbamoyl, sulfamoyl, oxo, silyl, silyloxyalkyl and the like.

[0111]

The artificial match-type miRNA of the present invention may include, for example, a labeling substance, and may be labeled with the aforementioned labeling substance. The aforementioned labeling substance is not particularly limited, and may be, for example, a fluorescent substance, a dye, an isotope, or the like. Examples of the aforementioned labeling substance include: fluorophores such as pyrene, TAMRA, fluorescein, a Cy3 dye, a Cy5 dye and the like. Examples of the aforementioned dye include Alexa dyes such as Alexa 488 and the like. Examples of the aforementioned isotope include stable isotopes and radioisotopes. Among them, stable isotopes are preferable. Moreover, for example, the aforementioned stable isotope does not change the physical properties of a compound labeled therewith and thus has an excellent property as a tracer. The aforementioned stable isotope is not particularly limited, and examples thereof include ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{33}S, ^{34}S and ^{36}S.

[0112]

As described above, the artificial match-type miRNA of the present invention can inhibit the aforementioned expression of a target gene. Thus, the artificial match-type miRNA of the present invention can be used, for example, as a therapeutic agent for treating a disease caused by a gene. When the artificial match-type miRNA of the present invention has a guide strand sequence of a mature miRNA that inhibits expression of a gene causing the aforementioned disease, for example, it is possible to treat the aforementioned disease by

inhibiting the expression of the aforementioned target gene. In the present invention, the term "treatment" encompasses prevention of the aforementioned diseases; improvement of the diseases; and improvement in prognosis, for example, and it can mean any of them. The aforementioned disease is not particularly limited and, for example, the aforementioned sequence that suppresses expression can be set appropriately according to the object disease. Examples of the aforementioned disease include cancer such as breast cancer, lung cancer, stomach cancer, colorectal cancer, liver cancer, pancreatic cancer, esophageal cancer, prostate cancer, gallbladder cancer, uterine body cancer, uterus cervix cancer, ovarian cancer, osteosarcoma, leukemia and the like, and diseases such as lung fibrosis, hepatic fibrosis and the like.

[0113]

The method of using the artificial match-type miRNA of the present invention is not particularly limited. For example, the aforementioned artificial match-type miRNA may be administered to a subject having the aforementioned target gene.

[0114]

Examples of the aforementioned subject include cells, tissues and organs. Examples of the aforementioned subject also include humans, nonhuman animals such as nonhuman mammals excluding humans. The aforementioned administration may be performed, for example, in vivo or in vitro. The aforementioned cells are not particularly limited, and examples thereof include: various cultured cells such as HeLa cells, 293 cells, NIH3T3 cells, COS cells and the like; stem cells such as ES cells, hematopoietic stem cells and the like; and cells isolated from living organisms, such as primary cultured cells and the like.

[0115]

In the present invention, the aforementioned target gene whose expression is to be inhibited is not particularly limited, and any desired gene can be set to the target gene. As

mentioned above, the aforementioned mature miRNA can be selected according to the kind of the aforementioned target gene.

[0116]

As to the use of the artificial match-type miRNA of the present invention, the following description regarding the composition, the expression inhibitory method, the treatment method, and the like according to the present invention to be describe below can be referred to.

[0117]

Since the artificial match-type miRNA of the present invention can inhibit the expression of a target gene as described above, for example, it is useful as a pharmaceutical product, a diagnostic agent, an agricultural chemical, and a tool for conducting research on agriculture, medical science, life science, and the like.

[0118]

The method for synthesizing the artificial match-type miRNA of the present invention is not particularly limited, and a conventionally known production method of nucleic acid can be employed. Examples of the aforementioned synthesis method include synthesis methods according to genetic engineering procedures, chemical synthesis methods and the like. Examples of the genetic engineering procedures include: synthesis methods utilizing in vitro transcription; methods using a vector; methods carried out using a PCR cassette and the like. The aforementioned vector is not particularly limited, and examples thereof include non-virus vectors such as plasmid and the like, and virus vectors and the like. The aforementioned chemical synthesis methods are not particularly limited, and examples thereof include a phosphoramidite method, an H-phosphonate method and the like. The aforementioned chemical synthesis methods can be carried out, for example, using a commercially available automated nucleic acid synthesizer. In the aforementioned chemical synthesis methods, an amidite is

generally used. The aforementioned amidite is not particularly limited. Examples of commercially available amidites include RNA Phosphoramidites (2'-O-TBDMSi, trade name, Samchully Pharm. Co., Ltd.), ACE amidite, TOM amidite, CEE amidite, CEM amidite, TEM amidite and the like.

[0119]

(2) composition

The expression inhibitory composition according to the present invention is, as described above, a composition for inhibiting the expression of a target gene, characteristically containing the aforementioned artificial match-type miRNA of the present invention. The composition of the present invention is characterized in that it contains the aforementioned artificial match-type miRNA of the present invention, and other configurations are by no means limited. The expression inhibitory composition of the present invention can also be referred to, for example, as an expression inhibitory reagent.

[0120]

According to the present invention, for example, by administering to a subject in which the aforementioned target gene is present, it is possible to inhibit the expression of the aforementioned target gene.

[0121]

Furthermore, as described above, the pharmaceutical composition according to the present invention characteristically contains the aforementioned artificial match-type miRNA of the present invention. The composition of the present invention is characterized in that it contains the aforementioned artificial match-type miRNA of the present invention, and other configurations are by no means limited. The pharmaceutical composition of the present invention can also be referred to, for example, as a pharmaceutical product.

[0122]

According to the present invention, for example,

administration to a patient with a disease caused by a gene can inhibit the expression of the aforementioned gene, thereby treating the aforementioned disease. In the present invention, the term "treatment" encompasses, as mentioned above, prevention of the aforementioned diseases; improvement of the diseases; and improvement in prognosis, for example, and it can mean any of them.

[0123]

In the present invention, a disease to be treated is not particularly limited, and examples thereof include diseases caused by the expression of genes. Depending on the kind of the aforementioned disease, a gene that causes the disease may be set as the aforementioned target gene, and further, depending on the aforementioned target gene, the aforementioned guide strand sequence of the aforementioned mature miRNA may be selected.

[0124]

The method of using the expression inhibitory composition and the pharmaceutical composition according to the present invention (hereinafter, both the compositions simply are referred to as "the compositions") are not particularly limited, and examples thereof include administering the aforementioned artificial match-type miRNA to a subject having the aforementioned target gene.

[0125]

Examples of the aforementioned subject include cells, tissues, and organs. Examples of the aforementioned subject also include humans, nonhuman animals such as nonhuman mammals excluding humans. The aforementioned administration may be performed, for example, in vivo or in vitro. The aforementioned cells are not particularly limited, and examples thereof include: various cultured cells such as HeLa cells, 293 cells, NIH3T3 cells, COS cells and the like; stem cells such as ES cells, hematopoietic stem cells and the like; and cells isolated from living organisms, such as primary cultured cells

and the like.

[0126]

The aforementioned administration method is not particularly limited, and can be determined, for example, as appropriate depending on the subject. When the aforementioned subject is a cultured cell, the administration method may be, for example, a method using a transfection reagent, an electroporation method, or the like.

[0127]

For example, each of the compositions of the present invention may contain only the artificial match-type miRNA of the present invention or further may contain an additive(s) in addition to the artificial match-type miRNA. The aforementioned additive is not particularly limited, and is preferably, for example, a pharmaceutically acceptable additive. The kind of the aforementioned additive is not particularly limited, and can be selected as appropriate depending on, for example, the kind of the subject.

[0128]

In the composition of the present invention, for example, the aforementioned artificial match-type miRNA may form a complex with the aforementioned additive. The aforementioned additive can also be referred to, for example, as a complexing agent. The aforementioned complex formation allows, for example, the aforementioned artificial match-type miRNA to be delivered efficiently.

[0129]

The aforementioned complexing agent is not particularly limited, and examples thereof include polymers, cyclodextrins, adamantine and the like. Examples of the aforementioned cyclodextrins include linear cyclodextrin copolymers, linear oxidized cyclodextrin copolymers and the like.

[0130]

Other examples of the aforementioned additive include a carrier, a binding substance that binds to a target cell, a

condensing agent, a fusogenic agent, an excipient and the like.

[0131]

(3) Expression inhibitory method

The expression inhibitory method according to the present invention is, as described above, a method for inhibiting the expression of a target gene, in which the aforementioned artificial match-type miRNA of the present invention is characteristically used. The expression inhibitory method of the present invention is characterized in that the aforementioned artificial match-type miRNA of the present invention is used therein, and other steps and conditions are by no means limited.

[0132]

In the expression inhibitory method of the present invention, the mechanism by which the aforementioned target gene expression is inhibited is not particularly limited, and examples thereof include inhibition of the expression by mature miRNA.

[0133]

The expression inhibitory method of the present invention includes, for example, the step of administering the aforementioned artificial match-type miRNA to a subject in which the aforementioned target gene is present. By the aforementioned administration step, for example, the aforementioned artificial match-type miRNA is brought into contact with the aforementioned subject. Examples of the aforementioned subject include cells, tissues, and organs. Examples of the aforementioned subject also include humans, nonhuman animals such as nonhuman mammals excluding humans. The aforementioned administration may be performed, for example, in vivo or in vitro.

[0134]

In the expression inhibitory method of the present invention, for example, the aforementioned artificial match-type miRNA alone may be administered, or the aforementioned

composition of the present invention containing the aforementioned artificial match-type miRNA may be administered. The aforementioned administration method is not particularly limited and, for example, can be selected as appropriate depending on the kind of the subject.

[0135]

(4) Treatment method

As described above, the method for treating a disease according to the present invention includes the step of administering the aforementioned artificial match-type miRNA of the present invention to a patient, and is characterized in that the aforementioned guide strand sequence in the aforementioned artificial match-type miRNA is the guide strand sequence of a mature miRNA that inhibits expression of a gene causing the aforementioned disease. The treatment method of the present invention is characterized by the use of the aforementioned artificial match-type miRNA of the present invention, and other steps and conditions are by no means limited.

[0136]

The aforementioned expression inhibitory method of the present invention also applies to, for example, the treatment method of the present invention. The aforementioned administration method is not particularly limited and may be, for example, any of oral administration and parenteral administration.

[0137]

(5) Use of artificial match-type miRNA

The use according to the present invention is the use of the aforementioned artificial match-type miRNA of the present invention for the aforementioned inhibition of the expression of a target gene.

[0138]

The single strand nucleic acid according to the present invention is a single strand nucleic acid for use in the

treatment of a disease. The aforementioned single strand nucleic acid is the aforementioned artificial match-type miRNA of the present invention, and is characterized in that the aforementioned guide strand sequence in the aforementioned artificial match-type miRNA is the guide strand sequence of a mature miRNA that inhibits expression of a gene causing the aforementioned disease.

[0139]

In the following, the present invention will be described in detail with reference to examples and the like. It is to be noted, however, the present invention is by no means limited thereto.

Examples

[0140]

(Example 1)

The artificial match-type miRNA of the present invention was synthesized based on the guide strand of a mature miR-34a, and suppression of the growth of H1299 cells was confirmed.

[0141]

(1) Synthesis of miRNA

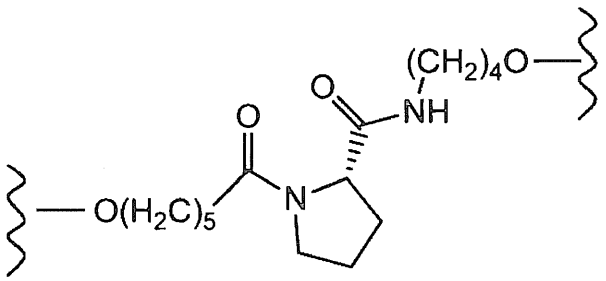
As miRNA of a positive control, human mature miR-34a composed of the guide strand (SEQ ID NO: 1) and the passenger strand (SEQ ID NO: 6) shown below was synthesized. As a negative control, mature miR-34a scramble composed of the guide strand scramble (SEQ ID NO: 7), wherein the base composition of the aforementioned guide strand is scrambled, and the corresponding passenger strand (SEQ ID NO: 8) was synthesized.

[0142]

As the artificial match-type miRNA in the Examples, match-type miR-34a, wherein X region composed of the aforementioned guide strand (SEQ ID NO: 1) and an additional sequence, and Y region composed of a sequence completely complementary to the aforementioned X region and an overhang, are linked via a non-nucleotide structure (shown by [P] in the sequences) of a proline derivative of the following formula,

was synthesized. In the following sequences, the underlined part corresponds to the aforementioned guide strand. The aforementioned non-nucleotide structure in the aforementioned match-type miRNA is shown by the following formula and introduced using L-prolinediamideamidite (see WO 2012/017919) in the aforementioned synthesis of match-type miRNA. In addition, as a negative control to the artificial match-type miRNA, match-type miR-34a scramble composed of the aforementioned guide strand wherein the base composition of the guide strand is scrambled, and a passenger strand corresponding thereto was synthesized.

[0143]



[0144]

The sequences and structures of these miRNAs are shown below. In the following, the sequences shown by underlined parts correspond to the guide strands.

mature miR-34a

guide strand (SEQ ID NO: 1)

5′-UGGCAGUGUCUUAGCUGGUUGU-3′

passenger strand (SEQ ID NO: 6)

5′-CAAUCAGCAAGUAUACUGCCCU-3′

mature miR-34a scramble

guide strand (SEQ ID NO: 7)

5′-UGUAUCGUUAUCGGGUCGGUUG-3′

passenger strand (SEQ ID NO: 8)

5′-CAACCGACCCGAUAACGAUACA-3′

match-type miR-34a(SEQ ID NO: 9)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[P]-

GGAACAACCAGCUAAGACACUGCCAUA-3′

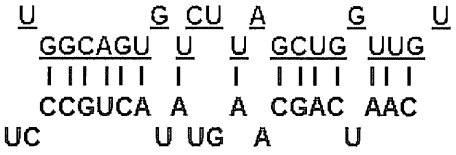
match-type miR-34a scramble (SEQ ID NO: 10)

5′-UGUAUCGUUAUCGGGUCGGUUGUCC-[P]-

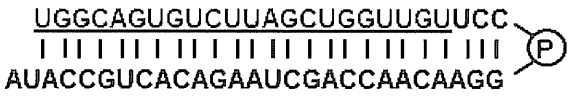
GGACAACCGACCCGAUAACGAUACAUA-3′

[0145]

mature miR-34a



match-type miR-34a



[0146]

(2) Influence of artificial match-type miRNA on cell derived from lung cancer

The aforementioned artificial match-type miRNA was introduced into human non-small cell type lung cancer cell line (NCI-H1299) and an influence on the aforementioned cells was confirmed.

[0147]

(2-1) Transfection

The aforementioned miRNA was dissolved in distilled water for injection (Otsuka Pharmaceutical Co., Ltd., hereinafter the same) to prepare 100 μmol/L miRNA solution. As the medium, RPMI-Medium 1640 (Invitrogen) containing 10% FBS was used. The culture conditions were set to 37°C, 5% CO_2.

[0148]

First, the cells were cultured in the aforementioned medium, and the cultured solution was dispensed to a 24-well plate so that each well contained 500 μL of the cultured solution to achieve a density of 1×10^4 cells/well. The cells in the aforementioned wells were cultured for 24 hours. The cells were transfected with the aforementioned miRNA using a transfection reagent RNAi MAX Transfection Reagent (trade name,

Life Technologies) according to the attached protocol. The transfection was carried out by setting the composition per well as follows. In the following composition, (B) is Opti-MEM (trade name, Life Technologies), (C) is the aforementioned RNA solution, and 49 μL in total of them was added. The final concentration of the aforementioned miRNA in the aforementioned well was set to 100 nmol/L. After the transfection, the cells in the aforementioned well were cultured for 3 days. After the aforementioned culture for 3 days, the cultured cells were confirmed as shown below.

[0149]

Table 1

(composition per well: μL)

cultured solution	450
(A) transfection reagent	1
(B) + (C)	49
	500

[0150]

(2-2) Count of cell number

The cell number per well of cultured cells was counted after culture. The results are shown in Fig. 2. Fig. 2 is a graph showing cell number per well. In Fig. 2, "Normal" shows the results of untreated cells, "Mock" shows cells introduced with a transfection reagent alone, "Scramble" shows miR-34a scramble as a negative control, "miR-34a" shows mature miR-34a as a positive control, "Scramble match" shows match-type miR-34a scramble as a negative control, and "miR-34a match" shows match-type miR-34a in the Examples (hereinafter the same). As shown in Fig. 2, the match-type miR-34a in the Examples could decrease the cell number to the same level as the positive control mature miR-34a.

[0151]

(2-3) MTT assay

After culture, the cultured cells were subjected to MTT assay by using a commercially available reagent kit (trade name

Cell Count Reagent SF, Nacalai Tesque), and cell proliferation was evaluated. Evaluation of cell proliferation was shown in relative values based on the results of Normal (non-treatment) as 1. The results are shown in Fig. 3. Fig. 3 is a graph showing the relative value of cell proliferation. As shown in Fig. 3, the match-type miR-34a in the Examples could decrease the cell number to the same level as the positive control mature miR-34a.

[0152]

(2-4) Apoptosis

After culture, the cultured cells were subjected to the detection of apoptosis by using a commercially available reagent kit (trade name Annexin V:PE Apoptosis Detection Kit, BD Biosciences). The results are shown in Fig. 4. Fig. 4 is a graph showing early stage apoptosis (%) and the latter stage apoptosis (%). As shown in Fig. 4, the match-type miR-34a in the Examples could promote apoptosis to the same level as the positive control mature miR-34a.

[0153]

(2-5) Suppression of expression of mRNA

RNA was recovered from the cultured cells after culture, by using ISOGEN reagent (trade name, NIPPON GENE) according to the attached protocol.

[0154]

Then, using reverse transcriptase (trade name M-MLV reverse transcriptase, Invitrogen) according to the attached protocol, cDNA was synthesized from the aforementioned RNA. Quantitative PCR was carried out using the aforementioned synthesized cDNA as a template, and the amounts of AXL cDNA and MET cDNA were measured. The cDNA amount thereof was also measured using GAPDH cDNA as an internal control.

[0155]

In the aforementioned quantitative PCR, FastStart Universal SYBR Green Master (trade name, Roche) was used as a reagent, MX3000P (trade name, Stratagene) was used as a

thermocycler, and MxPro (trade name, Stratagene) was used as an analysis instrument (hereinafter the same). For amplification of the aforementioned AXL cDNA, the aforementioned MET cDNA and the aforementioned GAPDH cDNA, the following primer sets were used. The total amount of the reaction mixture was 25 μL, and the measurement was performed 3 times for each.

[0156]

AXL primer set

5′-CTCAACCAGGACGACTCCAT-3′ (SEQ ID NO: 11)

5′-AGACCGCTTCACTCAGGAAA-3′ (SEQ ID NO: 12)

MET primer set

5′-CAGGCAGTGCAGCATGTAGT-3′ (SEQ ID NO: 13)

5′-TGTCCAACAAAGTCCCATGA-3′ (SEQ ID NO: 14)

GAPDH primer set

5′-ATGGGGAAGGTGAAGGTCG-3′ (SEQ ID NO: 15)

5′-GGGTCATTGATGGCAACAATATC-3′ (SEQ ID NO: 16)

[0157]

Relative values of AXL mRNA and MET mRNA in each transfected cell, when AXL mRNA or MET mRNA in miRNA non-added control is 1, were calculated. The results thereof are shown in Fig. 5. Fig. 5(A) shows the results of AXL mRNA, and Fig. 5(B) shows the results of MET mRNA.

[0158]

As shown in Fig. 5, the match-type miR-34a in the Examples decreased the amount of AXL mRNA and the amount of MET mRNA to the same level as the positive control mature miR-34a. Therefore, it can be said that the transcription of proteins encoded by AXL mRNA and MET mRNA is suppressed by the aforementioned artificial match-type miRNA.

[0159]

From these results, it was found that the match-type miR-34a in the Examples can suppress expression of AXL mRNA and MET mRNA and the like, and enables suppression of the growth of H1299 cells and promotion of apoptosis.

[0160]

Different from double stranded mature miR-34a, since the aforementioned artificial match-type miRNA is a single strand nucleic acid molecule, it does not require annealing of each single strand when in use and can avoid recognition by TLR3 and the like involved in natural immunity.

[0161]

(Example 2)

In match-type miR-34a of Example 1, the additional sequence of X region and the overhang of Y region were shortened.

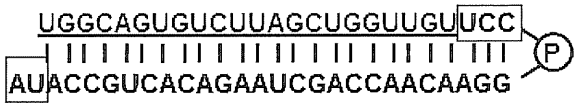
[0162]

(1) Synthesis of miRNA

As shown below, match-type miR-34a has a 3 base-length additional sequence (J) enclosed in rectangle on the 3'-side of the X region, and a 2 base-length overhang (O) enclosed in rectangle on the 5'-side of the Y region. Therefore, a molecule wherein the aforementioned additional sequence was deleted by 1 base from the 3'-side and the sequence corresponding thereto on the Y region side was deleted by 1 base from the 5'-side; a molecule wherein the overhang was deleted by 1 base from the 3'-side; and a molecule wherein the aforementioned additional sequence and the overhang were deleted by 1 base were synthesized, and suppression of the expression of AXL mRNA and MET mRNA was confirmed in the same manner as in the aforementioned Example 1. In the following sequences, the 5'-side region of [P] is X region; in the aforementioned X region, the underlined part is the aforementioned guide strand sequence, the rest is the aforementioned additional sequence, and the 3'-side region of [P] is Y region; and in the aforementioned Y region, the region enclosed in rectangle is the overhang.

[0163]

match-type miR-34a



[0164]

match-type miR-34a

O2J3 (SEQ ID NO: 9)

5' -UGGCAGUGUCUUAGCUGGUUGUUCC- [P] -GGAACAACCAGCUAAGACACUGCCAUA-3'

O2J2 (SEQ ID NO: 17)

5' -UGGCAGUGUCUUAGCUGGUUGUUC- [P] -GAACAACCAGCUAAGACACUGCCAUA-3'

O2J1 (SEQ ID NO: 18)

5' -UGGCAGUGUCUUAGCUGGUUGUU- [P] -AACAACCAGCUAAGACACUGCCAUA-3'

O2J0 (SEQ ID NO: 19)

5' -UGGCAGUGUCUUAGCUGGUUGU- [P] -ACAACCAGCUAAGACACUGCCAUA-3'

O1J3 (SEQ ID NO: 20)

5' -UGGCAGUGUCUUAGCUGGUUGUUCC- [P] -GGAACAACCAGCUAAGACACUGCCAU-3'

O1J2 (SEQ ID NO: 21)

5' -UGGCAGUGUCUUAGCUGGUUGUUC- [P] -GAACAACCAGCUAAGACACUGCCAU-3'

O1J1 (SEQ ID NO: 22)

5' -UGGCAGUGUCUUAGCUGGUUGUU- [P] -AACAACCAGCUAAGACACUGCCAU-3'

O1J0 (SEQ ID NO: 23)

5' -UGGCAGUGUCUUAGCUGGUUGU- [P] -ACAACCAGCUAAGACACUGCCAU-3'

O0J3 (SEQ ID NO: 24)

5' -UGGCAGUGUCUUAGCUGGUUGUUCC- [P] -GGAACAACCAGCUAAGACACUGCCA-3'

O0J2 (SEQ ID NO: 25)

5' -UGGCAGUGUCUUAGCUGGUUGUUC- [P] -GAACAACCAGCUAAGACACUGCCA-3'

O0J1 (SEQ ID NO: 26)

5' -UGGCAGUGUCUUAGCUGGUUGUU- [P] -AACAACCAGCUAAGACACUGCCA-3'

O0J0 (SEQ ID NO: 27)

5' -UGGCAGUGUCUUAGCUGGUUGU- [P] -ACAACCAGCUAAGACACUGCCA-3'

[0165]

The results are shown in Fig. 6 and Fig. 7. Fig. 6 shows the results of AXL mRNA, and Fig. 7 shows the results of MET mRNA. As shown in Fig. 6 and Fig. 7, the expression suppressive effect was maintained even when the additional sequence of the aforementioned X region and the overhang of the

aforementioned Y region were shortened.

[0166]

(Example 3)

In match-type miR-34a, the non-nucleotide structure of the linker was altered and the additional sequence of X region was increased or decreased, and a suppressive effect on the expression of AXL mRNA and MET RNA was examined.

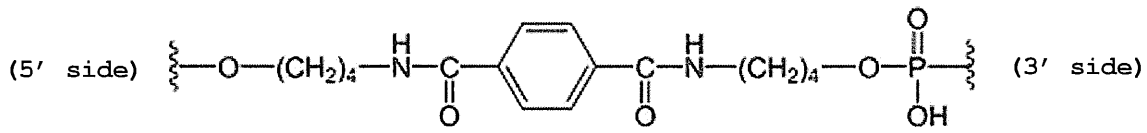
[0167]

(1) Synthesis of miRNA

As shown below, match-type miR-34a(PH-0039), wherein the base sequence of the overhang region is altered from that of match-type miR-34a of Example 1, was synthesized. Furthermore, a molecule wherein the additional sequence of PH-0039 and a sequence corresponding thereto on the Y region side were deleted (PH-0037), and a molecule wherein an additional sequence and a sequence corresponding thereto on the Y region side were extended to 5 bases length (PH-0093) was synthesized.

Also, molecules wherein the linker regions of PH-0037, PH-0039 and PH-0093 are substituted by a non-nucleotide structure (shown by [TP] in sequences) in the terephthalic acid derivative of the following formula were synthesized (XH-0016, XH-0025 and XH-0027, respectively). The non-nucleotide structure was introduced by using terephthalic acid amidite (see WO 2013/133221).

[0168]



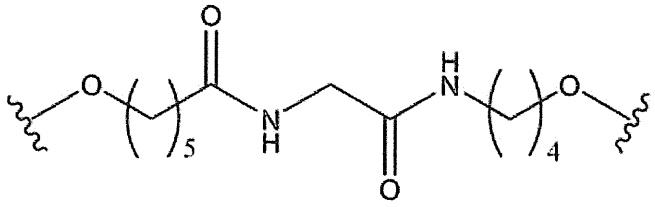
(TPA)

[0169]

Molecules wherein the linker regions of PH-0037 and PH-0039 are substituted by a non-nucleotide structure (shown by [Gly] in sequences) in the glycine derivative of the following

formula (XH-0012 and XH-0028, respectively), and

[0170]

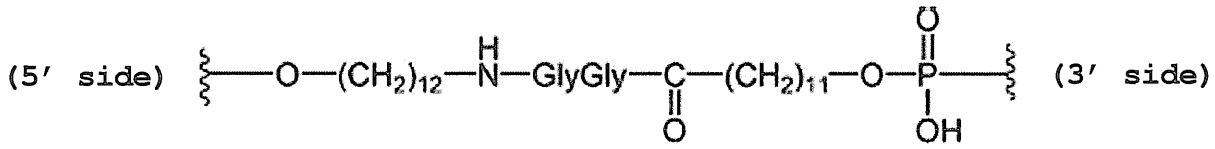


(I－1b)

[0171]

molecules wherein the linker regions of PH-0037 and PH-0039 are substituted by a non-nucleotide structure (shown by [GlyGly] in sequences) in the glycylglycine derivative of the following formula (XH-0014 and XH-0029, respectively).

[0172]



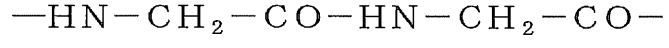
(G2)

[0173]

GlyGly in the aforementioned chemical formula (G2) is an atomic group represented by the following chemical formula (GlyGly), wherein the terminal carbonylcarbon is bonded to N atom in the above-mentioned chemical formula (G2), and the terminal nitrogen atom in the following chemical formula (GlyGly) is bonded to carbonylcarbon in the above-mentioned chemical formula (G2).

[0174]

(GlyGly)

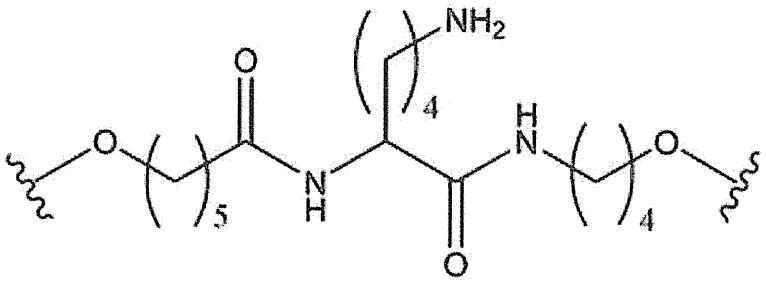


[0175]

Also, molecules wherein the linker regions of PH-0037 and PH-0039 are substituted by a non-nucleotide structure (shown by

[K] in sequences) in the lysine derivative of the following formula (KH-0007 and KH-0011, respectively) were synthesized.

[0176]



(I－7a)

[0177]

The non-nucleotide structure of the aforementioned glycine derivative was introduced by using glycine amideamidite (see WO 2013/103146), the non-nucleotide structure of the aforementioned glycylglycine derivative was introduced by using glycylglycine amideamidite (see WO 2013/133221), and the non-nucleotide structure of the lysine derivative was introduced by using L-lysinamideamidite (see WO 2013/103146).

[0178]

PH-0037 UGGCAGUGUCUUAGCUGGUUGU UCACCGUCACAGAAUCGACCAACA P PH-0039 UGGCAGUGUCUUAGCUGGUUGUUCC UCACCGUCACAGAAUCGACCAACAAGG P PH-0093 UGGCAGUGUCUUAGCUGGUUGUUCCGG UCACCGUCACAGAAUCGACCAACAAGGCC P	XH-0016 UGGCAGUGUCUUAGCUGGUUGU UCACCGUCACAGAAUCGACCAACA TP XH-0025 UGGCAGUGUCUUAGCUGGUUGUUCC UCACCGUCACAGAAUCGACCAACAAGG TP XH-0027 UGGCAGUGUCUUAGCUGGUUGUUCCGG UCACCGUCACAGAAUCGACCAACAAGGCC TP
XH-0012 UGGCAGUGUCUUAGCUGGUUGU UCACCGUCACAGAAUCGACCAACA Gly	XH-0028 UGGCAGUGUCUUAGCUGGUUGUUCC UCACCGUCACAGAAUCGACCAACAAGG Gly
XH-0014 UGGCAGUGUCUUAGCUGGUUGU UCACCGUCACAGAAUCGACCAACA GlyGly	XH-0029 UGGCAGUGUCUUAGCUGGUUGUUCC UCACCGUCACAGAAUCGACCAACAAGG GlyGly
KH-0007 UGGCAGUGUCUUAGCUGGUUGU UCACCGUCACAGAAUCGACCAACA K	KH-0011 UGGCAGUGUCUUAGCUGGUUGUUCC UCACCGUCACAGAAUCGACCAACAAGG K

[0179]

In the following sequences, the 5′-side region of each linker is X region; in the aforementioned X region, the underlined part is the aforementioned guide strand sequence, the rest is the aforementioned additional sequence, and the 3′-side region of each linker is Y region.

PH-0037 (SEQ ID NO: 28)

5′-UGGCAGUGUCUUAGCUGGUUGU-[P]-ACAACCAGCUAAGACACUGCCACU-3′

PH-0039 (SEQ ID NO: 29)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[P]-

GGAACAACCAGCUAAGACACUGCCACU-3′

PH-0093 (SEQ ID NO: 30)

5′-UGGCAGUGUCUUAGCUGGUUGUUCCGG-[P]-

CCGGAACAACCAGCUAAGACACUGCCACU-3′

XH-0016 (SEQ ID NO: 28)

5′-UGGCAGUGUCUUAGCUGGUUGU-[TP]-ACAACCAGCUAAGACACUGCCACU-

3′

XH-0025 (SEQ ID NO: 29)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[TP]-

GGAACAACCAGCUAAGACACUGCCACU-3′

XH-0027 (SEQ ID NO: 30)

5′-UGGCAGUGUCUUAGCUGGUUGUUCCGG-[TP]-

CCGGAACAACCAGCUAAGACACUGCCACU-3′

XH-0012 (SEQ ID NO: 28)

5′-UGGCAGUGUCUUAGCUGGUUGU-[Gly]-ACAACCAGCUAAGACACUGCCACU-

3′

XH-0028 (SEQ ID NO: 29)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[Gly]-

GGAACAACCAGCUAAGACACUGCCACU-3′

XH-0014 (SEQ ID NO: 28)

5′-UGGCAGUGUCUUAGCUGGUUGU-[GlyGly]-

ACAACCAGCUAAGACACUGCCACU-3′

XH-0029 (SEQ ID NO: 29)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[GlyGly]-

GGAACAACCAGCUAAGACACUGCCACU-3′

KH-0007(SEQ ID NO: 28)

5′-UGGCAGUGUCUUAGCUGGUUGU-[K]-ACAACCAGCUAAGACACUGCCACU-3′

KH-0011 (SEQ ID NO: 29)

5′-UGGCAGUGUCUUAGCUGGUUGUUCC-[K]-

GGAACAACCAGCUAAGACACUGCCACU-3′

As a negative control, match-type miRNA (PH-0000) comprising a guide strand composed of a sequence free of complementarity to all sequences recorded on nucleic acid

databases and a passenger strand corresponding thereto was synthesized.

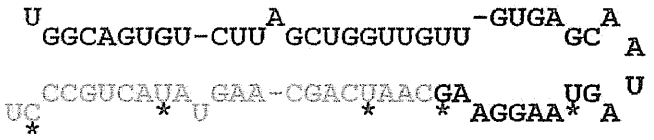
PH-0000 (SEQ ID NO: 31)

5′-UACUAUUCGACACGCGAAGUUCC-[P]-GGAACUUCGCGUGUCGAAUAGUAUU-3′

As a positive control, a molecule wherein the guide strand of a mature miR-34a and a passenger strand are linked via a loop region of natural type pre-miRNA (NM-0004) and a double stranded match-type RNA wherein the guide strand of a mature miRNA and a sequence completely complementary thereto are annealed (NI-0209) were synthesized.

[0180]

NM-0004 (64 mer)



NI-0209 (22/22 mer)



[0181]

NM-0004 (SEQ ID NO: 32)

5′-UGGCAGUGUCUUAGCUGGUUGUUGUGAGCAAUAGUAAGGAAGCAAUCAGCAAGUAUACUGCCCU-3′

NI-0209

guide strand (SEQ ID NO: 1)/ passenger strand (SEQ ID NO: 33)

5′-UGGCAGUGUCUUAGCUGGUUGU-3′/5′-AACCAGCUAAGACACUGCCACU-3′

[0182]

(2) Measurement of expression level of AXL gene

Each of the aforementioned RNAs was dissolved in distilled water for injection (Otsuka Pharmaceutical Co., Ltd.)

at 4 μmol/L, whereby an RNA solution was prepared.

[0183]

H1299 cells (ATCC) were used as the cell. As the medium, RPMI Medium 1640 (Life Technologies) containing 10% FBS was used. The culture conditions were set to 37°C, 5% CO_2.

[0184]

First, the cells were cultured in the aforementioned medium, and the cultured solution was dispensed to a 24-well plate so that each well contained 400 μL of the cultured solution to achieve a density of 4×10^4 cells/well. The cells were transfected with the aforementioned RNA using a transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the protocol attached to the aforementioned transfection reagent. Specifically, the transfection was carried out by setting the composition per well as follows. In the following composition, (B) is Opti-MEM (Life Technologies), (C) is the aforementioned 0.4 μmol/L and 2 μmol/L RNA solution, 98.5 μL in total of them was added. The final concentration of the aforementioned RNA in the aforementioned well was set to 2 nmol/L.

[0185]

Table 2

(composition per well: μL)

cultured solution	450
(A) transfection reagent	1
(B) + (C)	49
	500

[0186]

After the transfection, the cells in the aforementioned wells were cultured for 24 hours, and then, the RNA was collected using an RNeasy Mini Kit (Qiagen, Netherlands) according to the protocol supplied therewith. Subsequently, cDNA was synthesized from the aforementioned RNA by using Transcriptor First Strand cDNA Synthesis Kit (Roche) according

to the protocol supplied therewith. Then, as shown below, PCR was carried out using the aforementioned synthesized cDNA as a template, and the expression levels of the AXL and MET genes and that of GAPDH gene as an internal standard were measured. The aforementioned expression levels of the AXL and MET genes were normalized with reference to that of the GAPDH gene mentioned above.

[0187]

The aforementioned PCR was carried out using LightCycler 480 SYBR Green I Master (trade name, Roche) as a reagent and LightCycler 480 Instrument II (trade name, Roche) as an instrument (hereinafter the same). The aforementioned AXL, MET and GAPDH genes were amplified using the following primer sets, respectively.

PCR primer set for AXL gene

(SEQ ID NO: 11) 5′-CTCAACCAGGACGACTCCAT-3′

(SEQ ID NO: 12) 5′-AGACCGCTTCACTCAGGAAA-3′

PCR primer set for MET gene

(SEQ ID NO: 13) 5′-CAGGCAGTGCAGCATGTAGT-3′

(SEQ ID NO: 14) 5′-TGTCCAACAAAGTCCCATGA-3′

primer set for GAPDH gene

(SEQ ID NO: 15) 5′-ATGGGGAAGGTGAAGGTCG-3′

(SEQ ID NO: 16) 5′-GGGTCATTGATGGCAACAATATC-3′

[0188]

As control 1, regarding the cells to which 100 μL of the aforementioned solution (B) alone had been added to the aforementioned cultured solution, the expression levels of the genes also were measured (-). Furthermore, as control 2, regarding the cells subjected to the same transfection procedures as in the above except that the aforementioned RNA solution was not added and that the aforementioned (B) and 1.5 μL of the aforementioned (A) were added so that the total amount of (A) and (B) would be 100 μL, the expression level of the gene also was measured (mock).

[0189]

As for the expression levels of normalized AXL and MET genes, the relative value of the expression level in the cell introduced with each RNA was determined based on the expression level in the cells of the control (mock) as 1.

[0190]

(3) Results

As shown in Figs. 6 and 7, the expression suppressive effect of AXL mRNA and MET RNA was maintained even when the non-nucleotide structure of the linker region was altered or the additional sequence of X region was deleted or extended.

[0191]

(Example 4)

Various artificial match-type miRNAs of the present invention were synthesized based on the guide strand of mature let-7a, and a suppressive effect on the expression of the target gene HMGA2 mRNA was examined.

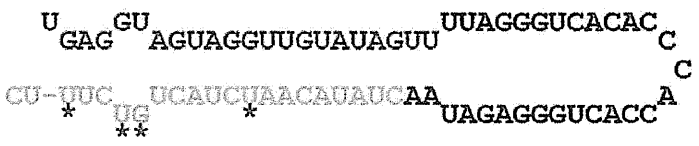
[0192]

(1) Synthesis of miRNA

As a positive control, a molecule wherein the guide strand (SEQ ID NO: 2) of mature let-7a and a passenger strand (SEQ ID NO: 34) are linked via a loop region of natural type pre-let-7a (NM-0003) and a double stranded match-type RNA wherein the guide strand of mature let-7a and a sequence completely complementary thereto are annealed (NI-0207) were synthesized.

[0193]

NM-0003 (72 mer)



NI-0207 (22/22 mer)



[0194]

In the following sequences, the underlined part shows the aforementioned guide strand sequence.

NM-0003 (SEQ ID NO: 35)

5′-

UGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACACCCACCACUGGGAGAUAACUAUACAAUCUA CUGUCUUUC-3′

NI-0207

guide strand (SEQ ID NO: 2)/ passenger strand (SEQ ID NO: 34)

5′-UGAGGUAGUAGGUUGUAUAGUU-3′/5′-CUAUACAACCUACUACCUCAUC-3′

[0195]

As shown below, various artificial match-type let-7a wherein linkers of proline derivative ([P]), terephthalic acid derivative ([TP]), glycine derivative ([Gly]), glycylglycine derivative ([GlyGly]) and lysine derivative ([K]) are introduced between X region comprising the guide strand sequence of mature let-7a and an additional sequence (0, 3 or 5 base length) on the 3′-side thereof, and Y region which is completely complementary to the X region and having a 2 base-length overhang on the 5′-side, as in Example 3, were synthesized.

[0196]

PH-0013
UGAGGUAGUAGGUUGUAUAGUU P
CUACUCCAUCAUCCAACAUAUCAA

PH-0015
UGAGGUAGUAGGUUGUAUAGUUUCC P
CUACUCCAUCAUCCAACAUAUCAAAGG

PH-0094
UGAGGUAGUAGGUUGUAUAGUUUCCGG P
CUACUCCAUCAUCCAACAUAUCAAAGGCC

XH-0010
UGAGGUAGUAGGUUGUAUAGUU TP
CUACUCCAUCAUCCAACAUAUCAA

XH-0030
UGAGGUAGUAGGUUGUAUAGUUUCC TP
CUACUCCAUCAUCCAACAUAUCAAAGG

XH-0031
UGAGGUAGUAGGUUGUAUAGUUUCCGG TP
CUACUCCAUCAUCCAACAUAUCAAAGGCC

XH-0008
UGAGGUAGUAGGUUGUAUAGUU Gly
CUACUCCAUCAUCCAACAUAUCAA

XH-0032
UGAGGUAGUAGGUUGUAUAGUUUCC Gly
CUACUCCAUCAUCCAACAUAUCAAAGG

XH-0009
UGAGGUAGUAGGUUGUAUAGUU GlyGly
CUACUCCAUCAUCCAACAUAUCAA

XH-0033
UGAGGUAGUAGGUUGUAUAGUUUCC GlyGly
CUACUCCAUCAUCCAACAUAUCAAAGG

KH-0005
UGAGGUAGUAGGUUGUAUAGUU K
CUACUCCAUCAUCCAACAUAUCAA

KH-0012
UGAGGUAGUAGGUUGUAUAGUUUCC K
CUACUCCAUCAUCCAACAUAUCAAAGG

[0197] In the following sequences, the 5′-side region of each linker is X region; in the aforementioned X region, the underlined part is the aforementioned guide strand sequence, the rest is the aforementioned additional sequence, and the 3′-side region of each linker is Y region.

PH-0013 (SEQ ID NO: 36)

5′-UGAGGUAGUAGGUUGUAUAGUU-[P]-AACUAUACAACCUACUACCUCAUC-3′

PH-0015 (SEQ ID NO: 37)

5′-UGAGGUAGUAGGUUGUAUAGUUUCC-[P]-

GGAAACUAUACAACCUACUACCUCAUC-3′

PH-0094 (SEQ ID NO: 38)

5′-UGAGGUAGUAGGUUGUAUAGUUUCCGG-[P]-

CCGGAAACUAUACAACCUACUACCUCAUC-3′

XH-0010 (SEQ ID NO: 36)

5′-UGAGGUAGUAGGUUGUAUAGUU-[TP]-AACUAUACAACCUACUACCUCAUC-3′

XH-0030 (SEQ ID NO: 37)

5′-UGAGGUAGUAGGUUGUAUAGUUUCC-[TP]-

GGAAACUAUACAACCUACUACCUCAUC-3′

XH-0031 (SEQ ID NO: 38)

5′-UGAGGUAGUAGGUUGUAUAGUUUCCGG-[TP]-

CCGGAAACUAUACAACCUACUACCUCAUC-3′

XH-0008 (SEQ ID NO: 36)

5′-UGAGGUAGUAGGUUGUAUAGUU-[Gly]-AACUAUACAACCUACUACCUCAUC-3′

XH-0032 (SEQ ID NO: 37)

5′-UGAGGUAGUAGGUUGUAUAGUUUCC-[Gly]-

GGAAACUAUACAACCUACUACCUCAUC-3′

XH-0009 (SEQ ID NO: 36)

5′-UGAGGUAGUAGGUUGUAUAGUU-[GlyGly]-

AACUAUACAACCUACUACCUCAUC-3′

XH-0033(SEQ ID NO: 37)

5′-UGAGGUAGUAGGUUGUAUAGUUUCC-[GlyGly]-

GGAAACUAUACAACCUACUACCUCAUC-3′

KH-0005 (SEQ ID NO: 36)

5′-UGAGGUAGUAGGUUGUAUAGUU-[K]-AACUAUACAACCUACUACCUCAUC-3′

KH-0012 (SEQ ID NO: 37)

5′-UGAGGUAGUAGGUUGUAUAGUUUCC-[K]-

GGAAACUAUACAACCUACUACCUCAUC-3′

As a negative control, PH-0000 synthesized in Example 3 was used.

[0198]

(2) Measurement of expression level of HMGA2 gene

Each of the aforementioned RNAs was dissolved in distilled water for injection (Otsuka Pharmaceutical Co., Ltd.) at 0.4 μmol/L, whereby an RNA solution was prepared.

[0199]

A549 cells (DS Pharma Biomedical Co., Ltd.) were used as the cells. A 10% FBS-containing DMEM (Life Technologies) was used as the medium. The culture conditions were set to 37°C and 5% CO_2.

[0200]

First, the cells were cultured in the aforementioned medium, and the cultured solution was dispensed to a 24-well plate so that each well contained 400 μL of the cultured solution to achieve a density of 4×10^4 cells/well. The cells were transfected with the aforementioned RNA using a transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the protocol attached to the aforementioned transfection reagent. Specifically, the transfection was carried out by setting the composition per well as follows. In the following composition, (B) is Opti-MEM (Life Technologies), and (C) is 0.1 μmol/L or 0.2 μmol/L aforementioned RNA solution and 98.5 μL in total of them was added. The final concentration of the aforementioned RNA in the aforementioned well was set to 0.2 nmol/L.

[0201]

Table 3

(composition per well: μL)

cultured solution	400
transfection reagent	1.5
(B) + (C)	98.5
total	500

[0202]

After the transfection, the cells in the aforementioned

wells were cultured for 24 hours, and then, the RNA was collected using an RNeasy Mini Kit (Qiagen, Netherlands) according to the protocol supplied therewith. Subsequently, cDNA was synthesized from the aforementioned RNA by using Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the protocol supplied therewith. Then, as shown below, PCR was carried out using the aforementioned synthesized cDNA as a template, and the expression level of the HMGA2 gene and that of GAPDH gene as an internal standard were measured. The aforementioned expression level of the HMGA2 gene was normalized with reference to that of the GAPDH gene mentioned above.

[0203]

The aforementioned PCR was carried out using LightCycler 480 SYBR Green I Master (trade name, Roche) as a reagent and LightCycler 480 Instrument II (trade name, Roche) as an instrument (hereinafter the same). The aforementioned HMGA2 and GAPDH genes were amplified using the following primer sets, respectively.

PCR primer set for HMGA2 gene

(SEQ ID NO: 39) 5′-GAAGCCACTGGAGAAAAACG-3′

(SEQ ID NO: 40) 5′-CTTCGGCAGACTCTTGTGAG-3′

primer set for GAPDH gene

(SEQ ID NO: 15) 5′-ATGGGGAAGGTGAAGGTCG-3′

(SEQ ID NO: 16) 5′-GGGTCATTGATGGCAACAATATC-3′

[0204]

As control 1, regarding the cells to which 100 μL of the aforementioned solution (B) alone had been added to the aforementioned cultured solution, the expression levels of the genes also were measured (-). Furthermore, as control 2, regarding the cells subjected to the same transfection procedures as in the above except that the aforementioned RNA solution was not added and that the aforementioned (B) and 1.5 μL of the aforementioned (A) were added so that the total amount of (A) and (B) would be 100 μL, the expression level of

the gene also was measured (mock).

[0205]

As for the expression level of normalized HMGA2 gene, the relative value of the expression level in the cell introduced with each RNA was determined based on the expression level in the cells of the control (mock) as 1.

[0206]

(3) Results

As shown in Fig. 10, match-type let-7a of the Example suppressed expression of HMGA2 mRNA at the same level as or not less than that by the positive control mature let-7a and double stranded match-type let-7a. In addition, the expression suppressive effect of HMGA2 mRNA was maintained even when the non-nucleotide structure of the linker region or the base length of the additional sequence of X region was altered.

[0207]

(Example 5)

Various artificial match-type miRNAs of the present invention were synthesized based on the guide strand of a mature miR-29b, and suppression effect on the expression of the target gene COLA1 mRNA was examined.

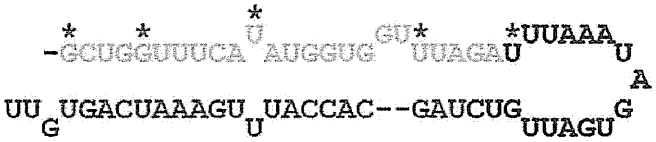
[0208]

(1) Synthesis of miRNA

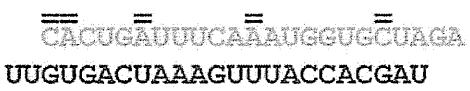
As a positive control, a molecule wherein the guide strand (SEQ ID NO: 5) of a mature miR-29b and a passenger strand (SEQ ID NO: 41) are linked via a loop region of natural type pre-miR-29b (NM-0005) and a double stranded match-type RNA wherein the guide strand of a mature miR-29b and a sequence completely complementary thereto are annealed (NI-0211) were synthesized.

[0209]

NM-0005 (64 mer)



NI-0211 (23/23 mer)



[0210]

In the following sequences, the underlined parts show the aforementioned guide strand sequences.

NM-0005 (SEQ ID NO: 42)

5′-

GCUGGUUUCAUAUGGUGGUUUAGAUUUAAAUAGUGAUUGUCUAGCACCAUUUGAAAUCAGUGUU-3′

NI-0211

passenger strand (SEQ ID NO: 41)/ guide strand (SEQ ID NO: 5)

5′-CACUGAUUUCAAAUGGUGCUAGA-3′/5′-UAGCACCAUUUGAAAUCAGUGUU-3′

[0211]

As shown below, various artificial match-type miR-29bs wherein linkers of proline derivative ([P]), terephthalic acid derivative ([TP]), glycine derivative ([Gly]), glycylglycine derivative ([GlyGly]) and lysine derivative ([K]) are introduced between X region comprising the guide strand sequence of a mature miR-29b and an additional sequence (0, 3 or 5 base length) on the 3′-side thereof, and Y region which is completely complementary to the X region and having a 2 base-length overhang on the 5′-side, as in Example 3, were synthesized.

[0212]

PH-0071 P AACACUGAUUUCAAAUGGUGCUAGA UUGUGACUAAAGUUUACCACGAU	XH-0034 TP AACACUGAUUUCAAAUGGUGCUAGA UUGUGACUAAAGUUUACCACGAU
PH-0073 P GGAAACACUGAUUUCAAAUGGUGCUAGA CCUUUGUGACUAAAGUUUACCACGAU	XH-0035 TP GGAAACACUGAUUUCAAAUGGUGCUAGA CCUUUGUGACUAAAGUUUACCACGAU
PH-0095 P CCGGAAACACUGAUUUCAAAUGGUGCUAGA GGCCUUUGUGACUAAAGUUUACCACGAU	XH-0036 TP CCGGAAACACUGAUUUCAAAUGGUGCUAGA GGCCUUUGUGACUAAAGUUUACCACGAU
XH-0037 Gly AACACUGAUUUCAAAUGGUGCUAGA UUGUGACUAAAGUUUACCACGAU	XH-0038 Gly GGAAACACUGAUUUCAAAUGGUGCUAGA CCUUUGUGACUAAAGUUUACCACGAU
XH-0039 GlyGly AACACUGAUUUCAAAUGGUGCUAGA UUGUGACUAAAGUUUACCACGAU	XH-0040 GlyGly GGAAACACUGAUUUCAAAUGGUGCUAGA CCUUUGUGACUAAAGUUUACCACGAU
KH-0013 K AACACUGAUUUCAAAUGGUGCUAGA UUGUGACUAAAGUUUACCACGAU	KH-0014 K GGAAACACUGAUUUCAAAUGGUGCUAGA CCUUUGUGACUAAAGUUUACCACGAU

[0213]

In the following sequences, the 5′-side region of each linker is X region; in the aforementioned X region, the underlined part is the aforementioned guide strand sequence, the rest is the aforementioned additional sequence, and the 3′-side region of each linker is Y region.

PH-0071 (SEQ ID NO: 43)

5′-UAGCACCAUUUGAAAUCAGUGUU-[P]-AACACUGAUUUCAAAUGGUGCUAGA-3′

PH-0073 (SEQ ID NO: 44)

5′-UAGCACCAUUUGAAAUCAGUGUUUCC-[P]-

GGAAACACUGAUUUCAAAUGGUGCUAGA-3′

PH-0095 (SEQ ID NO: 45)

5′-UAGCACCAUUUGAAAUCAGUGUUUCCGG-[P]-

CCGGAAACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0034 (SEQ ID NO: 43)

5′-UAGCACCAUUUGAAAUCAGUGUU-[TP]-

AACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0035 (SEQ ID NO: 44)

5′-UAGCACCAUUUGAAAUCAGUGUUUCC-[TP]-

GGAAACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0036 (SEQ ID NO: 45)

5′-UAGCACCAUUUGAAAUCAGUGUUUCCGG-[TP]-

CCGGAAACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0037(SEQ ID NO: 43)

5′-UAGCACCAUUUGAAAUCAGUGUU-[Gly]-

AACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0038 (SEQ ID NO: 44)

5′-UAGCACCAUUUGAAAUCAGUGUUUCC-[Gly]-

GGAAACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0039 (SEQ ID NO: 43)

5′-UAGCACCAUUUGAAAUCAGUGUU-[GlyGly]-

AACACUGAUUUCAAAUGGUGCUAGA-3′

XH-0040 (SEQ ID NO: 44)

5′-UAGCACCAUUUGAAAUCAGUGUUUCC-[GlyGly]-

GGAAACACUGAUUUCAAAUGGUGCUAGA-3′

KH-0013 (SEQ ID NO: 43)

5′-UAGCACCAUUUGAAAUCAGUGUU-[K]-AACACUGAUUUCAAAUGGUGCUAGA-

3′

KH-0014 (SEQ ID NO: 44)

5′-UAGCACCAUUUGAAAUCAGUGUUUCC-[K]-

GGAAACACUGAUUUCAAAUGGUGCUAGA-3′

As a negative control, PH-0000 synthesized in Example 3 was used.

[0214]

(2) Measurement of expression level of COL1A1 gene

Each of the aforementioned RNAs was dissolved in distilled water for injection (Otsuka Pharmaceutical Co., Ltd.) at 1 μmol/L, whereby an RNA solution was prepared.

[0215]

A549 cells (DS PHARMA BIOMEDICAL) were used as the cell. As the medium, DMEM (Life Technologies) containing 10% FBS was used. The culture conditions were set to 37°C, 5% CO_2.

[0216]

First, the cells were cultured in the aforementioned medium, and the cultured solution was dispensed to a 24-well plate so that each well contained 400 μL of the cultured solution to achieve a density of 4×10^4 cells/well. The cells were transfected with the aforementioned RNA using a transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the protocol attached to the aforementioned transfection reagent. Specifically, the transfection was carried out by setting the composition per well as follows. In the following composition, (B) is Opti-MEM (Life Technologies), (C) is the aforementioned 0.4 μmol/L and 2 μmol/L RNA solution, 98.5 μL in total of them was added. The final concentration of the aforementioned RNA in the aforementioned well was set to 0.5 nmol/L.

[0217]

[Table 4]

(composition per well: μL)

cultured solution	400
transfection reagent	1.5
(B) + (C)	98.5
total	500

[0218]

After the transfection, the cells in the aforementioned wells were cultured for 24 hours, and then, the RNA was

collected using an RNeasy Mini Kit (Qiagen, Netherlands) according to the protocol supplied therewith. Subsequently, cDNA was synthesized from the aforementioned RNA by using Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the protocol supplied therewith. Then, as shown below, PCR was carried out using the aforementioned synthesized cDNA as a template, and the expression level of the COL1A1 gene and that of GAPDH gene as an internal standard were measured. The aforementioned expression level of the COL1A1 gene was normalized with reference to that of the GAPDH gene mentioned above.

[0219]

The aforementioned PCR was carried out using LightCycler 480 SYBR Green I Master (trade name, Roche) as a reagent and LightCycler 480 Instrument II (trade name, Roche) as an instrument (hereinafter the same). The aforementioned COL1A1 and GAPDH genes were amplified using the following primer sets, respectively.

PCR primer set for COL1A1 gene

(SEQ ID NO: 46) 5′-CCCAAGGACAAGAGGCATGT-3′

(SEQ ID NO: 47) 5′-CCGCCATACTCGAACTGGAA-3′

primer set for GAPDH gene

(SEQ ID NO: 15) 5′-ATGGGGAAGGTGAAGGTCG-3′

(SEQ ID NO: 16) 5′-GGGTCATTGATGGCAACAATATC-3′

[0220]

As control 1, regarding the cells to which 100 μL of the aforementioned solution (B) alone had been added to the aforementioned cultured solution, the expression levels of the genes also were measured (-). Furthermore, as control 2, regarding the cells subjected to the same transfection procedures as in the above except that the aforementioned RNA solution was not added and that the aforementioned (B) and 1.5 μL of the aforementioned (A) were added so that the total amount of (A) and (B) would be 100 μL, the expression level of the gene also was measured (mock).

[0221]

As for the expression level of normalized COL1A1 gene, the relative value in the cell introduced with each RNA was determined based on the expression level in the cells of the control (mock) as 1.

[0222]

(3) Results

As shown in Fig. 11, match-type miR-29b of the Example suppressed expression of COLA1 mRNA at the same level as or not less than that by the positive control mature miR-29b and double stranded match-type miR-29b. In addition, the expression suppressive effect of COLA1 mRNA was maintained even when the non-nucleotide structure of the linker region or the base length of the additional sequence of X region was altered.

[0223]

While the present invention has been described above with reference to illustrative embodiments, the present invention is by no means limited thereto. Various changes that may become apparent to those skilled in the art may be made in the configuration and specifics of the present invention without departing from the scope of the present invention. In addition, the contents disclosed in any publication cited herein, including patents and patent applications, are hereby incorporated in their entireties by reference, to the extent that they have been disclosed herein.

[0224]

This application is based on a patent application No. 2013-273033 filed in Japan (filing date: December 27, 2013), the contents of which are incorporated in full herein.

Industrial Applicability

[0225]

Since the artificial match-type miRNA of the present invention can be easily synthesized at a low cost, and can suppress the translation of a protein encoded by the aforementioned gene. Therefore, an artificial match-type miRNA

of the present invention is useful as, for example, a pharmaceutical product, a diagnostic agent, an agricultural chemical, and a tool for conducting research on agriculture, medical science, life science, and the like.

Claims

1. An artificial match-type miRNA, which is a single strand nucleic acid comprising X region and Y region,
wherein the 3'-terminal of said X region and the 5'-terminal of said Y region are linked via a linker region of a non-nucleotide structure,
said X region comprises a guide strand sequence of a mature miRNA, and
said Y region comprises a sequence completely complementary to said X region.

2. The artificial match-type miRNA according to claim 1, wherein when said Y region and said X region are aligned, said Y region has an overhang on the 3'-terminal.

3. The artificial match-type miRNA according to claim 2, wherein said overhang has a 0 - 4 base length.

4. The artificial match-type miRNA according to any one of claims 1 to 3, wherein said X region comprises said guide strand sequence and an additional sequence, and said additional sequence is linked to the 3'-terminal of said guide strand sequence.

5. The artificial match-type miRNA according to claim 4, wherein said additional sequence in said X region has a length of 0 - 5 bases.

6. The artificial match-type miRNA according to any one of claims 1 to 5, wherein said X region has a length of 19 - 33 bases.

7. The artificial match-type miRNA according to any one of claims 1 to 6, wherein said Y region has a length of 21 - 35

bases.

8. The artificial match-type miRNA according to any one of claims 1 to 7, wherein a full-length is 40 - 68 base length.

9. The artificial match-type miRNA according to any one of claims 1 to 8, wherein said linker region comprises at least one selected from the group consisting of an amino acid residue, a polyamine residue, and a polycarboxylic acid residue.

10. The artificial match-type miRNA according to claim 9, wherein said linker region comprises a polycarboxylic acid residue.

11. The artificial match-type miRNA according to claim 10, wherein said polycarboxylic acid residue is a terephthalic acid residue.

12. The artificial match-type miRNA according to claim 9, wherein said linker region comprises an amino acid residue.

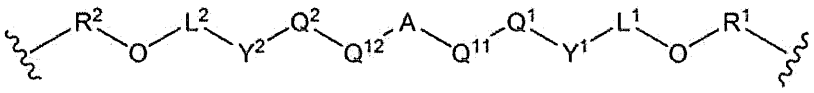
13. The artificial match-type miRNA according to claim 12, wherein said amino acid residue is a glycine residue, a terephthalic acid amide residue, a proline residue or a lysin residue.

14. The artificial match-type miRNA according to claim 12 or 13, wherein said amino acid residue comprises a plurality of amino acid residues linked to each other.

15. The artificial match-type miRNA according to claim 14, wherein said plurality of amino acid residues are linked to form a residue of a glycine dimer or trimer.

16. The artificial match-type miRNA according to any one of

claims 1 to 15, wherein said linker residue is represented by the following chemical formula (I-0):



(I-0)

in said chemical formula (I-0),

Q^{11} and Q^{12} are each independently a single bond, CH_2 (a methylene group), NH (an imino group), C=O (a carbonyl group), C=S (a thiocarbonyl group), C=NH (an iminomethylene group), O, or S,

Q^1 and Q^2 are each independently a single bond, CH_2 (a methylene group), NH (an imino group), C=O (a carbonyl group), C=S (a thiocarbonyl group), C=NH (an iminomethylene group), O, or S,

Y^1 and Y^2 are each independently a single bond, CH_2, NH, O, or S;

L^1 is an alkylene chain having n carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a, or,

L^1 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom, provided that: when Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other;

L^2 is an alkylene chain having m carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c, or

L^2 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom, provided that: when Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other;

R^a, R^b, R^c, and R^d are each independently a substituent or a protecting group;

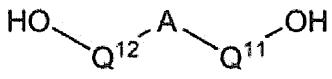
m is an integer in the range from 0 to 30;

n is an integer in the range from 0 to 30;
said regions X and Y are each linked to said linker residue via $-OR^1-$ or $-OR^2-$,
wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or said structure (I-0); and
A is any atomic group.

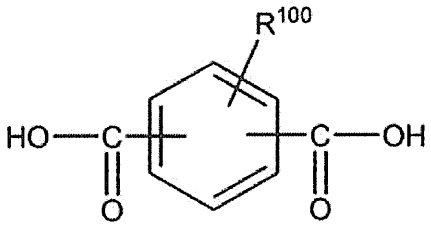
17. The artificial match-type miRNA according to claim 16, wherein Q^{11} and Q^{12} in said chemical formula (I-0) are each a carbonyl group.

18. The artificial match-type miRNA according to claim 17, wherein Q^1 and Q^2 in said chemical formula (I-0) are each an imino group.

19. The artificial match-type miRNA according to claim 17 or 18, wherein the following chemical formula (Iα) has a structure represented by the following chemical formula (Iα2):



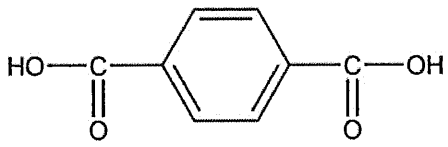
(Iα)



(Iα2)

in said chemical formula (Iα2),
R^{100} is any substituent, which may or may not be present, when it is present, it may be present singly or in plurality, and when it is present in plurality, they may be the same or different from each other.

20. The artificial match-type miRNA according to claim 14, wherein said chemical formula (Iα2) has a structure represented by the following chemical formula (Iα3):

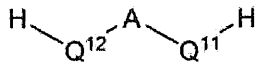


(Iα3) .

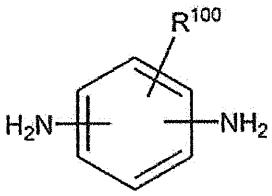
21. The artificial match-type miRNA according to claim 16, wherein Q^{11} and Q^{12} in said chemical formula (I-0) are each an imino group.

22. The artificial match-type miRNA according to claim 21, wherein Q^{1} and Q^{2} in said chemical formula (I-0) are each a carbonyl group.

23. The artificial match-type miRNA according to claim 16 or 17, wherein the following chemical formula (Iβ) has a structure represented by the following chemical formula (Iβ2):



(Iβ)

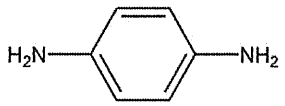


(Iβ2)

in said chemical formula (Iβ2),

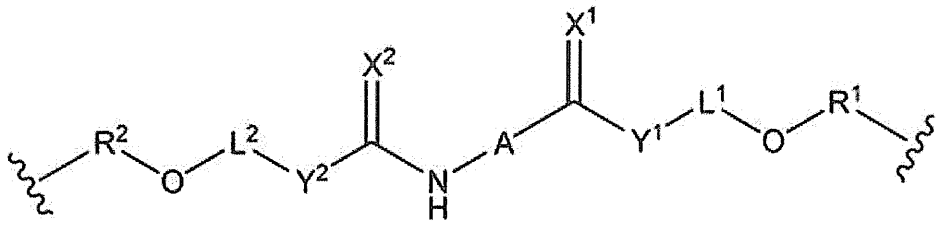
R^{100} is any substituent, which may or may not be present, when it is present, it may be present singly or in plurality, and when it is present in plurality, they may be the same or different from each other.

24. The artificial match-type miRNA according to claim 23, wherein said chemical formula (Iβ2) has a structure represented by the following chemical formula (Iβ3):



(Iβ3) .

25. The artificial match-type miRNA according to any one of claims 1 to 3 and 6 to 10, wherein said linker region comprises an amino acid residue, and said amino acid residue is represented by the following chemical formula (I):



(I)

in said chemical formula (I),

X^1 and X^2 are each independently H_2, O, S or NH;

Y^1 and Y^2 are each independently a single bond, CH_2, NH, O or S;

L^1 is an alkylene chain having n carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a, or,

L^1 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom, provided that: when Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other;

L^2 is an alkylene chain having m carbon atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c, or

L^2 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom,

provided that: when Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other;

R^a, R^b, R^c, and R^d are each independently a substituent or a protecting group;

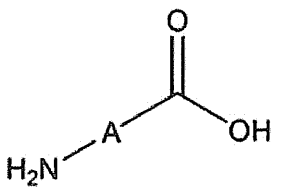
m is an integer in the range from 0 to 30;

n is an integer in the range from 0 to 30;

said regions X and Y are each linked to said amino acid residue via $-OR^1-$ or $-OR^2-$,

wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or said structure (I); and

A is any atomic group, provided that the following chemical formula (Ia) is an amino acid or peptide:



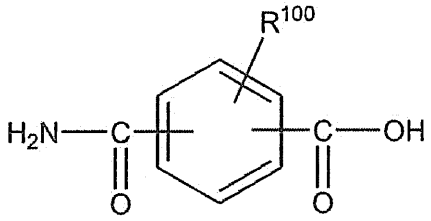
(Ia)

26. The artificial match-type miRNA according to any one of claims 1 to 3, 6 to 10 and 20, wherein said amino acid in said amino acid residue is at least one of a natural amino acid and an artificial amino acid.

27. The artificial match-type miRNA according to claim 26, wherein said natural amino acid is an amino acid that constitutes a protein.

28. The artificial match-type miRNA according to any one of claims 1 to 9, 12 to 15, 25 and 26, wherein said amino acid in said natural amino acid residue is at least one kind selected from the group consisting of glycine, α-alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine,

hydroxylysine, methionine, phenylalanine, serine, threonine, tyrosine, valine, proline, 4-hydroxyproline, tryptophan, β-alanine, 1-amino-2-carboxycyclopentane, aminobenzoic acid, aminopyridinecarboxylic acid and amino acid represented by the following chemical formula (Ia2), and optionally further has a substituent or a protecting group:

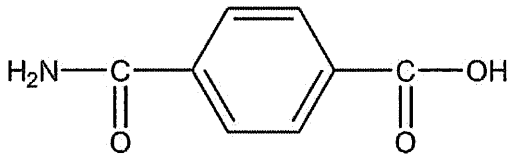


(Ia2)

in said chemical formula (Ia2),

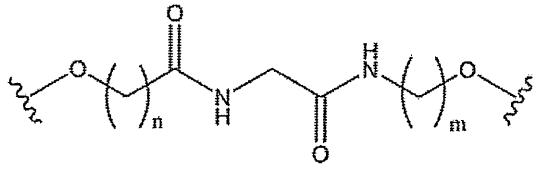
R^{100} is any substituent, which may or may not be present, when it is present, it may be present singly or in plurality, and when it is present in plurality, they may be the same or different from each other.

29. The artificial match-type miRNA according to claim 28, wherein said chemical formula (Ia2) has a structure represented by the following chemical formula (Ia3):

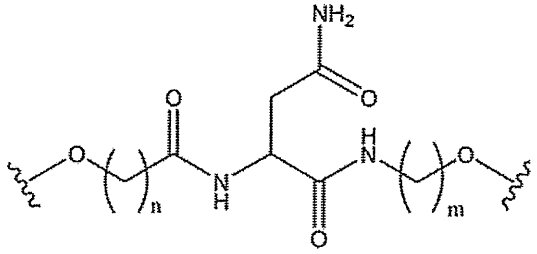


(Ia3)

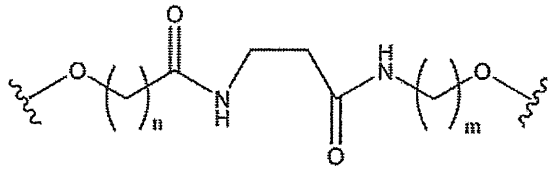
30. The artificial match-type miRNA according to claim 16 or 25, wherein said chemical formula (I-0) or (I) has a structure of the following chemical formulae (I-1) - (I-7) and, in the following chemical formulae (I-1) - (I-7), n is an integer of 0 - 30, and m is an integer of 0 - 30:



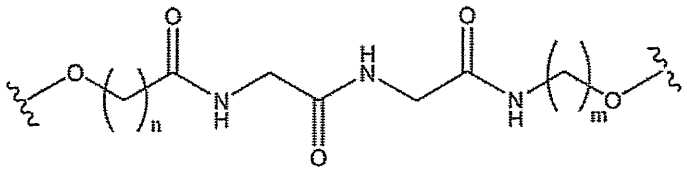
(I－1)



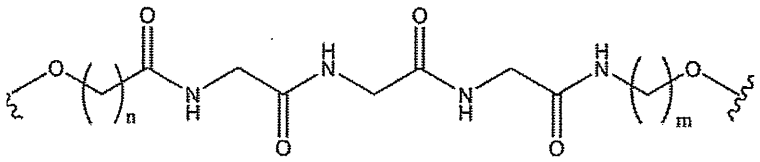
(I－2)



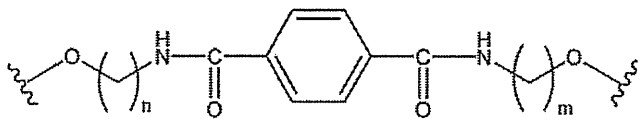
(I－3)



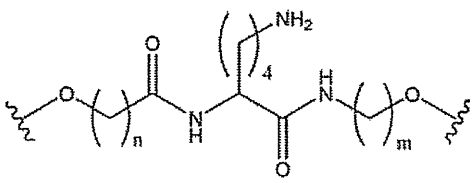
(I－4)



(I－5)



(I－6)



(I－7)

31. The artificial match-type miRNA according to claim 30, wherein, in said chemical formula (I-1), n=11 and m=12, or n=5 and m=4.

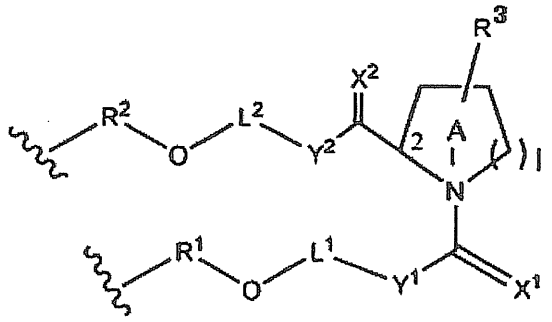
32. The artificial match-type miRNA according to claim 30, wherein, in said chemical formula (I-4), n=5 and m=4.

33. The artificial match-type miRNA according to claim 30, wherein, in said chemical formula (I-6), n=4 and m=4.

34. The artificial match-type miRNA according to claim 30, wherein, in said chemical formula (I-7), n=5 and m=4.

35. The artificial match-type miRNA according to any one of claims 1 to 9, 12 and 13, wherein a non-nucleotide structure of said linker region comprises at least one of a pyrrolidine skeleton and a piperidine skeleton.

36. The artificial match-type miRNA according to any one of claims 1 to 8, wherein said non-nucleotide structure is represented by the following formula (II):



... (II)

wherein,

X^1 and X^2 are each independently H_2, O, S, or NH;

Y^1 and Y^2 are each independently a single bond, CH_2, NH, O, or S;

R^3 is a hydrogen atom or a substituent which is bonded to C-3,

C-4, C-5 or C-6 on ring A,

L^1 is an alkylene chain having n atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^a, NH_2, NHR^a, NR^aR^b, SH, or SR^a, or,

L^1 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom, provided that: when Y^1 is NH, O, or S, an atom bound to Y^1 in L^1 is carbon, an atom bound to OR^1 in L^1 is carbon, and oxygen atoms are not adjacent to each other;

L^2 is an alkylene chain having m atoms, and a hydrogen atom on an alkylene carbon atom may or may not be substituted with OH, OR^c, NH_2, NHR^c, NR^cR^d, SH, or SR^c, or

L^2 is a polyether chain obtained by substituting at least one carbon atom on said alkylene chain with an oxygen atom, provided that: when Y^2 is NH, O, or S, an atom bound to Y^2 in L^2 is carbon, an atom bound to OR^2 in L^2 is carbon, and oxygen atoms are not adjacent to each other;

R^a, R^b, R^c, and R^d are each independently a substituent or a protecting group;

l is 1 or 2;

m is an integer in the range from 0 to 30;

n is an integer in the range from 0 to 30; and

in ring A, one carbon atom other than said C-2 on ring A may be substituted by nitrogen, oxygen or sulfur, and may contain, in said ring A, a carbon-carbon double bond or a carbon-nitrogen double bond,

said regions (X) and (Y) are each linked to said non-nucleotide structure via $-OR^1-$ or $-OR^2-$,

wherein R^1 and R^2 may or may not be present, and when they are present, R^1 and R^2 are each independently a nucleotide residue or said structure (I).

37. The artificial match-type miRNA according to any one of claims 1 to 36, wherein said X region comprises a guide strand sequence of a mature miRNA selected from the group consisting

of hsa-miR-34, hsa-miR-29 and hsa-let-7.

38. The artificial match-type miRNA according to claim 37, wherein said mature miRNA is hsa-miR-34a.

39. The artificial match-type miRNA according to claim 38, wherein the nucleotide sequence is a nucleotide sequence selected from the group consisting of SEQ ID NO: 9 and 17 - 28.

40. The artificial match-type miRNA according to claim 37, wherein said mature miRNA is hsa-let-7a.

41. The artificial match-type miRNA according to claim 40, wherein the nucleotide sequence is a nucleotide sequence selected from the group consisting of SEQ ID NO: 36 - 38.

42. The artificial match-type miRNA according to claim 37, wherein said mature miRNA is hsa-miR-29b.

43. The artificial match-type miRNA according to claim 42, wherein the nucleotide sequence is a nucleotide sequence selected from the group consisting of SEQ ID NO: 43 - 45.

44. A composition for suppressing expression of a target gene, comprising the artificial match-type miRNA according to any one of claims 1 to 43.

45. A pharmaceutical composition comprising the artificial match-type miRNA according to any one of claims 1 to 43.

46. A method of suppressing expression of a target gene, comprising using the artificial match-type miRNA according to any one of claims 1 to 43.

47. The method according to claim 46, comprising a step of

administering said artificial match-type miRNA to a cell, a tissue or an organ.

48. The method according to claim 46 or 47, wherein said artificial match-type miRNA is administered in vivo or in vitro.

49. The method according to claim 46 or 47, wherein said artificial match-type miRNA is administered to a non-human animal.

50. A method of treating a disease, comprising a step of administering the artificial match-type miRNA according to any one of claims 1 to 43 to patients, wherein said guide strand sequence of said artificial match-type miRNA is a guide strand sequence of a mature miRNA that suppresses expression of a gene involved in said disease.

51. A single strand nucleic acid for use in the treatment of a disease, which is the artificial match-type miRNA according to any one of claims 1 to 43, wherein said guide strand sequence of said artificial match-type miRNA is a guide strand sequence of a mature miRNA that suppresses expression of a gene involved in said disease.

SEQUENCE LISTING

<110> BONAC CORPORATION
TOKYO MEDICAL UNIVERSITY

<120> Artificial match-type miRNA for controlling gene expression and use therefor

<130> 092265

<150> JP-2013-273033
<151> 2013-12-27

<160> 47

<170> PatentIn version 3.5

<210> 1
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> mature miRNA (hsa-miR-34a)

<400> 1
uggcaguguc uuagcugguu gu 22

<210> 2
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> mature miRNA (hsa-let-7a)

<400> 2
ugagguagua gguuguauag uu 22

<210> 3
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> mature miRNA (hsa-let-7f)

<400> 3
ugagguagua gauuguauag uu 22

<210> 4
<211> 22
<212> RNA

<213> Artificial Sequence

<220>
<223> mature miRNA (hsa-miR-150)

<400> 4
ucucccaacc cuuguaccag ug 22

<210> 5
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> mature miRNA (hsa-miR-29b)

<400> 5
uagcaccauu ugaaaucagu guu 23

<210> 6
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> passenger strand (mature miR-34a)

<400> 6
caaucagcaa guauacugcc cu 22

<210> 7
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> guide strand (mature miR-34a scramble)

<400> 7
uguaucguua ucgggucggu ug 22

<210> 8
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> passenger strand (mature miR-34a scramble)

<400> 8
caaccgaccc gauaacgaua ca 22

<210> 9
<211> 52
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 9
uggcaguguc uuagcugguu guuccggaac aaccagcuaa gacacugcca ua 52

<210> 10
<211> 52
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 10
uguaucguua ucgggucggu uguccggaca accgacccga uaacgauaca ua 52

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 11
ctcaaccagg acgactccat 20

<210> 12
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 12
agaccgcttc actcaggaaa 20

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 13
caggcagtgc agcatgtagt 20

<210> 14
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 14
tgtccaacaa agtcccatga 20

<210> 15
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 15
atggggaagg tgaaggtcg 19

<210> 16
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 16
gggtcattga tggcaacaat atc 23

<210> 17
<211> 50
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 17
uggcaguguc uuagcugguu guucgaacaa ccagcuaaga cacugccaua 50

<210> 18
<211> 48
<212> RNA

<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 18
uggcaguguc uuagcugguu guuaacaacc agcuaagaca cugccaua 48

<210> 19
<211> 46
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 19
uggcaguguc uuagcugguu guacaaccag cuaagacacu gccaua 46

<210> 20
<211> 51
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 20
uggcaguguc uuagcugguu guuccggaac aaccagcuaa gacacugcca u 51

<210> 21
<211> 49
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 21
uggcaguguc uuagcugguu guucgaacaa ccagcuaaga cacugccau 49

<210> 22
<211> 47
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 22
uggcaguguc uuagcugguu guuaacaacc agcuaagaca cugccau 47

<210> 23
<211> 45
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 23
uggcaguguc uuagcugguu guacaaccag cuaagacacu gccau 45

<210> 24
<211> 50
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 24
uggcaguguc uuagcugguu guuccggaac aaccagcuaa gacacugcca 50

<210> 25
<211> 48
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 25
uggcaguguc uuagcugguu guucgaacaa ccagcuaaga cacugcca 48

<210> 26
<211> 46
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 26
uggcaguguc uuagcugguu guuaacaacc agcuaagaca cugcca 46

<210> 27
<211> 44
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 27
uggcaguguc uuagcugguu guacaaccag cuaagacacu gcca 44

<210> 28
<211> 46
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotid

<400> 28
uggcaguguc uuagcugguu guacaaccag cuaagacacu gccacu 46

<210> 29
<211> 52
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotid

<400> 29
uggcaguguc uuagcugguu guuccggaac aaccagcuaa gacacugcca cu 52

<210> 30
<211> 56
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 30
uggcaguguc uuagcugguu guuccggccg gaacaaccag cuaagacacu gccacu 56

<210> 31
<211> 48
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 31
uacuauucga cacgcgaagu uccggaacuu cgcgugucga auaguauu 48

<210> 32
<211> 64
<212> RNA

<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 32
uggcaguguc uuagcugguu guugugagca auaguaagga agcaaucagc aaguauacug 60

cccu 64

<210> 33
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 33
aaccagcuaa gacacugcca cu 22

<210> 34
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 34
cuauacaacc uacuaccuca uc 22

<210> 35
<211> 72
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 35
ugagguagua gguuguauag uuuuaggguc acacccacca cugggagaua acuauacaau 60

cuacugucuu uc 72

<210> 36
<211> 46
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 36
ugagguagua gguuguauag uuaacuauac aaccuacuac cucauc 46

<210> 37
<211> 52
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 37
ugagguagua gguuguauag uuuccggaaa cuauacaacc uacuaccuca uc 52

<210> 38
<211> 56
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 38
ugagguagua gguuguauag uuuccggccg gaaacuauac aaccuacuac cucauc 56

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 39
gaagccactg gagaaaaacg 20

<210> 40
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 40
cttcggcaga ctcttgtgag 20

<210> 41
<211> 23
<212> RNA

<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 41
cacugauuuc aaauggugcu aga 23

<210> 42
<211> 64
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 42
gcugguuuca uaugguggguu uagauuuaaa uagugauugu cuagcaccau uugaaaucag 60

uguu 64

<210> 43
<211> 48
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 43
uagcaccauu ugaaaucagu guuaacacug auuucaaaug gugcuaga 48

<210> 44
<211> 54
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 44
uagcaccauu ugaaaucagu guuuccggaa acacugauuu caaauggugc uaga 54

<210> 45
<211> 58
<212> RNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<400> 45

uagcaccauu ugaaaucagu guuuccggcc ggaaacacug auuucaaaug gugcuaga 58

<210> 46
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 46
cccaaggaca agaggcatgt 20

<210> 47
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 47
ccgccatact cgaactggaa 20