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(72) Inventeurs/Inventors:
HONG, SUN, WOO, KR;
HONG, ISU, KR;
KIM, JI, HYUN, KR

(73) Propriétaire/Owner:
OLIX PHARMACEUTICALS, INC., KR

(74) Agent: BORDEN LADNER GERVAIS LLP

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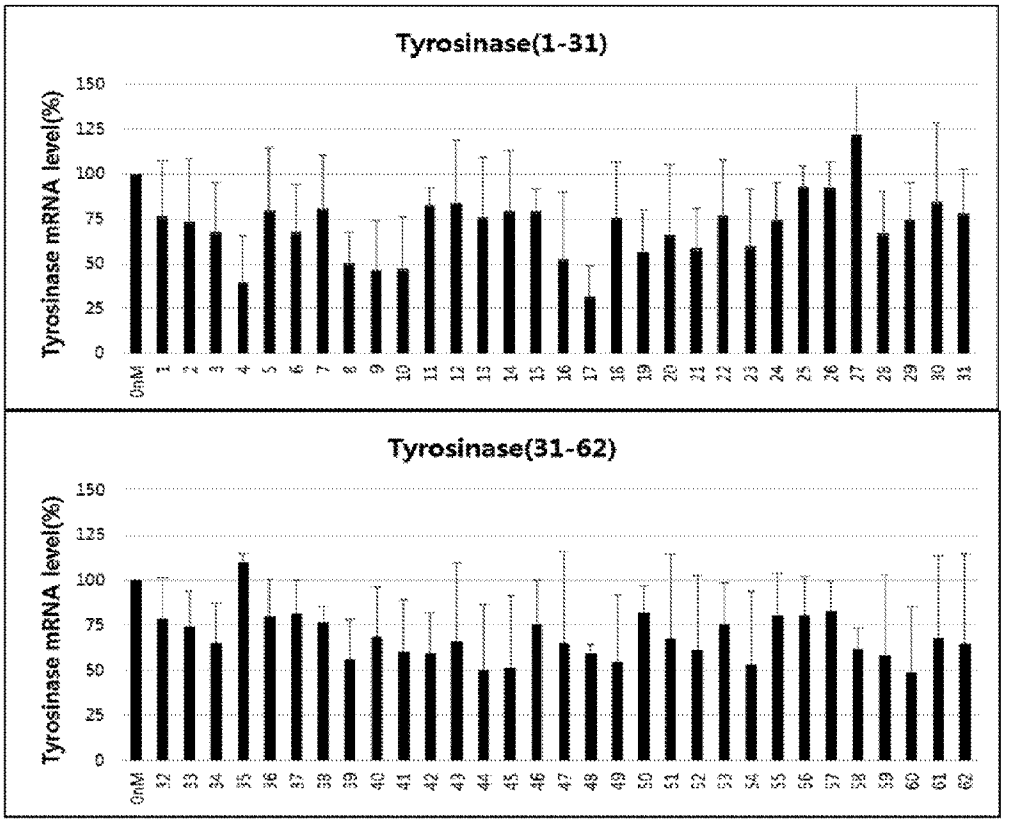
(54) Title: RNA COMPLEXES THAT INHIBIT MELANIN PRODUCTION



(57) Abrégé/Abstract:
In certain aspects, provided herein are RNA complexes (e.g., asymmetric RNA complexes, such as asiRNAs and lasiRNAs) that inhibit tyrosinase expression and are therefore useful for reducing melanin production and for treating pigmentation-related disorders associated with excessive melanin production, such as melasma and age spots.

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(71) Applicant: **OLIX PHARMACEUTICALS, INC.** [KR/KR]; 60-11 Star Valley, Suite 1305, Gasan-dong, Geumcheon-gu, Seoul 153-777 (KR).

(72) Inventors: **HONG, Sun, Woo**; LG Village Apt., #211-1905, Geumgok-ro, Gwonseon-gu, Suwon-si, Gyeonggi-do 45 (KR). **HONG, Isu**; SK HUB SU 27, Rm. 703, Guil-ro 10-gil, Seoul 27 (KR). **KIM, Ji, Hyun**; GreenM House, Room 203, 4254 Singlil 6-dong, Seoul 4254 (KR).

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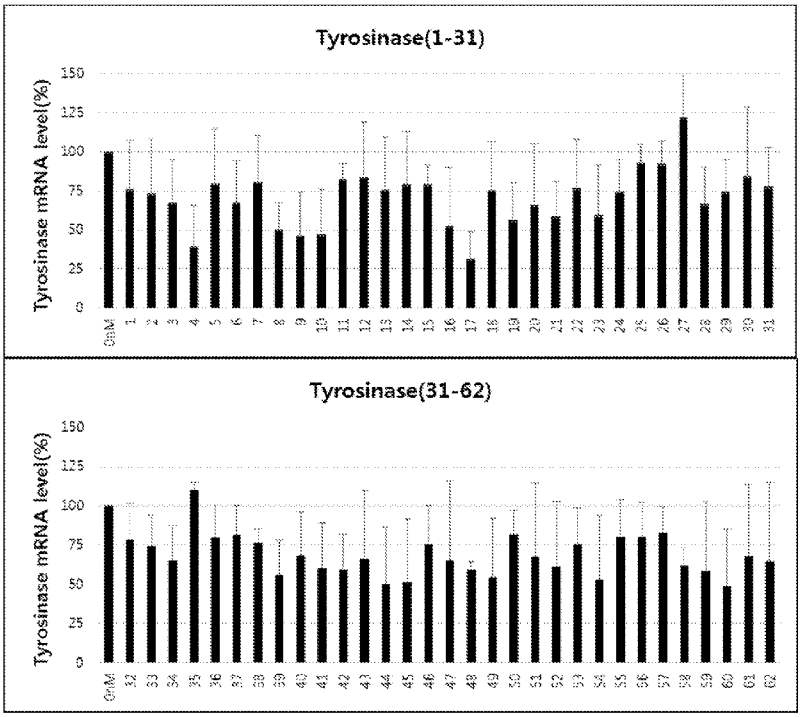
[Continued on next page]

(54) Title: RNA COMPLEXES THAT INHIBIT MELANIN PRODUCTION

Figure 1



(57) Abstract: In certain aspects, provided herein are RNA complexes (e.g., asymmetric RNA complexes, such as asiRNAs and lasiRNAs) that inhibit tyrosinase expression and are therefore useful for reducing melanin production and for treating pigmentation-related disorders associated with excessive melanin production, such as melasma and age spots.

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RNA COMPLEXES THAT INHIBIT MELANIN PRODUCTION

BACKGROUND

Excess melanin production by melanocytes is associated with a variety of skin pigmentation-related disorders, including melasma and age spots. In melasma, excessive production of melanin results in black deposits in melanocytes present in the epidermal skin layer. Melasma is one of the leading refractory diseases occurring in the skin of women. Melasma often occurs in pregnant women and in women who are taking oral or patch contraceptives or undergoing hormone replacement therapy.

Tyrosinase is an oxidase that is the rate limiting enzyme in the synthesis of melanin and is therefore an important therapeutic target for agents that reduce hyperpigmentation and treat skin pigmentation-related disorders. In humans, the tyrosinase enzyme is encoded by the TYR gene. Mutations in the TYR gene that result in impaired tyrosinase production lead to type I oculocutaneous albinism.

Currently available treatments of skin pigmentation-related disorders associated with excessive melanin production include hydroquinone, arbutin, tretinoin, azelaic acid, kojic acid, chemical peels and microdermabrasion. However, such treatments are often ineffective and can have significant side-effects. Individuals with such disorders often need to resort to cosmetics to hide the areas of excessive skin pigmentation.

Thus, there is a need for improved compositions and methods for the inhibition of melanin production and the treatment of skin pigmentation-related disorders, including melasma and age spots.

SUMMARY

In certain aspects, provided herein are RNA complexes that inhibit tyrosinase and are useful for reducing melanin production and the treatment of pigmentation-related disorders, including melasma and age spots. In certain aspects, provided herein are pharmaceutical compositions comprising such RNA complexes and methods of using such RNA complexes and pharmaceutical compositions.

In certain aspects, provided herein is an RNA complex comprising an antisense strand having sequence complementarity to a tyrosinase mRNA sequence (*e.g.,* a human

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tyrosinase mRNA sequence) and a sense strand having sequence complementarity to the antisense strand. In some embodiments, the RNA complex is capable of inhibiting tyrosinase expression by a cell (*e.g.*, a melanocyte). In certain embodiments, the RNA complex is capable of inhibiting melanin production by a cell (*e.g.*, a melanocyte). In some embodiments, the RNA complex is an asymmetric short interfering RNA (an asiRNA). In some embodiments, the RNA complex is a long asymmetric short interfering RNA (a lasiRNA). In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 4, Table 5 and Table 6.

In some embodiments, the RNA complex provided herein comprises a chemical modification, wherein the modification facilitates the penetration of a cellular membrane in the absence of a delivery vehicle. In some embodiments, the modification is a 2′-O-methylated nucleoside, a phosphorothioate bond or a cholesterol moiety. In some embodiments, the RNA complex is a modified RNA complex listed in Table 2 or Table 4. In certain embodiments, the RNA complex is not cytotoxic.

In certain aspects, provided herein is a pharmaceutical composition comprising an RNA complex provided herein and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is formulated for topical delivery. In some embodiments, the pharmaceutical composition is a cream or a lotion. In some embodiments, the pharmaceutical composition further comprises a second skin lightening agent (*e.g.*, hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid or tranexamic acid).

In certain aspects, provided herein is a method of inhibiting tyrosinase expression by a cell (*e.g.*, a melanocyte) comprising contacting the cell with an RNA complex provided herein. In certain aspects, provided herein is a method of inhibiting melanin production by a cell (*e.g.*, a melanocyte) comprising contacting the cell with an RNA complex provided herein.

In certain aspects, provided herein is a method of inhibiting melanin production in the skin of a human subject comprising administering to the subject an RNA complex or pharmaceutical composition provided herein. In certain aspects, provided herein is a method of treating a human subject for a skin pigmentation disorder associated with excessive melanin production (*e.g.*, melasma or age spots) comprising administering to the subject an RNA complex or pharmaceutical composition provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the gene silencing efficiency of 62 exemplary asiRNAs that target tyrosinase. The asiRNAs were transfected in A375P at a concentration of 0.3 nM, and, after 24 hours, the degree of tyrosinase mRNA expression was measured using real-time PCR. The graph depicts the mean and standard deviation of three repeat experiments.

Figure 2 shows the gene silencing efficiency of exemplary tyrosinase-targeting cell penetrating asiRNAs (cp-asiRNAs, or cp-asiTYRs) to which various chemical modifications have been applied. The cp-asiRNAs were incubated without transfection vehicle in the presence of MNT-1 cells at a concentration of 1 μM, and, after 48 hours, the degree of tyrosinase mRNA expression was measured using real-time PCR. The graph depicts the mean and standard deviation of three repeat experiments.

Figure 3 shows the inhibition of tyrosinase protein expression by exemplary cp-asiRNAs. The cp-asiRNAs were contacted with MNT-1 cells without transfection vehicle and, after 72 hours, proteins were extracted and a western blot was performed (NT = no treatment).

Figure 4 shows the result of a melanin content assay performed 72 hours after the treatment of MNT-1 cells with exemplary cp-asiRNAs without transfection vehicle (NT = no treatment).

Figure 5 shows pigmentation inhibition by exemplary cp-asiRNAs. Panel (a) depicts the color change of MNT-1 cells 72 hours after treatment with cp-asiRNA without transfection vehicle. Panel (b) depicts the color change of the melanin obtained from the MNT-1 cell line 72 hours after treatment of the MNT-1 cells with cp-asiRNA without vehicle (NT = no treatment).

Figure 6 shows the cytotoxicity of cells treated with exemplary cp-asiRNAs without transfection vehicle using a LDH assay and a CCK-8 assay. Panel (a) depicts the cytotoxicity in MNT-1 cells 24 hours after the treatment with the exemplary cp-asiRNAs or the indicated controls as determined by an LDH assay. Panel (b) depicts the cytotoxicity in MNT-1 cells 24 hours after the treatment with cp-asiRNAs or the indicated controls as determined by a CCK-8 assay. Panel (c) depicts the cytotoxicity in HaCaT cells 24 hours after treatment with cp-asiRNAs or controls as determined by an LDH assay. Panel (d) depicts the cytotoxicity of HaCaT cells 24 hours after treatment with cp-asiRNAs or controls as determined by a CCK-8 assay (NT = no treatment).

Figure 7 shows the gene silencing effects of exemplary cp-asiRNAs of different antisense strand lengths (21 or 19 nucleotides) and containing 2′-O-Methylation

modifications. Each cp-asiRNA was contacted to MNT-1 without transfection vehicle at 1 μM concentration and the resulting tyrosinase mRNA production was measured by Real-Time PCR after 48 hours.

Figure 8 shows the inhibition of tyrosinase protein expression by exemplary cp-asiRNAs. The indicated cp-asiRNAs were contacted to MNT-1 cells without transfection vehicle and, after 72 hours, proteins were extracted and a western blot was performed (NT = no treatment).

Figure 9 shows the results produced by a melanin content assay performed 72 hours after the treatment of MNT-1 cells with exemplary cp-asiRNAs without transfection vehicle. (NT = no treatment).

Figure 10 shows the inhibition of tyrosinase protein expression by exemplary cp-asiRNAs. The indicated cp-asiRNAs were contacted to MNT-1 cells without transfection vehicle and, after 72 hours, proteins were extracted and a western blot was performed (NT = no treatment).

Figure 11 shows the effect of treatment of MNT-1 cells with exemplary asiRNAs and lasiRNAs. Each complex identified was incubated with MNT-1 cells for 48 hours at the indicated concentration and tyrosinase mRNA expression was determined by real-time RT-PCR.

Figure 12 shows the effect of treatment of MNT-1 cells with exemplary asiRNAs and lasiRNAs. Panel (a) depicts the results produced by a western blot for tyrosinase expression by MNT-1 cells 72 hours after the treatment with exemplary asiRNAs, lasiRNAs or controls. Panel (b) depicts the melanin content of MNT-1 cells 72 hours after treatment with exemplary asiRNAs, lasiRNAs or controls (NT = no treatment).

Figure 13 provides the human tyrosinase mRNA sequence.

Figure 14 shows inhibition of melanin synthesis in reconstructed skin model by an exemplary cp-asiRNA. Panel (a) depicts the experimental scheme for the study in which cp-MEL-300-B samples were treated every day for 13 days with asiTYR#4-1 in medium (13 times). Panel (b) shows light microscopy analysis of melanocyte in a no treatment control sample (NT), a cp-asiTYR#4-1 treated sample, and a kojic acid treated sample. Panel (c) shows Fontana-Massons staining for melanin analysis in a no treatment control sample (NT), a cp-asiTYR#4-1 treated sample, and a kojic acid treated sample. Panel (d) shows the tyrosinase mRNA level at day 14 as measured using real-time PCR. Panel (e) shows

tyrosinase protein level at day 14 as measured by western blot. Panel (f) shows the melanin content of samples at day 14 as measured using a melanin contents assay.

DETAILED DESCRIPTION

General

In certain aspects, provided herein are asymmetric RNA complexes (*e.g.,* asiRNAs or lasiRNAs) that inhibit tyrosinase expression and are therefore useful for reducing melanin production and the treatment of pigmentation-related disorders associated with excessive melanin production, such as melasma and age spots. In some embodiments, the RNA complexes are chemically modified to be capable of penetrating a cell without need for a transfection vehicle. In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 4, Table 5 and Table 6. In certain aspects, provided herein are pharmaceutical compositions comprising such RNA complexes and methods of using such RNA complexes and pharmaceutical compositions.

Tyrosinase is a protein that plays a key role in melanin synthesis. Various small molecule inhibitors targeting tyrosinase, including hydroquinone, retinoic acid and kojic acid, have been used as active ingredients of skin-whitening products. However, such treatments are often ineffective and often result in serious side effects, such as itching and skin browning.

In certain embodiments, the RNA complexes provided herein have reduced risk for side effects compared to the conventional small molecules currently in use for skin whitening. As described herein, exemplary RNA complexes provided herein have significant tyrosinase inhibitory effect, even at a 1000-fold lower concentration than current skin-whitening agents. Thus, the RNA complexes provided herein can replace or supplement currently available small molecule products for improved skin-whitening effects.

In some embodiments, the RNA complexes described herein are asiRNAs or lasiRNAs. As used herein, the term asiRNA refers to double-stranded asymmetrical short interfering RNA molecules that have a 19-21 nt antisense strand and a 13-17 nt sense strand. Additional information on asiRNAs can be found in U.S. Pat. Pub. No. 2012/0238017 and in Chang *et al., Mol. Ther.* 17:725-732 (2009). As used herein, the term lasiRNA refers to double-stranded long asymmetrical interfering RNA molecules that have a 13-21 nt sense strand and an antisense strand of greater than 24 nt. Additional information on lasiRNAs can be

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found in U.S. Pat. Pub. No. 2013/0273657.

In some embodiments, the RNA complexes described herein are delivered to cells using a delivery vehicle, such as liposomes, cationic polymers, cell penetrating peptides (CPPs), protein transduction domains (PTDs), antibodies and/or aptamers. In some embodiments, the RNA complex described herein is chemically modified so as to not require the use of such delivery vehicles to mediate tyrosinase inhibition in a cell. Such RNA complexes are referred to herein as cell-penetrating asiRNAs (cp-asiRNAs) or cell-penetrating lasiRNAs (cp-lasiRNAs).

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The articles "*a*" and "*an*" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "*administering*" means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

As used herein, the terms "*interfering nucleic acid,*" "*inhibiting nucleic acid*" are used interchangeably. Interfering nucleic acids generally include a sequence of cyclic subunits, each bearing a base-pairing moiety, linked by intersubunit linkages that allow the base-pairing moieties to hybridize to a target sequence in a nucleic acid (typically an RNA) by Watson-Crick base pairing, to form a nucleic acid:oligomer heteroduplex within the target sequence. Interfering RNA molecules include, but are not limited to, antisense molecules, siRNA molecules, asiRNA molecules, lasiRNA molecules, single-stranded siRNA molecules, miRNA molecules and shRNA molecules. Such an interfering nucleic acids can be designed to block or inhibit translation of mRNA or to inhibit natural pre-mRNA splice processing, or induce degradation of targeted mRNAs, and may be said to be "directed to" or "targeted against" a target sequence with which it hybridizes. Interfering nucleic acids may include, for example, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), 2′-O-Methyl oligonucleotides and RNA interference agents (siRNA agents). RNAi molecules generally act by forming a heteroduplex with the target molecule, which is selectively degraded or "knocked down," hence inactivating the target RNA. Under some

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conditions, an interfering RNA molecule can also inactivate a target transcript by repressing transcript translation and/or inhibiting transcription of the transcript. An interfering nucleic acid is more generally said to be "targeted against" a biologically relevant target, such as a protein, when it is targeted against the nucleic acid of the target in the manner described above.

The terms "*polynucleotide*", and "*nucleic acid*" are used interchangeably. They refer to a polymeric form of nucleotides, whether deoxyribonucleotides, ribonucleotides, or analogs thereof, in any combination and of any length. Polynucleotides may have any three-dimensional structure, and may perform any function. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. A polynucleotide may be further modified, such as by conjugation with a labeling component. In all nucleic acid sequences provided herein, U nucleotides are interchangeable with T nucleotides.

The phrase "*pharmaceutically-acceptable carrier*" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material.

An oligonucleotide "*specifically hybridizes*" to a target polynucleotide if the oligomer hybridizes to the target under physiological conditions, with a Tm substantially greater than 45° C, or at least 50° C, or at least 60° C-80° C or higher. Such hybridization corresponds to stringent hybridization conditions. At a given ionic strength and pH, the Tm is the temperature at which 50% of a target sequence hybridizes to a complementary polynucleotide. Again, such hybridization may occur with "near" or "substantial" complementarity of the antisense oligomer to the target sequence, as well as with exact complementarity.

As used herein, the term "*subject*" means a human or non-human animal selected for treatment or therapy.

The phrases "*therapeutically-effective amount*" and "*effective amount*" as used herein means the amount of an agent which is effective for producing the desired therapeutic effect in at least a sub-population of cells in a subject at a reasonable benefit/risk ratio applicable to any medical treatment.

"*Treating*" a disease in a subject or "*treating*" a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease is decreased or prevented from worsening.

RNA Complexes

In certain aspects, provided herein are RNA complexes that target tyrosinase mRNA and inhibit tyrosinase expression by a cell. Tyrosinase is an oxidase that is the rate-limiting enzyme for controlling production of melanin. The nucleic acid sequence of human tyrosinase mRNA is available at NCBI accession numbers NM_000372 and is provided in Figure 13.

In certain aspects, provided herein is an RNA complex comprising an antisense strand having sequence complementarity to a tyrosinase mRNA sequence (*e.g.*, a human tyrosinase mRNA sequence) and a sense strand having sequence complementarity to the antisense strand. In some embodiments, the RNA complex is capable of inhibiting tyrosinase expression by a cell (*e.g.*, a melanocyte). In certain embodiments, the RNA complex is capable of inhibiting melanin production by a cell (*e.g.*, a melanocyte). In some embodiments, the RNA complex is an asymmetric short interfering RNA (an asiRNA). In some embodiments, the RNA complex is a long asymmetric short interfering RNA (a lasiRNA). In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 4, Table 5 and Table 6. The RNA complexes described herein can contain RNA bases, non-RNA bases or a mixture of RNA bases and non-RNA bases. For example, certain RNA complexes provided herein can be primarily composed of RNA bases but also contain DNA bases or non-naturally occurring nucleotides.

In some embodiments, the antisense strand is at least 19 nucleotides (nt) in length. In some embodiments, the antisense strand is 19 to 21 nt in length (*i.e.*, 19, 20 or 21 nt in length). In some embodiments, at least 13, 14, 15, 16, 17, 18, 19, 20 or 21 nt of the antisense strand are complementary to the tyrosinase mRNA sequence. Perfect complementarity is not necessary. In some embodiments, the antisense strand is perfectly complementary to the tyrosinase mRNA sequence.

In some embodiments, the antisense strand is at least 24 nt in length (*e.g.*, at least 25 nt in length, at least 26 nt in length, at least 27 nt in length, at least 28 nt in length, at least 29 nt in length, at least 30 nt in length or at least 31 nt in length). In some embodiments, the antisense strand is no greater than 124 nt in length (*e.g.*, no greater than 100 nt in length, no greater than 90 nt in length, no greater than 80 nt in length, no greater than 70 nt in length, no greater than 60 nt in length, no greater than 50 nt in length or no greater than 40 nt in length. In some embodiments, the antisense strand is 31 nt in length. In some embodiments, at least 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 29, 30 or 31 nt of the antisense strand are complementary to the tyrosinase mRNA sequence. Perfect complementarity is not necessary. In some embodiments, the antisense strand is perfectly complementary to the tyrosinase mRNA sequence.

In some embodiments, the sense strand is 15 to 17 nt in length (*i.e.*, 15 nt in length, 16 nt in length or 17 nt in length). In some embodiments, at least 15 nt, at least 16 nt or at least 17 nt of the sense strand are complementary to the sequence of the antisense strand. In some embodiments the sense strand is perfectly complementary to the sequence of the antisense strand.

In some embodiments, the antisense strand and the sense strand form a complex in which the 5′ end of the antisense strand and the 3′ end of the sense strand form a blunt end. In some embodiments, the antisense strand and the sense strand form a complex in which the 5′ end of the antisense strand overhangs the 3′ end of the sense strand (*e.g.*, by 1, 2, 3, 4 or 5 nt). In some embodiments, the antisense strand and the sense strand form a complex in which the 5′ end of the sense strand overhangs the 3′ end of the antisense strand (*e.g.*, by 1, 2, 3, 4 or 5 nt).

In some embodiments, the antisense strand and/or the sense strand of the RNA complex has a sense strand sequence and/or an antisense strand sequence selected from the sequences listed in Table 1, Table 2, Table 4, Table 5 and Table 6. In some embodiments, the sense strand has a sequence of SEQ ID NO: 1 and the antisense strand has a sequence of SEQ ID NO: 2. In some embodiments, the sense strand has a sequence of SEQ ID NO: 3 and the antisense strand has a sequence of SEQ ID NO: 4. In some embodiments, the sense strand has a sequence of SEQ ID NO: 5 and the antisense strand has a sequence of SEQ ID NO: 6. In some embodiments, the sense strand has a sequence of SEQ ID NO: 7 and the antisense strand has a sequence of SEQ ID NO: 8. In some embodiments, the sense strand has a sequence of SEQ ID NO: 9 and the antisense strand has a sequence of SEQ ID NO:

10. In some embodiments, the sense strand has a sequence of SEQ ID NO: 11 and the antisense strand has a sequence of SEQ ID NO: 12. In some embodiments, the sense strand has a sequence of SEQ ID NO: 13 and the antisense strand has a sequence of SEQ ID NO: 14. In some embodiments, the sense strand has a sequence of SEQ ID NO: 15 and the antisense strand has a sequence of SEQ ID NO: 16. In some embodiments, the sense strand has a sequence of SEQ ID NO: 17 and the antisense strand has a sequence of SEQ ID NO: 18. In some embodiments, the sense strand has a sequence of SEQ ID NO: 19 and the antisense strand has a sequence of SEQ ID NO: 20. In some embodiments, the sense strand has a sequence of SEQ ID NO: 21 and the antisense strand has a sequence of SEQ ID NO: 22. In some embodiments, the sense strand has a sequence of SEQ ID NO: 23 and the antisense strand has a sequence of SEQ ID NO: 24. In some embodiments, the sense strand has a sequence of SEQ ID NO: 25 and the antisense strand has a sequence of SEQ ID NO: 26. In some embodiments, the sense strand has a sequence of SEQ ID NO: 27 and the antisense strand has a sequence of SEQ ID NO: 28. In some embodiments, the sense strand has a sequence of SEQ ID NO: 29 and the antisense strand has a sequence of SEQ ID NO: 30. In some embodiments, the sense strand has a sequence of SEQ ID NO: 31 and the antisense strand has a sequence of SEQ ID NO: 32. In some embodiments, the sense strand has a sequence of SEQ ID NO: 33 and the antisense strand has a sequence of SEQ ID NO: 34. In some embodiments, the sense strand has a sequence of SEQ ID NO: 35 and the antisense strand has a sequence of SEQ ID NO: 36. In some embodiments, the sense strand has a sequence of SEQ ID NO: 37 and the antisense strand has a sequence of SEQ ID NO: 38. In some embodiments, the sense strand has a sequence of SEQ ID NO: 39 and the antisense strand has a sequence of SEQ ID NO: 40. In some embodiments, the sense strand has a sequence of SEQ ID NO: 41 and the antisense strand has a sequence of SEQ ID NO: 42. In some embodiments, the sense strand has a sequence of SEQ ID NO: 43 and the antisense strand has a sequence of SEQ ID NO: 44. In some embodiments, the sense strand has a sequence of SEQ ID NO: 45 and the antisense strand has a sequence of SEQ ID NO: 46. In some embodiments, the sense strand has a sequence of SEQ ID NO: 47 and the antisense strand has a sequence of SEQ ID NO: 48. In some embodiments, the sense strand has a sequence of SEQ ID NO: 49 and the antisense strand has a sequence of SEQ ID NO: 50. In some embodiments, the sense strand has a sequence of SEQ ID NO: 51 and the antisense strand has a sequence of SEQ ID NO: 2. In some embodiments, the sense strand has a sequence of SEQ ID NO: 53 and the antisense strand has a sequence of SEQ ID NO:

54. In some embodiments, the sense strand has a sequence of SEQ ID NO: 55 and the antisense strand has a sequence of SEQ ID NO: 56. In some embodiments, the sense strand has a sequence of SEQ ID NO: 57 and the antisense strand has a sequence of SEQ ID NO: 58. In some embodiments, the sense strand has a sequence of SEQ ID NO: 59 and the antisense strand has a sequence of SEQ ID NO: 60. In some embodiments, the sense strand has a sequence of SEQ ID NO: 61 and the antisense strand has a sequence of SEQ ID NO: 62. In some embodiments, the sense strand has a sequence of SEQ ID NO: 63 and the antisense strand has a sequence of SEQ ID NO: 64. In some embodiments, the sense strand has a sequence of SEQ ID NO: 65 and the antisense strand has a sequence of SEQ ID NO: 66. In some embodiments, the sense strand has a sequence of SEQ ID NO: 67 and the antisense strand has a sequence of SEQ ID NO: 68. In some embodiments, the sense strand has a sequence of SEQ ID NO: 69 and the antisense strand has a sequence of SEQ ID NO: 70. In some embodiments, the sense strand has a sequence of SEQ ID NO: 71 and the antisense strand has a sequence of SEQ ID NO: 72. In some embodiments, the sense strand has a sequence of SEQ ID NO: 73 and the antisense strand has a sequence of SEQ ID NO: 74. In some embodiments, the sense strand has a sequence of SEQ ID NO: 75 and the antisense strand has a sequence of SEQ ID NO: 76. In some embodiments, the sense strand has a sequence of SEQ ID NO: 77 and the antisense strand has a sequence of SEQ ID NO: 78. In some embodiments, the sense strand has a sequence of SEQ ID NO: 79 and the antisense strand has a sequence of SEQ ID NO: 80. In some embodiments, the sense strand has a sequence of SEQ ID NO: 81 and the antisense strand has a sequence of SEQ ID NO: 82. In some embodiments, the sense strand has a sequence of SEQ ID NO: 83 and the antisense strand has a sequence of SEQ ID NO: 84. In some embodiments, the sense strand has a sequence of SEQ ID NO: 85 and the antisense strand has a sequence of SEQ ID NO: 86. In some embodiments, the sense strand has a sequence of SEQ ID NO: 87 and the antisense strand has a sequence of SEQ ID NO: 88. In some embodiments, the sense strand has a sequence of SEQ ID NO: 89 and the antisense strand has a sequence of SEQ ID NO: 90. In some embodiments, the sense strand has a sequence of SEQ ID NO: 91 and the antisense strand has a sequence of SEQ ID NO: 92. In some embodiments, the sense strand has a sequence of SEQ ID NO: 93 and the antisense strand has a sequence of SEQ ID NO: 94. In some embodiments, the sense strand has a sequence of SEQ ID NO: 95 and the antisense strand has a sequence of SEQ ID NO: 96. In some embodiments, the sense strand has a sequence of SEQ ID NO: 97 and the antisense strand has a sequence of SEQ ID NO:

98. In some embodiments, the sense strand has a sequence of SEQ ID NO: 99 and the antisense strand has a sequence of SEQ ID NO: 100. In some embodiments, the sense strand has a sequence of SEQ ID NO: 101 and the antisense strand has a sequence of SEQ ID NO: 102. In some embodiments, the sense strand has a sequence of SEQ ID NO: 103 and the antisense strand has a sequence of SEQ ID NO: 104. In some embodiments, the sense strand has a sequence of SEQ ID NO: 105 and the antisense strand has a sequence of SEQ ID NO: 106. In some embodiments, the sense strand has a sequence of SEQ ID NO: 107 and the antisense strand has a sequence of SEQ ID NO: 108. In some embodiments, the sense strand has a sequence of SEQ ID NO: 109 and the antisense strand has a sequence of SEQ ID NO: 110. In some embodiments, the sense strand has a sequence of SEQ ID NO: 111 and the antisense strand has a sequence of SEQ ID NO: 112. In some embodiments, the sense strand has a sequence of SEQ ID NO: 113 and the antisense strand has a sequence of SEQ ID NO: 114. In some embodiments, the sense strand has a sequence of SEQ ID NO: 115 and the antisense strand has a sequence of SEQ ID NO: 116. In some embodiments, the sense strand has a sequence of SEQ ID NO: 117 and the antisense strand has a sequence of SEQ ID NO: 118. In some embodiments, the sense strand has a sequence of SEQ ID NO: 119 and the antisense strand has a sequence of SEQ ID NO: 120. In some embodiments, the sense strand has a sequence of SEQ ID NO: 121 and the antisense strand has a sequence of SEQ ID NO: 122. In some embodiments, the sense strand has a sequence of SEQ ID NO: 123 and the antisense strand has a sequence of SEQ ID NO: 124. In some embodiments, the sense strand has a sequence of SEQ ID NO: 125 and the antisense strand has a sequence of SEQ ID NO: 126.

In some embodiments, the RNA complex provided herein comprises a chemical modification, wherein the modification facilitates the penetration of a cellular membrane in the absence of a delivery vehicle. In some embodiments, the modification is a 2′-O-methylated nucleoside, a phosphorothioate bond or a cholesterol moiety. In some embodiments, the RNA complex is a modified RNA complex listed in Table 2 or Table 4. In certain embodiments, the RNA complex is not cytotoxic.

The RNA complexes described herein can employ a variety of oligonucleotide chemistries. Examples of oligonucleotide chemistries include, without limitation, peptide nucleic acid (PNA), linked nucleic acid (LNA), phosphorothioate, 2'O-Me-modified oligonucleotides, and morpholino chemistries, including combinations of any of the foregoing. In general, PNA and LNA chemistries can utilize shorter targeting sequences

because of their relatively high target binding strength relative to 2'O-Me oligonucleotides. Phosphorothioate and 2'O-Me-modified chemistries are often combined to generate 2'O-Me-modified oligonucleotides having a phosphorothioate backbone. See, *e.g.*, PCT Publication Nos. WO/2013/112053 and WO/2009/008725.

Peptide nucleic acids (PNAs) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone, consisting of N-(2-aminoethyl) glycine units to which pyrimidine or purine bases are attached. PNAs containing natural pyrimidine and purine bases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and mimic DNA in terms of base pair recognition. The backbone of PNAs is formed by peptide bonds rather than phosphodiester bonds, making them well-suited for antisense applications (see structure below). The backbone is uncharged, resulting in PNA/DNA or PNA/RNA duplexes that exhibit greater than normal thermal stability. PNAs are not recognized by nucleases or proteases.

Despite a radical structural change to the natural structure, PNAs are capable of sequence-specific binding in a helix form to DNA or RNA. Characteristics of PNAs include a high binding affinity to complementary DNA or RNA, a destabilizing effect caused by single-base mismatch, resistance to nucleases and proteases, hybridization with DNA or RNA independent of salt concentration and triplex formation with homopurine DNA. PANAGENE.TM. has developed its proprietary Bts PNA monomers (Bts; benzothiazole-2-sulfonyl group) and proprietary oligomerization process. The PNA oligomerization using Bts PNA monomers is composed of repetitive cycles of deprotection, coupling and capping. PNAs can be produced synthetically using any technique known in the art. See, *e.g.*, U.S. Pat. Nos. 6,969,766, 7,211,668, 7,022,851, 7,125,994, 7,145,006 and 7,179,896. See also U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262 for the preparation of PNAs. Further teaching of PNA compounds can be found in Nielsen *et al.*, *Science*, 254:1497-1500, 1991.

Interfering nucleic acids may also contain "locked nucleic acid" subunits (LNAs). "LNAs" are a member of a class of modifications called bridged nucleic acid (BNA). BNA is characterized by a covalent linkage that locks the conformation of the ribose ring in a C3-endo (northern) sugar pucker. For LNA, the bridge is composed of a methylene between the 2'-O and the 4'-C positions. LNA enhances backbone preorganization and base stacking to increase hybridization and thermal stability.

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The structures of LNAs can be found, for example, in Wengel, *et al.*, *Chemical Communications* (1998) 455; *Tetrahedron* (1998) 54:3607, and *Accounts of Chem. Research* (1999) 32:301); Obika, *et al.*, *Tetrahedron Letters* (1997) 38:8735; (1998) 39:5401, and *Bioorganic Medicinal Chemistry* (2008) 16:9230. Compounds provided herein may incorporate one or more LNAs; in some cases, the compounds may be entirely composed of LNAs. Methods for the synthesis of individual LNA nucleoside subunits and their incorporation into oligonucleotides are described, for example, in U.S. Pat. Nos. 7,572,582, 7,569,575, 7,084,125, 7,060,809, 7,053,207, 7,034,133, 6,794,499, and 6,670,461. Typical intersubunit linkers include phosphodiester and phosphorothioate moieties; alternatively, non-phosphorous containing linkers may be employed. One embodiment is an LNA-containing compound where each LNA subunit is separated by a DNA subunit. Certain compounds are composed of alternating LNA and DNA subunits where the intersubunit linker is phosphorothioate.

In certain embodiments, the RNA complex is linked to a cholesterol moiety. In some embodiments, the cholesterol moiety is attached to the 3′ terminus of the sense strand. In some embodiments, the cholesterol moiety is attached to the 3′ terminus of the antisense strand. In some embodiments, the cholesterol moiety is attached to the 5′ terminus of the sense strand. In some embodiments, the cholesterol moiety is attached to the 5′ terminus of the antisense strand.

In some embodiments, the RNA complex comprises a 2′-O-methylated nucleoside. 2′-O-methylated nucleosides carry a methyl group at the 2′-OH residue of the ribose molecule. 2′-O-Me-RNAs show the same (or similar) behavior as RNA, but are protected against nuclease degradation. 2′-O-Me-RNAs can also be combined with phosphothioate oligonucleotides (PTOs) for further stabilization. 2′-O-Me-RNAs (phosphodiester or phosphothioate) can be synthesized according to routine techniques in the art (see, *e.g.*, Yoo *et al.*, *Nucleic Acids Res.* 32:2008-16, 2004).

In some embodiments, the 2′-O-methyl nucleoside is positioned at the 3′ terminus of the sense strand. In some embodiments, 3′ terminal region of the sense strand comprises a plurality of 2′-O-methylated nucleosides (*e.g.,* 2, 3, 4, 5 or 6 2′-O-methylated nucleosides within 6 nucleosides of the 3′ terminus). In some embodiments, the 2′-O-methyl nucleoside is positioned at the 3′ terminus of the antisense strand. In some embodiments, 3′ terminal region of the antisense strand comprises a plurality of 2′-O-methylated nucleosides (*e.g.,* 2,

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3, 4, 5 or 6 2′-O-methylated nucleosides within 6 nucleosides of the 3′ terminus). In some embodiments, both the 3′ terminal region of the sense strand and the 3′ terminal region of the antisense strand comprise a plurality of 2′-O-methylated nucleosides. In some embodiments, the sense strand comprises 2′-O-methylated nucleosides that alternate with unmodified nucleosides. In some embodiments, the sense strand comprises a contiguous sequence of 2, 3, 4, 5, 6, 7 or 8 2′-O-methylated nucleosides that alternate with unmodified nucleosides. In some embodiments, the anti-sense strand comprises 2′-O-methylated nucleosides that alternate with unmodified nucleosides. In some embodiments, the anti-sense strand comprises a contiguous sequence of 2, 3, 4, 5, 6, 7 or 8 2′-O-methylated nucleosides that alternate with unmodified nucleosides.

In some embodiments, the RNA complex comprises a phosphorothioate bond. "Phosphorothioates" (or S-oligos) are a variant of normal DNA in which one of the nonbridging oxygens is replaced by a sulfur. The sulfurization of the internucleotide bond reduces the action of endo-and exonucleases including 5' to 3' and 3' to 5' DNA POL 1 exonuclease, nucleases S1 and P1, RNases, serum nucleases and snake venom phosphodiesterase. Phosphorothioates are made by two principal routes: by the action of a solution of elemental sulfur in carbon disulfide on a hydrogen phosphonate, or by the method of sulfurizing phosphite triesters with either tetraethylthiuram disulfide (TETD) or 3H-1,2-benzodithiol-3-one 1,1-dioxide (BDTD) (see, *e.g.*, Iyer *et al.*, *J. Org. Chem.* 55, 4693-4699, 1990). The latter methods avoid the problem of elemental sulfur's insolubility in most organic solvents and the toxicity of carbon disulfide. The TETD and BDTD methods also yield higher purity phosphorothioates.

In some embodiments, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds. In some embodiments, all of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds.

In some embodiments, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds. In some embodiments, all of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds.

The RNA complexes described herein may be contacted with a cell or administered to an organism (*e.g.,* a human). Alternatively, constructs and/or vectors encoding the RNA complexes may be contacted with or introduced into a cell or organism. In certain embodiments, a viral, retroviral or lentiviral vector is used.

The RNA complexes described herein can be prepared by any appropriate method known in the art. For example, in some embodiments, the RNA complexes described herein are prepared by chemical synthesis or in vitro transcription.

Pharmaceutical Compositions

In certain aspects, provided herein is a pharmaceutical composition comprising an RNA complex provided herein and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is formulated for topical delivery. In some embodiments, the pharmaceutical composition is a cream or a lotion. In some embodiments, the pharmaceutical composition further comprises a second skin lightening agent (*e.g.,* hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid or tranexamic acid). In certain embodiments, the pharmaceutical composition does not comprise a transfection vehicle. In some embodiments, the pharmaceutical composition comprises a delivery vehicle (*e.g.,* liposomes, cationic polymers, cell penetrating peptides (CPPs), protein transduction domains (PTDs), antibodies and/or aptamers). In some embodiments, the composition includes a combination of multiple (*e.g.,* two or more) of the RNA complexes described herein.

As described in detail below, the pharmaceutical compositions disclosed herein may be specially formulated for administration in solid or liquid form, including those adapted for topical administration (*e.g.,* as a cream or lotion).

Methods of preparing these formulations or compositions include the step of bringing into association an RNA complex described herein with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association an agent described herein with liquid carriers.

The pharmaceutical compositions described herein can be provided in any cosmetically and/or dermatologically suitable form, for example, an emulsion, a cream, a mousse, a gel, a foam, a lotion, a mask, an ointment, a pomade, a solution, a serum, a spray, a stick, a patch, or a towelette. For example, pharmaceutical compositions for topical administration can be more or less fluid and have the appearance of a white or colored cream, of an ointment, of a milk, of a lotion, of a serum, of a paste, of a mousse or of a gel.

It can, where appropriate, be applied to the skin in the form of an aerosol. It can also be present in solid form and, for example, be in the form of a stick. It can be used as a care product and/or as a skin makeup product.

In some embodiments, the pharmaceutical compositions described herein can, in addition to the RNA complex, contain at least one compound selected from: hydroquinone, arbutin, tretinoin, azelaic acid, tranexamic acid, α-hydroxyacids; salicylic acid and its derivatives such as n-octanoyl-5-salicylic acid; HEPES; procysteine; O-octanoyl-6-D-maltose; the disodium salt of methylglycinediacetic acid; ceramides; steroids such as diosgenin and derivatives of DHEA; kojic acid; N-ethyloxycarbonyl-4-paraaminophenol; ascorbic acid and its derivatives; bilberry extracts; retinoids and, in particular, retinol and its esters; polypeptides and their acylated derivatives; phytohormones; extracts of the yeast Saccharomyces cerevisiae; algal extracts; extracts of Vitreoscilla filiformis; extracts of soybean, lupin, corn and/or pea; alverine and its salts, in particular alverine citrate; resveratrol; carotenoids and, in particular, lycopene; tocopherol and its esters; coenzyme Q10 or ubiquinone; xanthines and, in particular, caffeine and the natural extracts containing it; extracts of butcher's-broom and horse-chestnut; and their mixtures.

In some embodiments, the pharmaceutical compositions described herein can contain at least one UVA and/or UVB filter. The sunscreen filters can be selected from organic filters and inorganic filters and combinations thereof.

Examples of organic filters that block transmission of UV-A and/or the UV-B include: derivatives of paraminobenzoic acid (*e.g.*, PABA, ethyl PABA, ethyldihydroxypropyl PABA, ethylhexyldimethyl PABA, glyceryl PABA, PEG-25 PABA), salicylic derivatives (*e.g.*, homosalate, ethylhexyl salicylate, dipropyleneglycol salicylate, TEA salicylate) derivatives of dibenzoylmethane (*e.g.*, butylmethoxydibenzoylmethane, isopropyldibenzoylmethane), cinnamic derivatives (*e.g.*, ethylhexyl methoxycinnamate, isopropylmethoxycinnamate, isoamylmethoxycinnamate, cinoxate, DEA methoxycinnamate, diisopropyl methylcinnamate, glyceryl ethylhexanoate dimethoxycinnamate), derivatives of β,β'-diphenylacrylate (*e.g.*, octocrylene, etocrylene), derivatives of benzophenone (*e.g.*, benzophenone-1, benzophenone-2, benzophenone-3 or oxybenzone, benzophenone-4, benzophenone-5, benzophenone-6, benzophenone-8, benzophenone-9, and benzophenone-12), derivatives of benzylidene camphor (*e.g.*, 3-benzylidene camphor, 4-methylbenzylidene camphor, benzylidene camphor sulfonic acid, camphor benzalkonium methosulfate, terephthalylidene dicamphor sulfonic acid and

polyacrylamidomethyl benzylidene camphor), derivatives of phenyl benzimidazole (*e.g.*, phenylbenzimidazole sulfonic acid, and benzimidazilate), derivatives of triazine (*e.g.*, anisotriazine, ethylhexyl triazone, and diethylhexyl-butamidotriazone), derivatives of phenyl benzotriazole (*e.g.*, drometrizole trisiloxane), anthranilic derivatives (menthyl anthranilate), imidazoline derivatives (*e.g.*, ethylhexyldimethoxy-benzylidenedioxoimidazoline propionate), derivatives of benzalmalonate (polyorganosiloxane) and combinations thereof.

Examples of inorganic filters that block transmission of UV-A and/or the UV-B include: or uncoated metallic oxide nanopigments (mean size of the primary particles: in general, from 5 nm to 100 nm, preferably from 10 nm to 50 nm), such as nanopigments of titanium oxide (amorphous or crystallized in rutile and/or anatase form), of iron oxide, of zinc oxide, of zirconium oxide or of cerium oxide. Coating agents are, in addition, alumina and/or aluminum stearate.

In certain embodiments, the pharmaceutical compositions described herein also contain other cosmetic and dermatological ingredients, such as hydrophilic or lipophilic gelatinizing agents, preservatives, antioxidants, solvents, surfactants, thickeners, perfumes, fillers, pigments, odor absorbers and coloring substances.

In certain embodiments, the pharmaceutical compositions described herein also contain oils. Examples of oils that can be included in the pharmaceutical composition described herein include: hydrocarbonaceous oils of animal origin (*e.g.*, perhydrosqualene), hydrocarbonaceous oils of vegetable origin (*e.g.*, liquid fatty acid triglycerides which comprise from 4 to 10 carbon atoms and the liquid fraction of karite butter), synthetic esters and ethers of fatty acids (*e.g.*, the oils of the formulae R^1COOR^2 and R^1OR^2 in which R^1 represents the residue of a fatty acid comprising from 8 to 29 carbon atoms and R^2 represents a branched or unbranched hydrocarbon chain which contains from 3 to 30 carbon atoms, such as Purcellin's oil, isononyl isononanoate, isopropyl myristate, ethyl-2-hexyl palmitate, octyl-2-dodecyl stearate, octyl-2-dodecyl erucate, and isostearyl isostearate; hydroxylated esters such as isostearyl lactate, octylhydroxystearate, octyldodecyl hydroxystearate, diisostearylmalate, triisocetyl citrate, and heptanoates, octanoates and decanoates of fatty alcohols; polyol esters, such as propylene glycol dioctanoate, neopentylglycol diheptanoate and diethyleneglycol diisononanoate; and pentaerythritol esters, such as pentaerythrityl tetraisostearate), linear or branched hydrocarbons of mineral or synthetic origin (*e.g.*, volatile or nonvolatile paraffin oils and their derivatives,

petrolatum, polydecenes, and hydrogenated polyisobutene such as parleam oil), fatty alcohols having from 8 to 26 carbon atoms (*e.g.*, cetyl alcohol and stearyl alcohol and their mixture octyldodecanol, 2-butyloctanol, 2-hexyldecanol, 2-undecylpentadecanol, oleic alcohol or linoleic alcohol), partially hydrocarbonaceous and/or siliconaceous fluorinated oils, silicone oils (*e.g.*, volatile or nonvolatile polymethylsiloxanes (PDMS) which have a linear or cyclic siliconaceous chain and which are liquid or pasty at ambient temperature, in particular cyclopoly-dimethylsiloxanes (cyclomethicones) such as cyclohexasiloxane; polydimethylsiloxanes which comprise alkyl, alkoxy or phenyl groups which are pendent or at the end of the siliconaceous chain, with the groups having from 2 to 24 carbon atoms; phenylated silicones such as phenyltrimethicones, phenyldimethicones, phenyl-trimethylsiloxydiphenylsiloxanes, diphenyldimethicones, diphenylmethyldiphenyltrisiloxanes, 2-phenylethyltrimethylsiloxysilicates and polymethylphenylsiloxanes), and combinations thereof.

Examples of emulsifiers and coemulsifiers which can be included in the pharmaceutical compositions described herein include O/W emulsifiers, such as esters of fatty acid and polyethylene glycol, in particular PEG-100 stearate, and esters of fatty acid and glycerol, such as glyceryl stearate, as well as W/O emulsifiers such as the oxyethylenated poly(methylcetyl)(dimethyl)-methylsiloxane or the mixture of ethylene glycol acetyl stearate and glyceryl tristearate.

Hydrophilic gelatinizing agents that can be included in the pharmaceutical compositions described herein include carboxyvinylic polymers (carbomer), acrylic polymers such as acrylate/alkyl acrylate copolymers, polyacrylamides, polysaccharides, natural gums and clays, while lipophilic gelatinizing agents which may be mentioned are modified clays such as bentonites, metallic salts of fatty acids, hydrophobic silica and polyethylenes.

Examples of fillers that may be included in the pharmaceutical compositions described herein include pigments, silica powder, talc, starch which is crosslinked with octenylsuccinic anhydride, polyamide particles, polyethylene powders, microspheres based on acrylic copolymers, expanded powders such as hollow microspheres, silicone resin microbeads and combinations thereof.

In certain embodiments, the pharmaceutical compositions described herein are formulated for transdermal modes of delivery, such as patches and the like, with or without a suitable skin penetration enhancer. Accordingly, a transdermal means of delivering a

composition or formulation (often with a skin penetration enhancer composition) to the skin is that of the transdermal patch or a similar device as known and described in the art. Examples of such devices are disclosed in U.S. Pat. Nos. 5,146,846, 5,223,262, 4,820,724, 4,379,454 and 4,956,171. In some embodiments, the composition described herein is delivered by a microneedle patch. Exemplary microneedle patches are described in U.S. Pat. Nos. 5,697,901, 6,503,231, 6,611,707, 6,660,987, 8,162,901, 8,696,637 and 8,784,363.

Therapeutic Methods

In certain aspects, provided herein is a method of inhibiting tyrosinase expression by a cell (*e.g.,* a melanocyte) comprising contacting the cell with an RNA complex provided herein. In some embodiments, the RNA complex is a modified RNA complex and the cell is contacted with the RNA complex in the absence of a transfection vehicle. In some embodiments, the cell is contacted with the RNA complex in the presence of a delivery vehicle (*e.g.,* a liposome, cationic polymer, cell penetrating peptide (CPPs), protein transduction domain (PTDs), antibody and/or aptamer). In some embodiments, the cell is present in the skin of a human subject. In some embodiments, the subject has a skin pigmentation disorder associated with excessive melanin production (*e.g.,* melasma or age spots). In some embodiments, the subject is female. In some embodiments, the subject is pregnant or is taking oral or patch contraceptives or is undergoing hormone replacement therapy.

In certain aspects, provided herein is a method of inhibiting melanin production by a cell (*e.g.,* a melanocyte) comprising contacting the cell with an RNA complex provided herein. In some embodiments, the RNA complex is a modified RNA complex and the cell is contacted with the RNA complex in the absence of a transfection vehicle. In some embodiments, the cell is contacted with the RNA complex in the presence of a delivery vehicle (*e.g.,* a liposome, cationic polymer, cell penetrating peptide (CPPs), protein transduction domain (PTDs), antibody and/or aptamer). In some embodiments, the cell is present in the skin of a human subject. In some embodiments, the subject has a skin pigmentation disorder associated with excessive melanin production (*e.g.,* melasma or age spots). In some embodiments, the subject is female. In some embodiments, the subject is

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pregnant or is taking oral or patch contraceptives or is undergoing hormone replacement therapy.

In certain aspects, provided herein is a method of inhibiting melanin production in the skin of a human subject comprising administering to the subject an RNA complex or pharmaceutical composition provided herein. In some embodiments, the subject has a skin pigmentation disorder associated with excessive melanin production (*e.g.,* melasma or age spots). In some embodiments, the subject is female. In some embodiments, the subject is pregnant or is taking oral or patch contraceptives or is undergoing hormone replacement therapy. In certain embodiments, the RNA complex or pharmaceutical composition is administered topically to the skin of the subject. In some embodiments, the RNA complex or pharmaceutical composition is self-administered by the subject. In some embodiments, the method further comprises administering to the subject a second skin lightening agent (*e.g.,* hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid or tranexamic acid).

In certain aspects, provided herein is a method of treating a human subject for a skin pigmentation disorder associated with excessive melanin production (*e.g.,* melasma or age spots) comprising administering to the subject an RNA complex or pharmaceutical composition provided herein. In some embodiments, the subject is female. In some embodiments, the subject is pregnant or is taking oral or patch contraceptives or is undergoing hormone replacement therapy. In certain embodiments, the RNA complex or pharmaceutical composition is administered topically to the skin of the subject. In some embodiments, the RNA complex or pharmaceutical composition self-administered by the subject. In some embodiments, the method further comprises administering to the subject a second skin lightening agent (*e.g.,* hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid or tranexamic acid).

In the present methods, an RNA complex described herein can be administered to the subject, for example, as nucleic acid without delivery vehicle (*e.g.,* for cp-asiRNAs and cp-lasiRNAs), in combination with a delivery reagent, and/or as a nucleic acid comprising sequences that express the RNA complex described herein. In some embodiments, any nucleic acid delivery method known in the art can be used in the methods described herein. Suitable delivery reagents include, but are not limited to, *e.g.,* the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (*e.g.,* polylysine), atelocollagen, nanoplexes and liposomes. The use of atelocollagen as a delivery vehicle for nucleic acid molecules is described in Minakuchi *et al. Nucleic Acids Res.*, 32(13):e109

(2004); Hanai *et al. Ann NY Acad Sci.*, 1082:9-17 (2006); and Kawata *et al. Mol Cancer Ther.*, 7(9):2904-12 (2008). Exemplary interfering nucleic acid delivery systems are provided in U.S. Patent Nos. 8,283,461, 8,313,772, 8,501,930. 8,426,554, 8,268,798 and 8,324,366.

In some embodiments of the methods described herein, liposomes are used to deliver an RNA complex described herein to a subject. Liposomes suitable for use in the methods described herein can be formed from standard vesicle-forming lipids, which generally include neutral or negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of factors such as the desired liposome size and half-life of the liposomes in the blood stream. A variety of methods are known for preparing liposomes, for example, as described in Szoka *et al.* (1980), *Ann. Rev. Biophys. Bioeng.* 9:467; and U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

The liposomes for use in the present methods can also be modified so as to avoid clearance by the mononuclear macrophage system ("MMS") and reticuloendothelial system ("RES"). Such modified liposomes have opsonization-inhibition moieties on the surface or incorporated into the liposome structure.

Opsonization-inhibiting moieties for use in preparing the liposomes described herein are typically large hydrophilic polymers that are bound to the liposome membrane. As used herein, an opsonization inhibiting moiety is "bound" to a liposome membrane when it is chemically or physically attached to the membrane, *e.g.,* by the intercalation of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids. These opsonization-inhibiting hydrophilic polymers form a protective surface layer that significantly decreases the uptake of the liposomes by the MMS and RES; *e.g.,* as described in U.S. Pat. No. 4,920,016.

In some embodiments, opsonization inhibiting moieties suitable for modifying liposomes are water-soluble polymers with a number-average molecular weight from about 500 to about 40,000 daltons, or from about 2,000 to about 20,000 daltons. Such polymers include polyethylene glycol (PEG) or polypropylene glycol (PPG) derivatives; *e.g.,* methoxy PEG or PPG, and PEG or PPG stearate; synthetic polymers such as

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polyacrylamide or poly N-vinyl pyrrolidone; linear, branched, or dendrimeric polyamidoamines; polyacrylic acids; polyalcohols, *e.g.,* polyvinylalcohol and polyxylitol to which carboxylic or amino groups are chemically linked, as well as gangliosides, such as ganglioside GM1. Copolymers of PEG, methoxy PEG, or methoxy PPG, or derivatives thereof, are also suitable. In addition, the opsonization inhibiting polymer can be a block copolymer of PEG and either a polyamino acid, polysaccharide, polyamidoamine, polyethyleneamine, or polynucleotide. The opsonization inhibiting polymers can also be natural polysaccharides containing amino acids or carboxylic acids, *e.g.,* galacturonic acid, glucuronic acid, mannuronic acid, hyaluronic acid, pectic acid, neuraminic acid, alginic acid, carrageenan; aminated polysaccharides or oligosaccharides (linear or branched); or carboxylated polysaccharides or oligosaccharides, *e.g.,* reacted with derivatives of carbonic acids with resultant linking of carboxylic groups. In some embodiments, the opsonization-inhibiting moiety is a PEG, PPG, or derivatives thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called "PEGylated liposomes."

The pharmaceutical compositions disclosed herein may be delivered by any suitable route of administration, including topically, orally and parenterally. In certain embodiments the pharmaceutical compositions are delivered generally (*e.g.,* via oral or parenteral administration). In certain other embodiments the pharmaceutical compositions are delivered locally through direct administration to the skin.

Actual dosage levels of the RNA complexes in the pharmaceutical compositions may be varied so as to obtain an amount of RNA complex that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could prescribe and/or administer doses of the agents employed in the

pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Similarly, an individual user could apply increasing amounts of the composition until the desired level of whitening is achieved.

In general, a suitable daily dose of an RNA complex described herein will be that amount of the RNA complex which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

EXEMPLIFICATION

Example 1: Screening for tyrosinase-specific asymmetric small interfering RNAs

To identify asymmetric small interfering RNAs (asiRNAs) that inhibit tyrosinase with high efficiency, 62 asiRNAs were synthesized and screened. The nucleic acid sequences of the screened asiRNAs are provided in Table 1.

Table 1: Nucleic acid sequences for exemplary tyrosinase-targeting asiRNA.

SEQ ID NO.:	SEQUENCE
1	asiTYR(1)S : CAGGGCUUGUGAGCUU
2	asiTYR(1)AS : AAGCUCACAAGCCCUGCCAGC
3	asiTYR(2)S : AUAGAGUAGGGCCAAA
4	asiTYR(2)AS : UUUGGCCCUACUCUAUUGCCU
5	asiTYR(3)S : GAAAUCCAGAAGCUGA
6	asiTYR(3)AS : UCAGCUUCUGGAUUUCUUGUU
7	asiTYR(4)S : GCUGACAGGAGAUGAA
8	asiTYR(4)AS : UUCAUCUCCUGUCAGCUUCUG
9	asiTYR(5)S : AACAAGAAAUCCAGAA
10	asiTYR(5)AS : UUCUGGAUUUCUUGUUCCCAC
11	asiTYR(6)S : GAUUGGAGGAGUACAA
12	asiTYR(6)AS : UUGUACUCCUCCAAUCGGCUA
13	asiTYR(7)S : ACAAGCGAGUCGGAUC
14	asiTYR(7)AS : GAUCCGACUCGCUUGUUCCAA
15	asiTYR(8)S : GCCGAUUGGAGGAGUA
16	asiTYR(8)AS : UACUCCUCCAAUCGGCUACUA
17	asiTYR(9)S : UGAAGCACCAGCUUUU
18	asiTYR(9)AS : AAAAGCUGGUGCUUCAUGGGC
19	asiTYR(10)S : AAUGAAAAAUGGAUCA
20	asiTYR(10)AS : UGAUCCAUUUUUCAUUUGGCC
21	asiTYR(11)S: ACAAGAAAUCCAGAAG
22	asiTYR(11)AS : CUUCUGGAUUUCUUGUUCCCA

23	asiTYR(12)S : CCGAUUGGAGGAGUAC
24	asiTYR(12)AS : GUACUCCUCCAAUCGGCUACA
25	asiTYR(13)S : CAGCUGAUGUAGAAUU
26	asiTYR(13)AS : AAUUCUACAUCAGCUGAAGAG
27	asiTYR(14)S : CUGGCGGGAUGCAGAA
28	asiTYR(14)AS : UUCUGCAUCCCGCCAGUCCCA
29	asiTYR(15)S : AGGAGUACAACAGCCA
30	asiTYR(15)AS : UGGCUGUUGUACUCCUCCAAU
31	asiTYR(16)S : GCUAUGACUAUAGCUA
32	asiTYR(16)AS : UAGCUAUAGUCAUAGCCCAGA
33	asiTYR(17)S : CCCAUGUUUAACGACA
34	asiTYR(17)AS : UGUCGUUAAACAUGGGUGUUG
35	asiTYR(18)S : UAGACUCUUCUUGUUG
36	asiTYR(18)AS : CAACAAGAAGAGUCUAUGCCA
37	asiTYR(19)S : CUGUGGAGUUUCCAGA
38	asiTYR(19)AS : UCUGGAAACUCCACAGCAGGC
39	asiTYR(20)S : CAGGCAGAGGUUCCUG
40	asiTYR(20)AS : CAGGAACCUCUGCCUGAAAGC
41	asiTYR(21)S : GGACCUGCCAGUGCUC
42	asiTYR(21)AS : GAGCACUGGCAGGUCCUAUUA
43	asiTYR(22)S : UACUCAGCCCAGCAUC
44	asiTYR(22)AS : GAUGCUGGGCUGAGUAAGUUA
45	asiTYR(23) S : UCAGUCUUUAUGCAAU
46	asiTYR(23) AS : AUUGCAUAAAGACUGAUGGCU
47	asiTYR(24) S : ACAAGAUUCAGACCCA
48	asiTYR(24) AS : UGGGUCUGAAUCUUGUAGAUA
49	asiTYR(25) S : CAAGCGAGUCGGAUCU
50	asiTYR(25) AS : AGAUCCGACUCGCUUGUUCCA
51	asiTYR(26) S : UAAAAGGCUUAGGCAA
52	asiTYR(26) AS : UUGCCUAAGCCUUUUAUAAAU
53	asiTYR(27) S : CUAUAUGAAUGGAACA
54	asiTYR(27) AS : UGUUCCAUUCAUAUAGAUGUG
55	asiTYR(28) S : AAGAUCUGGGCUAUGA
56	asiTYR(28) AS : UCAUAGCCCAGAUCUUUGGAU
57	asiTYR(29) S : GUCCAAUGCACCACUU
58	asiTYR(29) AS : AAGUGGUGCAUUGGACAGAAG
59	asiTYR(30) S : UCACAGGGGUGGAUGA
60	asiTYR(30) AS : UCAUCCACCCCUGUGAAGGGA
61	asiTYR(31) S : GGCCUUCCGUCUUUUA
62	asiTYR(31) AS : UAAAAGACGGAAGGCCACGAC

63	asiTYR(32) S : CUGCAAGUUUGGCUUU
64	asiTYR(32) AS : AAAGCCAAACUUGCAGUUUCC
65	asiTYR(33) S : CAGAGAAGGACAAAUU
66	asiTYR(33) AS : AAUUUGUCCUUCUCUGGGGCA
67	asiTYR(34) S : GCAUACCAUCAGCUCA
68	asiTYR(34) AS : UGAGCUGAUGGUAUGCUUUGC
69	asiTYR(35) S : UUGGGGGAUCUGAAAU
70	asiTYR(35) AS : AUUUCAGAUCCCCCAAGCAGU
71	asiTYR(36) S : UCAGCACCCCACAAAU
72	asiTYR(36) AS : AUUUGUGGGGUGCUGACCUCC
73	asiTYR(37) S : GCCCGAGGGACCUUUA
74	asiTYR(37) AS : UAAAGGUCCCUCGGGCGUUCC
75	asiTYR(38) S : CCAUGUUUAACGACAU
76	asiTYR(38) AS : AUGUCGUUAAACAUGGGUGUU
77	asiTYR(39) S : UGACAGGAGAUGAAAA
78	asiTYR(39) AS : UUUUCAUCUCCUGUCAGCUUC
79	asiTYR(40) S : CAACUUCAUGGGAUUC
80	asiTYR(40) AS : GAAUCCCAUGAAGUUGCCAGA
81	asiTYR(41) S : GUUCCUGUCAGAAUAU
82	asiTYR(41) AS : AUAUUCUGACAGGAACCUCUG
83	asiTYR(42) S : CCUAUGGCCAAAUGAA
84	asiTYR(42) AS : UUCAUUUGGCCAUAGGUCCCU
85	asiTYR(43) S : UUCCUGUCAGAAUAUC
86	asiTYR(43) AS : GAUAUUCUGACAGGAACCUCU
87	asiTYR(44) S : AGGUUCCUGUCAGAAU
88	asiTYR(44) AS : AUUCUGACAGGAACCUCUGCC
89	asiTYR(45) S : GGCAACUUCAUGGGAU
90	asiTYR(45) AS : AUCCCAUGAAGUUGCCAGAGC
91	asiTYR(46) S : AACUUCAUGGGAUUCA
92	asiTYR(46) AS : UGAAUCCCAUGAAGUUGCCAG
93	asiTYR(47) S : ACCUAUGGCCAAAUGA
94	asiTYR(47) AS : UCAUUUGGCCAUAGGUCCCUA
95	asiTYR(48) S : UAUGGCCAAAUGAAAA
96	asiTYR(48) AS : UUUUCAUUUGGCCAUAGGUCC
97	asiTYR(49) S : CUGACAGGAGAUGAAA
98	asiTYR(49) AS : UUUCAUCUCCUGUCAGCUUCU
99	asiTYR(50) S : AGCUGACAGGAGAUGA
100	asiTYR(50) AS : UCAUCUCCUGUCAGCUUCUGG
101	asiTYR(51) S : ACCCAUGUUUAACGAC
102	asiTYR(51) AS : GUCGUUAAACAUGGGUGUUGA

103	asiTYR(52) S : AACACCCAUGUUUAAC
104	asiTYR(52) AS : GUUAAACAUGGGUGUUGAUCC
105	asiTYR(53) S : CAGUCUUUAUGCAAUG
106	asiTYR(53) AS : CAUUGCAUAAAGACUGAUGGC
107	asiTYR(54) S : AUCAGUCUUUAUGCAA
108	asiTYR(54) AS : UUGCAUAAAGACUGAUGGCUG
109	asiTYR(55) S : CUUGGUGAGAAGAAAC
110	asiTYR(55) AS : GUUUCUUCUCACCAAGAGUCG
111	asiTYR(56) S : CUGCCAACGAUCCUAU
112	asiTYR(56) AS : AUAGGAUCGUUGGCAGAUCCC
113	asiTYR(57) S : UCCUACAUGGUUCCUU
114	asiTYR(57) AS : AAGGAACCAUGUAGGAUUCCC
115	asiTYR(58) S : CUUUGUCUGGAUGCAU
116	asiTYR(58) AS : AUGCAUCCAGACAAAGAGGUC
117	asiTYR(59) S : ACAUUUGCACAGAUGA
118	asiTYR(59) AS : UCAUCUGUGCAAAUGUCACAC
119	asiTYR(60) S : GCGGAUGCCUCUCAAA
120	asiTYR(60) AS : UUUGAGAGGCAUCCGCUAUCC
121	asiTYR(61) S : AACCGGGAAUCCUACA
122	asiTYR(61) AS : UGUAGGAUUCCCGGUUAUGUC
123	asiTYR(62) S : GGACAUAACCGGGAAU
124	asiTYR(62) AS : AUUCCCGGUUAUGUCCAAUGG

The asiRNAs listed in Table 1 were incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in 1x siRNA duplex buffer (STpharm). Proper strand annealing was confirmed via gel electrophoresis. For the screen, 1.6×10^4 A375 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco) and 100 μg/ml penicillin / streptomycin in a 100 mm cell culture dish were seeded in 24-well plates. The A375 cells were transfected with 0.3 nM of the asiRNAs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

The tyrosinase mRNA levels in the transfected cells were measured 24 hours after transfection using real-time RTPCR. Specifically, total RNA were extracted using Isol-RNA lysis reagent (5PRIME), and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. The synthesized cDNA was diluted and then quantitative real-time PCR was performed using the StepOne real-time PCR system (Applied Biosystems) according to manufacturer's instructions. Amplification of the

tyrosinase gene was detected using a power SYBR green PCR master Mix (Applied Biosystems).GAPDH was amplified as an internal control. The following primer sequences were used:

Human GAPDH-forward 5′-GAG TCA ACG GAT TTG GTC GT-3′ (SEQ ID NO:125)
Human GAPDH-reverse 5′-GAC AAG CTT CCC GTT CTC AG-3′ (SEQ ID NO: 126)
Human Tyrosinase-forward : 5′-GGA TCT GGT CAT GGC TCC TT-3′ (SEQ ID NO: 127)
Human Tyrosinase)-reverse : 5′-GTC AGG CTT TTT GGC CCT AC-3′ (SEQ ID NO: 128)

The level of tyrosinase inhibition by each of the 62 asiRNAs is provided in Figure 1. Six of the asiRNA sequences, asiTYR(4), asiTYR(9), asiTYR(10), asiTYR(17), asiTYR(44) and asiTYR(45), were selected for use in follow-up studies.

Example 2: Chemical modification of asiRNAs for self-delivery

Chemical modifications were applied to the six asiRNAs selected in Example 1 and the cellular delivery of the modified asiRNAs was tested in the absence of other delivery vehicle. As described below, certain of the modifications improved endocytosis and stability of the asiRNAs. Such cell penetrating asiRNAs (cp-asiRNAs) are able to be delivered into the cell in the absence of a delivery vehicle.

Thirty-eight potential cp-asiRNAs (Table 2) were screened for tyrosinase mRNA inhibition in MNT-1 cells. Each potential cp-asiRNA was incubated with MNT-1 cells, a human melanoma cell line, at 1 μM without a delivery vehicle and tyrosinase mRNA levels were measured by Real-Time PCR.

Table 2. Modified asiRNA sequences tested for self-delivery and tyrosinase inhibition. m = 2'-O-Methyl RNA. * = phosphorothioate bond.

asiTYR(4)-1 S : GCUGACAGGAGAUG*A*A*cholesterol
asiTYR(4)-1 AS : UUCAUCUCCUGUCAGCU*U*C*U*G
asiTYR(4)-2 S : GCUGACAGGAGAUG*A*A*cholesterol
asiTYR(4)-2 AS : UUCAUCUCCUGUCAGCU*U*mC*mU*mG
asiTYR(4)-3 S : GCUGACAGGAGAUG*A*A*cholesterol
asiTYR(4)-3 AS : UUCAUCUCCUGUCAGCmU*mU*mC*mU*mG
asiTYR(4)-4 S : mGCmUGmACmAGmGAmGAmUG*mA*A*cholesterol
asiTYR(4)-4 AS : UUCAUCUCCUGUCAGCU*U*C*U*G
asiTYR(4)-5 S : mGCmUGmACmAGmGAmGAmUG*mA*A*cholesterol
asiTYR(4)-5 AS : UUCAUCUCCUGUCAGCU*U*mC*mU*mG
asiTYR(4)-6 S : mGCmUGmACmAGmGAmGAmUG*mA*A*cholesterol
asiTYR(4)-6 AS : UUCAUCUCCUGUCAGCmU*mU*mC*mU*mG

asiTYR(9)-1 S : UGAAGCACCAGCUU*U*U*cholesterol
asiTYR(9)-1 AS : AAAAGCUGGUGCUUCAU*G*G*G*C
asiTYR(9)-3 S : UGAAGCACCAGCUU*U*U*cholesterol
asiTYR(9)-3 AS : AAAAGCUGGUGCUUCAmU*mG*mG*mG*mC
asiTYR(9)-4 S : mUGmAAmGCmACmCAmGCmUU*mU*U*cholesterol
asiTYR(9)-4 AS : AAAAGCUGGUGCUUCAU*G*G*G*C
asiTYR(9)-6 S : mUGmAAmGCmACmCAmGCmUU*mU*U*cholesterol
asiTYR(9)-6 AS : AAAAGCUGGUGCUUCAmU*mG*mG*mG*mC
asiTYR(10)-1 S : AAUGAAAAAUGGAU*C*A*cholesterol
asiTYR(10)-1 AS : UGAUCCAUUUUUCAUUU*G*G*C*C
asiTYR(10)-3 S : AAUGAAAAAUGGAU*C*A*cholesterol
asiTYR(10)-3 AS : UGAUCCAUUUUUCAUUmU*mG*mG*mC*mC
asiTYR(10)-4 S : mAAmUGmAAmAAmAUmGGmAU*mC*A*cholesterol
asiTYR(10)-4 AS : UGAUCCAUUUUUCAUUU*G*G*C*C
asiTYR(10)-6 S : mAAmUGmAAmAAmAUmGGmAU*mC*A*cholesterol
asiTYR(10)-6 AS : UGAUCCAUUUUUCAUUmU*mG*mG*mC*mC
asiTYR(17)-1 S : CCCAUGUUUAACGA*C*A*cholesterol
asiTYR(17)-1 AS : UGUCGUUAAACAUGGGU*G*U*U*G
asiTYR(17)-2 S : CCCAUGUUUAACGA*C*A*cholesterol
asiTYR(17)-2 AS : UGUCGUUAAACAUGGGU*G*mU*mU*mG
asiTYR(17)-3 S : CCCAUGUUUAACGA*C*A*cholesterol
asiTYR(17)-3 AS : UGUCGUUAAACAUGGGmU*mG*mU*mU*mG
asiTYR(17)-4 S : mCCmCAmUGmUUmUAmACmGA*mC*A*cholesterol
asiTYR(17)-4 AS : UGUCGUUAAACAUGGGU*G*U*U*G
asiTYR(17)-5 S : mCCmCAmUGmUUmUAmACmGA*mC*A*cholesterol
asiTYR(17)-5 AS : UGUCGUUAAACAUGGGU*G*mU*mU*mG
asiTYR(17)-6 S : mCCmCAmUGmUUmUAmACmGA*mC*A*cholesterol
asiTYR(17)-6 AS : UGUCGUUAAACAUGGGmU*mG*mU*mU*mG
asiTYR(44)-1 S : AGGUUCCUGUCAGA*A*U*cholesterol
asiTYR(44)-1 AS : AUUCUGACAGGAACCUC*U*G*C*C
asiTYR(44)-3 S : AGGUUCCUGUCAGA*A*U*cholesterol
asiTYR(44)-3 AS : AUUCUGACAGGAACCUmC*mU*mG*mC*mC
asiTYR(44)-4 S : mAGmGUmUCmCUmGUmCAmGA*mA*U*cholesterol
asiTYR(44)-4 AS : AUUCUGACAGGAACCUC*U*G*C*C
asiTYR(44)-6 S : mAGmGUmUCmCUmGUmCAmGA*mA*U*cholesterol
asiTYR(44)-6 AS : AUUCUGACAGGAACCUmC*mU*mG*mC*mC
asiTYR(45)-1 S : GGCAACUUCAUGGG*A*U*cholesterol
asiTYR(45)-1 AS : AUCCCAUGAAGUUGCCA*G*A*G*C
asiTYR(45)-3 S : GGCAACUUCAUGGG*A*U*cholesterol
asiTYR(45)-3 AS : AUCCCAUGAAGUUGCCmA*mG*mA*mG*mC
asiTYR(45)-4 S : mGGmCAmACmUUmCAmUGmGG*mA*U*cholesterol
asiTYR(45)-4 AS : AUCCCAUGAAGUUGCCA*G*A*G*C
asiTYR(45)-6 S : mGGmCAmACmUUmCAmUGmGG*mA*U*cholesterol
asiTYR(45)-6 AS : AUCCCAUGAAGUUGCCmA*mG*mA*mG*mC

MNT-1 cells (obtained from Sungkyunkwan University) were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100 μg/ml penicillin/streptomycin, 10% 200 mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene).

The potential cp-asiRNAs listed in Table 2 were incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

One day prior to cp-asiRNA treatment, 2.0 x 10^4 cells were seeded 24 well plates. Immediately before treatment, the MNT-1 cells were washed with 1 x DPBS buffer (Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media. Twenty-four hours later, tyrosinase mRNA levels were in the MNT-1 cells were determined.

The level of tyrosinase inhibition by each of the 38 potential cp-asiRNAs is provided in Figure 2. From among the potential cp-asiRNAs tested, cp-asiTYR(4)-1 was selected for further study.

Example 3: Inhibition of tyrosinase protein and melanin using tyrosine-specific cp-asiRNAs

The efficacy of cp-asiTYR(4)-1 for the inhibition of tyrosinase protein and the suppression of melanin production was tested. To test for non-specific effects, a mutated cp-asiTYR that lacked sequence complementarity to the tyrosinase mRNA sequence (referred to as cp-asiTYR (seed mutation)) was also tested. The sequences of the cp-asiTYR (seed mutation) are provided in Table 3.

Table 3. Sequences used in cp-asiRNA(4)-1 (seed mutation) m = 2'-O-Methyl RNA. * = phosphorothioate bond.

cp-asiTYR(4)-1(seed mutation) S : GCUGACAGGUCUAC*U*A*chol.
cp-asiTYR(4)-1(seed mutation) AS : UAGUAGACCUGUCAGCU*U*C*U*G

The cp-asiRNA was incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

MNT-1 cells were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100 μg/ml penicillin/streptomycin, 10% 200 mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene). One day prior to

treatment, 6.5 x 10^4 MNT-1 cells were seeded in 12-well plates. Immediately before treatment, the MNT-1 cells were washed with 1 x DPBS buffer (Gibco), and then cultured in the presence of 1 μM or 3 μM of cp-asiRNATYR(4)-1 in OPTI-MEM buffer for 24 hours, at which point the OPTI-MEM media was replaced with a serum-containing media.

After 72 hours of cp-asiTYR(4)-1 incubation, the level of tyrosinase protein expression was determined via western blot. Briefly, the treated MNT-1 cells were lysed with RIPA buffer (GE). Fifteen μg of the total protein extract were loaded onto a 12% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and then incubated overnight at 4°C in 5% skim milk containing anti-tyrosinase antibody (Santa Cruz) and anti-β-actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL for 1 minute. The tyrosinase and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad).

The results of the western blot assay are depicted in Figure 3. As a result, in all cp-asiTYR #4-1 incubated cell lines, 70% or more of tyrosinase protein inhibition were confirmed. In addition, the cp-asiTYR was shown to have a higher efficiency in the tyrosinase inhibition ability than other tyrosinase inhibitors such as Hydroquinone and Arbutin (Figure 3).

MNT-1 cells treated with cp-asiTYR(4)-1 as described above were tested for melanin content. After 72 hours of incubation in the presence of cp-asiTYR, the MNT-1 cells were collected, lysed with RIPA buffer (GE) and centrifuged at 13000 rpm. The resulting melanin pellet was dissolved in 100 μL of 1N NaOH (containing 10% DMSO) at 85 °C for 15 minutes and light absorption and melanin production were measured.

As shown in Figure 4, MNT-1 cells treated with 1 μM cp-asiTYR(4)-1 showed greater than 60% inhibition in melanin production, which is higher than when treated with compounds commonly used for melanin production, including hydroquinone (20 μM) and arbutin (2 mM).

Example 4: MNT-1 cell lightening following treatment with cp-asiRNAs

The ability of cp-asiRNA(4)-1 to lighten the color of MNT-1 cells was tested.

As in Example 3, MNT-1 cells were cultured in the presence of 1 μM or 3 μM cp-asiTYR(4)-1. After 72 hours, cells were pelleted and the color change of the cells was observed. As shown in Figure 5, the color of the cp-asiTYR(4)-1 treated cells was lighter than untreated MNT-1 control cells (NT), hydroquinone treated cells and arbutin treated cells.

Example 5: Cytotoxicity of cp-asiRNAs

To test the cytotoxicity of cp-asiRNAs, MNT-1, a human melanoma cell line, and HaCaT, a human keratinocyte cell line were treated with cp-asiTYR #4-1 and hydroquinone.

The cp-asiRNA was incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

One day before treatment with cp-asiRNA(4)-1, 5.0×10^3 MNT-1 cells or 1.0×10^4 HaCaT cells were seeded into 96 well plates. Immediately before treatment, the cells were washed with 1 x DPBS buffer (Gibco), and then cultured in the presence of 1 μM or 3 μM of cp-asiRNATYR(4)-1 in OPTI-MEM buffer for 24 hours, at which point the cytotoxicity level was measured using a CytoTox96 Non-Radio Cytotoxicity assay (Promega) according to manufacturer's instructions. The media was then replaced with the serum-containing media and cell viability was measured using a cell counting kit-8 (Enzo) according to manufacturer's instructions.

As shown in Figure 6, no cytotoxicity or loss of cell viability was observed in either MNT-1 or HaCaT due to treatment with cp-asiRNA. On the other hand, cytotoxicity was observed in HaCaT cells treated with hydroquinone or arbutin.

Example 6: Additional cp-asiRNA structures

A variety of potential cp-asiTYR structures having different strand lengths and numbers of 2'-O-methylation modifications were synthesized and tested for their ability to inhibit tyrosinase expression (Table 4).

Table 4. Additional cp-asiRNA sequences. m = 2'-O-Methyl RNA. * = phosphorothioate bond.

cp-asiTYR(4) S : GCUGACAGGAGAUG*A*A*cholesterol
cp-asiTYR(4) 21AS-1 : UUCAUCUCCUGUCAGCU*U*C*U*G
cp-asiTYR(4) 21AS-2 : UUCAUCUCCUGUCAGCU*U*mC*mU*mG
cp-asiTYR(4) 21AS-3 : UUCAUCUCCUGUCAGCmU*mU*mC*mU*mG
cp-asiTYR(4) 19AS-4 : UUCAUCUCCUGUCAG*C*U*U*C
cp-asiTYR(4) 19AS-5 : UUCAUCUCCUGUCAG*C*mU*mU*mC
cp-asiTYR(4) 19AS-6 : UUCAUCUCCUGUCAmG*mC*mU*mU*mC

The ability of 1 μM of each of the potential cp-asiRNAs listed in Table 4 to inhibit tyrosinase mRNA in MNT-1 cells was tested.

MNT-1 cells were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100 μg/ml penicillin/streptomycin, 10% 200mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene).

The potential cp-asiRNAs listed in Table 4 were incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

One day prior to treatment, 2.0 x 10^4 MNT-1 cells were seeded in 24-well plates. Immediately before treatment, the MNT-1 cells were washed with 1 x DPBS buffer (Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media. Twenty-four hours later, tyrosinase mRNA levels were in the MNT-1 cells were determined.

As seen in Figure 7, tyrosinase mRNA potential cp-asiRNAs containing 4 phosphorothioate bonds on 21 nucleotide antisense strands and potential cp-asiRNAs containing three 2'-O-Methylation and four phosphorothioate bonds on 19 nucleotide antisense strands exhibited the highest levels of tyrosinase inhibition. The cp-asiTYR(4) 21AS-1 and cp-asiTYR(4) 19AS-5 were selected for further experimentation.

The effect of cp-asiTYR(4) 21AS-1 and cp-asiTYR(4) 19AS-5 on the production tyrosinase protein and melanin production was tested.

The cp-asiRNA was incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

MNT-1 cells were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100 µg/ml penicillin/streptomycin, 10% 200 mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene). One day prior to treatment, 6.5 x 10^4 MNT-1 cells were seeded in 12-well plates. Immediately before treatment, the MNT-1 cells were washed with 1 x DPBS buffer (Gibco), and then cultured in the presence of 1 µM or 3 µM of cp-asiRNATYR(4)-1 in OPTI-MEM buffer for 24 hours, at which point the OPTI-MEM media was replaced with a serum-containing media.

The level of tyrosinase protein expression by MNT-1 cells after treatment with 1 µM and 3 µM cp-asiRNAs was determined via western blot. Briefly, the transfected MNT-1 cells were lysed with RIPA buffer (GE). Fifteen µg of the total protein extract were loaded onto a 12% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and then incubated overnight at 4°C in 5% skim milk containing anti-tyrosinase antibody (Santa Cruz) and anti-β-actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL for 1 minute. The tyrosinase and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad).

As seen in Figure 8, treatment with cp-asiTYR(4) 21AS-1 or cp-asiTYR(4) 19AS-5 resulted in a greater than 70% inhibition in the level of tyrosinase protein. In addition, with cp-asiTYR(4) 19AS-5 exhibiting a slightly higher inhibitory activity than cp-asiTYR(4) 21AS-1.

MNT-1 cells treated with cp-asiTYR(4) 21AS-1 or cp-asiTYR(4) 19AS-5 as described above were tested for melanin content. After 72 hours of incubation in the presence of cp-asiTYR, the MNT-1 cells were collected, lysed with RIPA buffer (GE) and centrifuged at 13000 rpm. The resulting melanin pellet was dissolved in 100 µL of 1N NaOH (containing 10% DMSO) at 85 °C for 15 minutes and light absorption and melanin production were measured.

As shown in Figure 9, MNT-1 cells treated with 1 µM cp-asiTYR(4) 21AS-1 or cp-asiTYR(4) 19AS-5 showed about a 50% inhibition in melanin production, which is higher than the inhibition shown in MNT-1 cells treated with 2 mM arbutin.

Additional potential cp-asiTYR structures having different strand lengths, numbers of 2′-O-methylation modifications and numbers of phosphorothioate bond were synthesized and tested for their ability to inhibit tyrosinase expression (Table 5).

Table 5. Additional cp-asiRNA sequences. m = 2'-O-Methyl RNA. * = phosphorothioate bond.

cp-asiTYR(4) S : GCUGACAGGAGAUG*A*A*cholesterol
cp-asiTYR(4) 21AS-1 : UUCAUCUCCUGUCAGCU*U*C*U*G
cp-asiTYR(4) 19AS-7 : UUCAUCUCCUGUC*A*G*C*mU*mU*mC

The effect of cp-asiTYR(4) 21AS-1 and cp-asiTYR(4) 19AS-7 on the tyrosinase protein production was tested.

The cp-asiRNA was incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis.

MNT-1 cells were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100 μg/ml penicillin/streptomycin, 10% 200 mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene). One day prior to treatment, 6.5 x 10^4 MNT-1 cells were seeded in 12-well plates. Immediately before treatment, the MNT-1 cells were washed with 1 x DPBS buffer (Gibco), and then cultured in the presence of 1 μM, 0.6 μM, 0.3 μM and 0.1 μM of cp-asiTYR(4) 21AS-1 and cp-asiTYR(4) 19AS-7 in OPTI-MEM buffer for 24 hours, at which point the OPTI-MEM media was replaced with a serum-containing media.

The level of tyrosinase protein expression by MNT-1 cells after treatment with 1 μM, 0.6 μM, 0.3 μM and 0.1 μM cp-asiRNAs was determined via western blot. Briefly, the transfected MNT-1 cells were lysed with RIPA buffer (GE). Fifteen μg of the total protein extract were loaded onto a 12% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and then incubated overnight at 4°C in 5% skim milk containing anti-tyrosinase antibody (Santa Cruz) and anti-β-actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes

and treated with 1x ECL for 1 minute. The tyrosinase and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad).

As seen in Figure 10, treatment with cp-asiTYR(4) 21AS-1 or cp-asiTYR(4) 19AS-7 resulted in a greater than 70% inhibition in the level of tyrosinase protein. In addition, with cp-asiTYR(4) 21AS-1 exhibiting a slightly higher inhibitory activity than cp-asiTYR(4) 19AS-7.

Example 7: Use of cell penetrating peptide with asiRNAs and lasiRNAs

The combination of asiRNAs or lasiRNA with Pepfect 6 (PF6) cell penetrating peptide was tested for inhibition of tyrosinase mRNA and protein level without use of another transfection reagent.

asiTYR(4) and lasiTYR(21) (Table 6) were incubated at 95 °C for 2 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing of the asiRNA and lasiRNA was confirmed by gel electrophoresis. Annealed RNA and PF6 in DEPC was diluted in 100 µl 0.6 x DPBS with a molar ratio of RNA complex:PF6 of 1:10 and then incubated at room temperature for 30 minutes for complex formation. Proper complex formation was confirmed by gel electrophoresis.

Table 6. Nucleic acid sequence of asiTYR(4) and lasiTYR(21).

asiTYR(4)S : GCUGACAGGAGAUGAA (SEQ ID NO: 7)
asiTYR(4)AS : UUCAUCUCCUGUCAGCUUCUG (SEQ ID NO: 8)
lasiTYR(21)S : GGUUCCUGUCAGAAUA (SEQ ID NO: 125)
lasiTYR(21)AS : UAUUCUGACAGGAACCUCUGCCUGAAAGCUG (SEQ ID NO: 126)

MNT-1 cells were cultured in Minimum Essential Media (Welgene) containing 20% fetal bovine serum (Gibco), 100µg/ml penicillin/streptomycin, 10% 200mM HEPES (Welgene) and 10% Dulbecco's modified Eagle's medium (Welgene). One day prior to treatment, 6.5 x 10^4 MNT-1 cells were seeded in 12-well plates. Four hours prior to treatment, the cell media was replaced with 900 µL of FBS-containing media. The PF6-complexed asiRNA or lasiRNA was added to the cells and the cells were incubated for 24 hours, at which point the media was replaced. Tyrosinase mRNA levels were measured using real-time RT-PCR 24 hours after media replacement.

As seen in Figure 11, MNT-1 cell lines treated with the PF6-complexed asiRNA or lasiRNA had significantly reduced levels of tyrosinase mRNA compared to control.

To test the treated MNT-1 cells for tyrosinase protein expression and melanin production, western blot and melanin content assays were performed as described above 48 hours after media replacement.

As seen in Figure 12, cell lines treated with asiTYR(4)/PF6 complex and lasiTYR(21)/PF6 complex exhibited at least 70% tyrosinase protein inhibition compared to control. Additionally, cells treated with asiTYR(4)/PF6 complex and lasiTYR(21)/PF6 complex exhibited less melanin production than control.

Example 8: Inhibition of melanin synthesis in reconstructed skin model using an exemplary cp-asiRNA

Tyrosinase expression and melanin level was analyzed in an cp-asiTYR#4-1 treated 3-D skin model. MEL-300-B (MatTek), a reconstructed skin model, was used in this study. MEL-300-B was stabilized in EPI-100-NMM-113 media 24 hours before treatment with cp-asiTYR#4-1. For annealing, cp-asiTYR#4-1 dissolved in DEPC-treated water was incubated at 95 °C for 2 minutes and at 37 °C for 1 hour. MEL-300-B samples were treated with cp-asiTYR#4-1 every day for 13 days (final concentration = 5 μM) by adding cp-asiTYR#4-1 directly to the media. As a control, other MEL-300-B samples were treated with kojic acid (Sigma, 2% final) as depicted in Figure 14(a). The samples were harvested at day 14 and the melanocytes in the sample were analyzed using light microscopy. As seen in Figure 14(c), cp-asiTYR#4-1 treatment reduced the level of melanocytes in the treated reconstructed skin model samples. Melanin level in each sample was analyzed using Fontana-Massons staining. As shown in Figure 14(c), ci-asiTYR#4-1 treatment reduced the level of melanin in the treated reconstructed skin model samples.

In order to analyze mRNA level at day 14, samples were harvested in Isol-RNA lysis reagent (5PRIME) and homogenized by using a homogenizer (IKA). Total RNA from the each sample was extracted. For each sample, 500 ng of the extracted RNA was used for cDNA synthesis using the high-capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Quantitative real-time PCR was then performed using the StepOne real-time PCR system (Applied Biosystems). Amplification of the tyrosinase cDNA was detected using a power SYBR green PCR master Mix (Applied Biosystems). GAPDH was amplified as an internal control. As shown in Figure 14(d), ci-asiTYR#4-1 treatment reduced the level of tyrosinase mRNA in the treated reconstructed skin model samples.

Protein level analysis was conducted as using western blot. Harvested samples in RIPA buffer (GE) were homogenized by using homogenizer (IKA) and protein from the each sample was obtained. Fifteen micrograms of the total protein extract were loaded onto a 12% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) that had been previously activated with methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and then incubated overnight at 4 °C in 5% skim milk containing anti-tyrosinase antibody (Santa Cruz) and anti-β-actin antibody (Santa Cruz). The membrane was then washed three times with 1x TBST for 10 minutes and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL (Thermo) for 1 minute. The tyrosinase and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad). As shown in Figure 14e, potent knockdown of tyrosinase protein was observed in the cp-asiTYR#4-1 treated reconstructed skin model sample.

To test melanin content, samples were harvested at day 14, lysed with RIPA buffer (GE) and centrifuged at 13000 rpm. The resulting pellet was dissolved in 100 μL of 1N NaOH (containing 10% DMSO) at 85 °C for 15 minutes and light absorption and melanin production were measured. As shown in Figure 14(f), cp-asiTYR#4-1 treatment reduced melanin level in the treated reconstructed skin model samples.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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CLAIMS

1. An RNA complex comprising:

an antisense strand of at least 19 nucleotides (nt) in length having sequence complementarity to a tyrosinase mRNA sequence and comprising a nucleotide sequence of SEQ ID NO: 8, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 32, SEQ ID NO: 90, or SEQ ID NO: 120; and

a sense strand of 15 to 17 nt in length having sequence complementarity to the antisense strand and comprising a nucleotide sequence of SEQ ID NO: 7, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 31, SEQ ID NO: 89, or SEQ ID NO: 119,

wherein the antisense strand and the sense strand form a complex in which the 5′ end of the antisense strand and the 3′ end of the sense strand form a blunt end.

2. The RNA complex of claim 1, wherein the antisense strand is 19 to 21 nt in length.

3. The RNA complex of claim 1, wherein the antisense strand is 24 to 121 nt in length.

4. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 7 and the antisense strand has a sequence of SEQ ID NO: 8.

5. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 17 and the antisense strand has a sequence of SEQ ID NO: 18.

6. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 19 and the antisense strand has a sequence of SEQ ID NO: 20.

7. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 31 and the antisense strand has a sequence of SEQ ID NO: 32.

8. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 89 and the antisense strand has a sequence of SEQ ID NO: 90.

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9. The RNA complex of claim 1, wherein the sense strand has a sequence of SEQ ID NO: 119 and the antisense strand has a sequence of SEQ ID NO: 120.

10. The RNA complex of any one of claims 1 to 9, wherein the RNA complex further comprises a 2′-O-methylated nucleoside, a phosphorothioate bond or a cholesterol moiety.

11. The RNA complex of claim 10, wherein the cholesterol moiety is attached to the 3′ terminus of the sense strand.

12. The RNA complex of claim 10, wherein the RNA complex comprises a 2′-O-methylated nucleoside positioned at the 3′ terminus of the sense strand or at the 3′ terminus of the antisense strand.

13. The RNA complex of claim 10, wherein the RNA complex comprises a phosphorothioate bond.

14. The RNA complex of claim 4, wherein:
(a) the sense strand is GCUGACAGGAGAUG*A*A*cholesterol; and
(b) the antisense strand is UUCAUCUCCUGUCAGCU*U*C*U*G,
wherein * indicates a phosphorothioate bond.

15. The RNA complex of any one of claims 10 to 14, wherein the RNA complex is capable of penetrating the cellular membrane of a cell in the absence of a delivery vehicle.

16. The RNA complex of any one of claims 1 to 15 for use in reducing melanin production in the skin of a subject.

17. The RNA complex for use of claim 16, wherein the RNA complex is for topical administration to the skin of the subject.

18. The RNA complex of any one of claims 1 to 15 for use in treating a skin pigmentation disorder associated with excessive melanin production in a subject.

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19. The RNA complex for use of claim 18, wherein the skin pigmentation disorder is melasma or age spots.

20. The RNA complex for use of any one of claims 18 to 19, wherein the RNA complex is for topical administration to the skin of the subject.

21. A pharmaceutical composition comprising the RNA complex of any one of claims 1 to 15 and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of claim 21, wherein the composition is formulated for topical delivery.

23. The pharmaceutical composition of claim 22, wherein the pharmaceutical composition is a cream or a lotion.

24. The pharmaceutical composition of any one of claims 21 to 23, wherein the pharmaceutical composition further comprises a second skin lightening agent.

25. The pharmaceutical composition of claim 24, wherein the second skin lightening agent is selected from hydroquinone, arbutin, tretinoin, kojic acid, azelaic acid and tranexamic acid.

26. The pharmaceutical composition of any one of claims 21 to 25 for use in reducing melanin production in the skin of a subject.

27. The pharmaceutical composition for use of claim 26, wherein the pharmaceutical composition is for topical administration to the skin of the subject.

28. The pharmaceutical composition of any one of claims 21 to 25 for use in treating a skin pigmentation disorder associated with excessive melanin production in a subject.

29. The pharmaceutical composition for use of claim 28, wherein the skin pigmentation disorder is melasma or age spots.

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30. The pharmaceutical composition for use of any one of claims 28 to 29, wherein the pharmaceutical composition is for topical administration to the skin of the subject.

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Figure 1

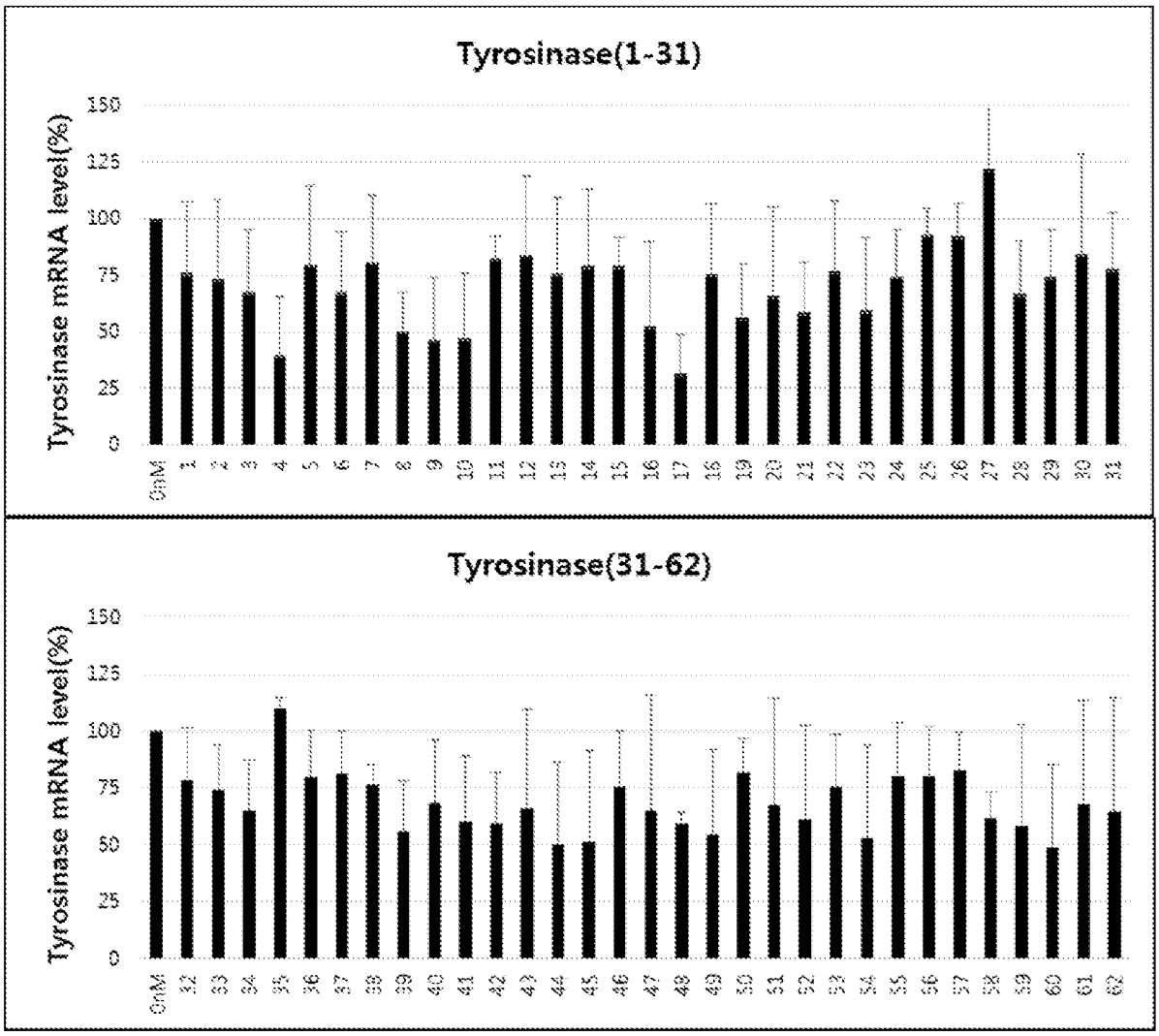

Tyrosinase(1-31)
Tyrosinase mRNA level(%)
150
125
100
75
50
25
0
0nM 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Tyrosinase(31-62)
Tyrosinase mRNA level(%)
150
125
100
75
50
25
0
0nM 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62

Figure 2

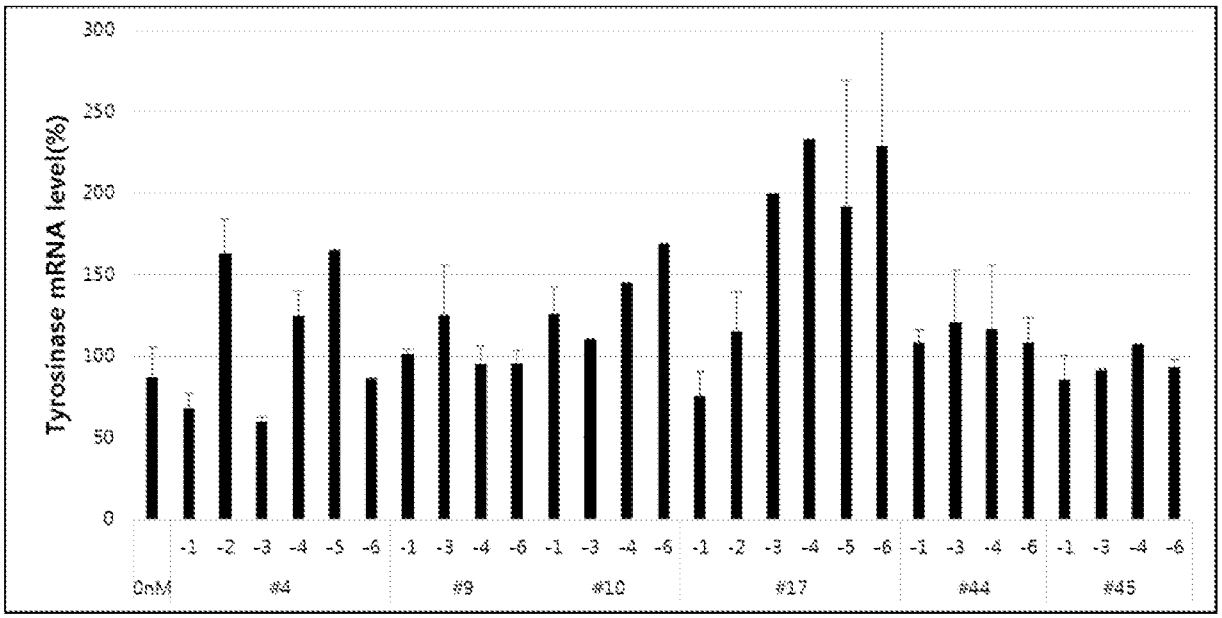

Tyrosinase mRNA level(%)
300
250
200
150
100
50
0
0nM
-1 -2 -3 -4 -5 -6
#4
-1 -3 -4 -6
#9
-1 -3 -4 -6
#10
-1 -2 -3 -4 -5 -6
#17
-1 -3 -4 -6
#44
-1 -3 -4 -6
#45

Figure 3

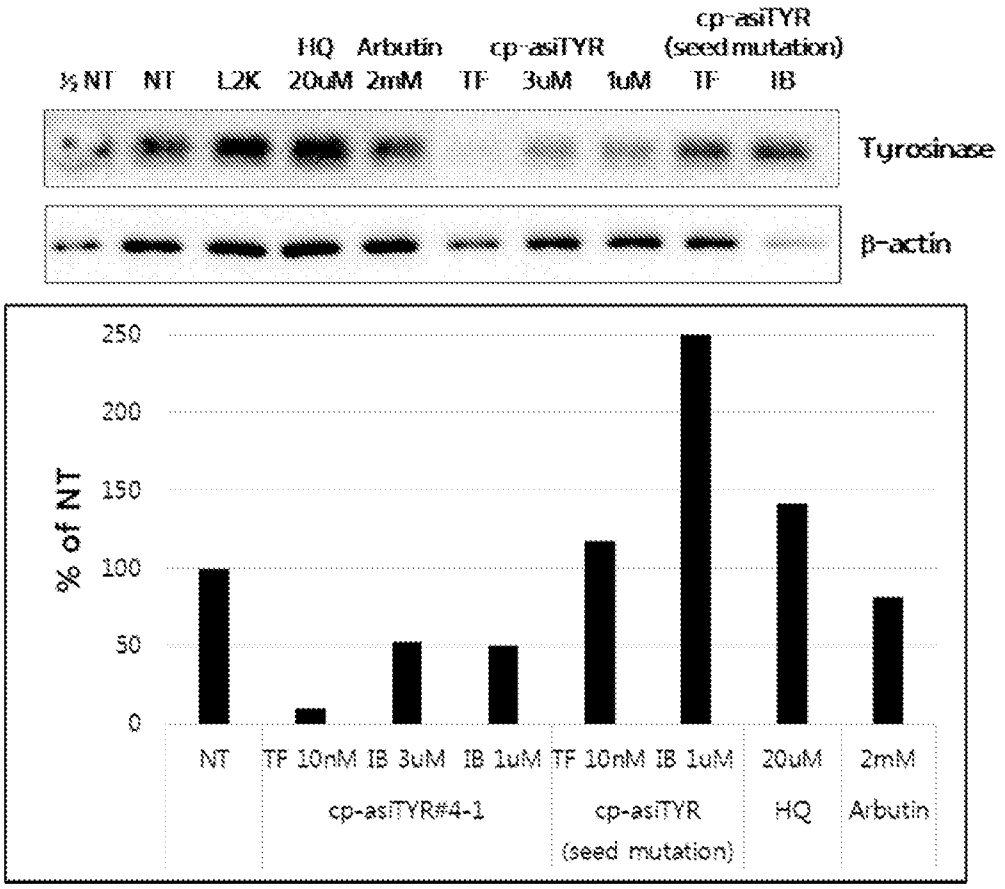

½ NT
NT
L2K
HQ 20uM
Arbutin 2mM
cp-asiTYR TF
cp-asiTYR 3uM
cp-asiTYR 1uM
cp-asiTYR (seed mutation) TF
cp-asiTYR (seed mutation) IB
Tyrosinase
β-actin
% of NT
250
200
150
100
50
0
NT
TF 10nM
IB 3uM
IB 1uM
TF 10nM
IB 1uM
20uM
2mM
cp-asiTYR#4-1
cp-asiTYR (seed mutation)
HQ
Arbutin

Figure 4

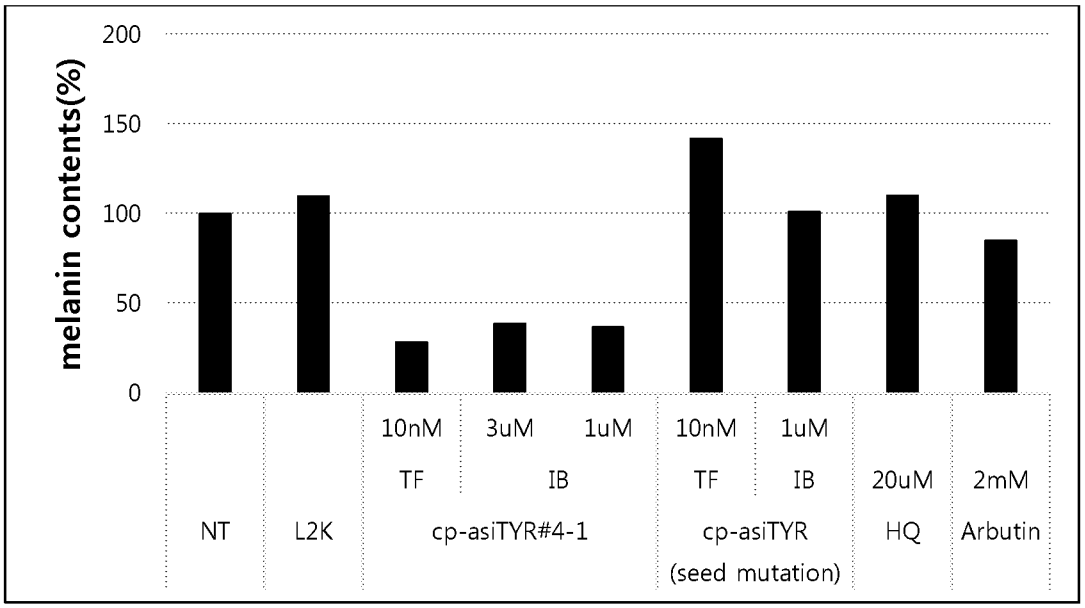
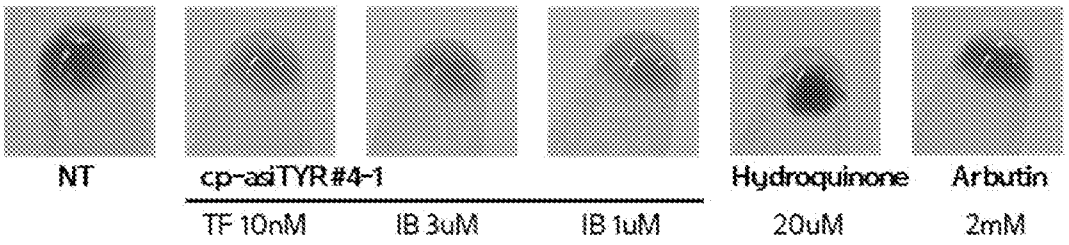

melanin contents(%)
200
150
100
50
0
10nM
3uM
1uM
10nM
1uM
TF
IB
TF
IB
20uM
2mM
NT
L2K
cp-asiTYR#4-1
cp-asiTYR
(seed mutation)
HQ
Arbutin

Figure 5

(a)


NT
cp-asiTYR#4-1
TF 10nM
IB 3uM
IB 1uM
Hydroquinone
20uM
Arbutin
2mM

(b)

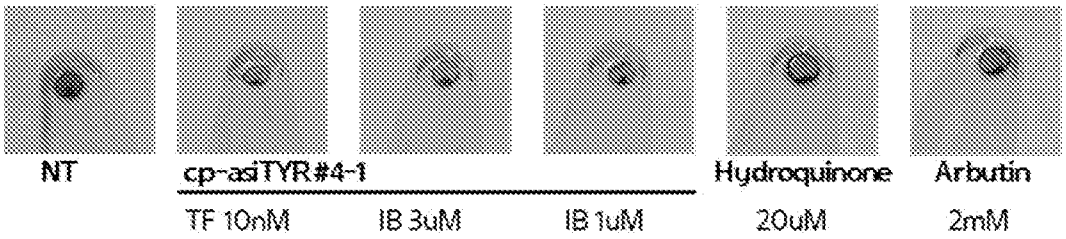

NT
cp-asiTYR#4-1
TF 10nM
IB 3uM
IB 1uM
Hydroquinone
20uM
Arbutin
2mM

Figure 6

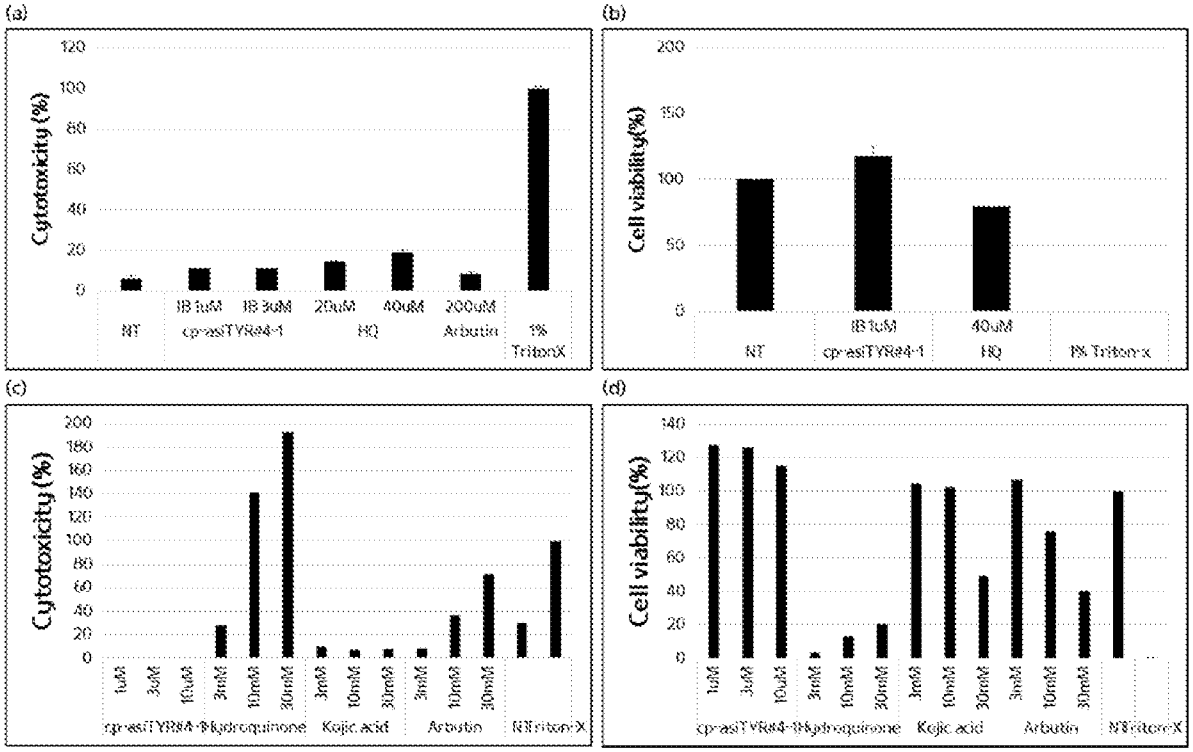

(a)
Cytotoxicity (%)
120
100
80
60
40
20
0
NT
IB 1uM
IB 3uM
cp-rasTYR84-1
20uM
40uM
HQ
200uM
Arbutin
1%
TritonX
(b)
Cell viability(%)
200
150
100
50
0
NT
IB 1uM
cp-rasTYR84-1
40uM
HQ
1% Triton-x
(c)
Cytotoxicity (%)
200
180
160
140
120
100
80
60
40
20
0
1uM
3uM
10uM
3mM
10mM
30mM
3mM
10mM
30mM
3mM
10mM
30mM
cp-rasTYR84-1
Hydroquinone
Kojic acid
Arbutin
NT
Triton-X
(d)
Cell viability(%)
140
120
100
80
60
40
20
0
1uM
3uM
10uM
3mM
10mM
30mM
3mM
10mM
30mM
3mM
10mM
30mM
cp-rasTYR84-1
Hydroquinone
Kojic acid
Arbutin
NT
Triton-X

Figure 7

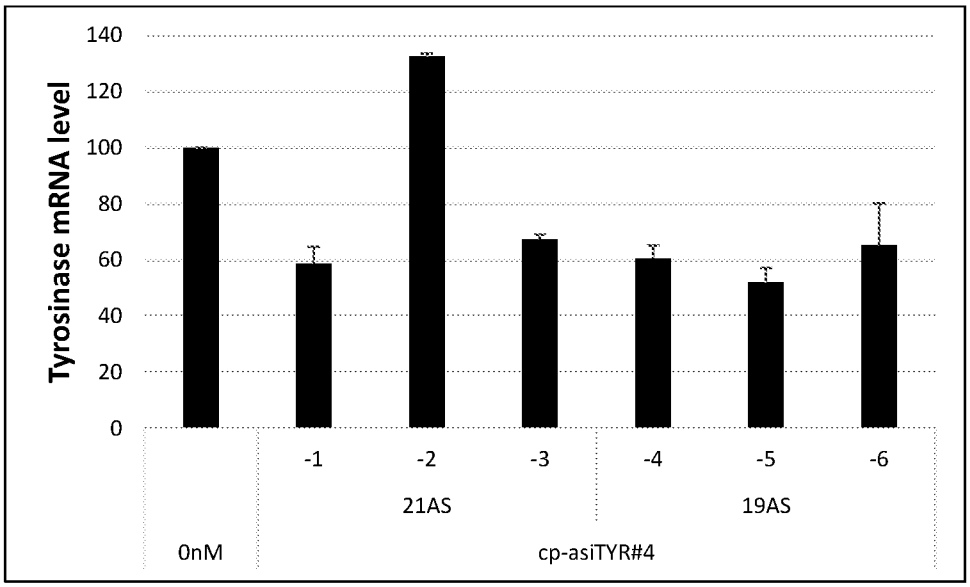

Tyrosinase mRNA level
140
120
100
80
60
40
20
0
-1
-2
-3
-4
-5
-6
21AS
19AS
0nM
cp-asiTYR#4

Figure 8

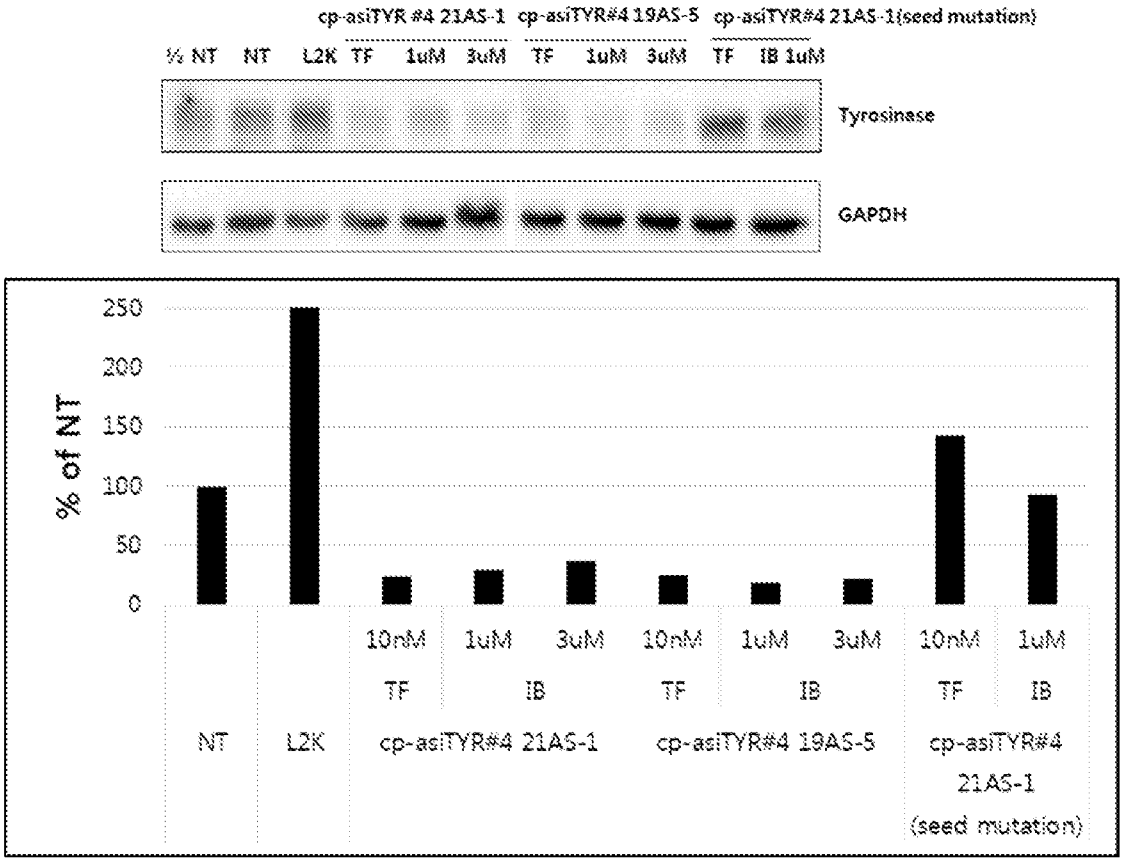

cp-asiTYR #4 21AS-1
cp-asiTYR#4 19AS-5
cp-asiTYR#4 21AS-1(seed mutation)
½ NT
NT
L2K
TF
1uM
3uM
TF
1uM
3uM
TF
IB 1uM
Tyrosinase
GAPDH
% of NT
250
200
150
100
50
0
10nM
1uM
3uM
10nM
1uM
3uM
10nM
1uM
TF
IB
TF
IB
TF
IB
NT
L2K
cp-asiTYR#4 21AS-1
cp-asiTYR#4 19AS-5
cp-asiTYR#4 21AS-1 (seed mutation)

Figure 9

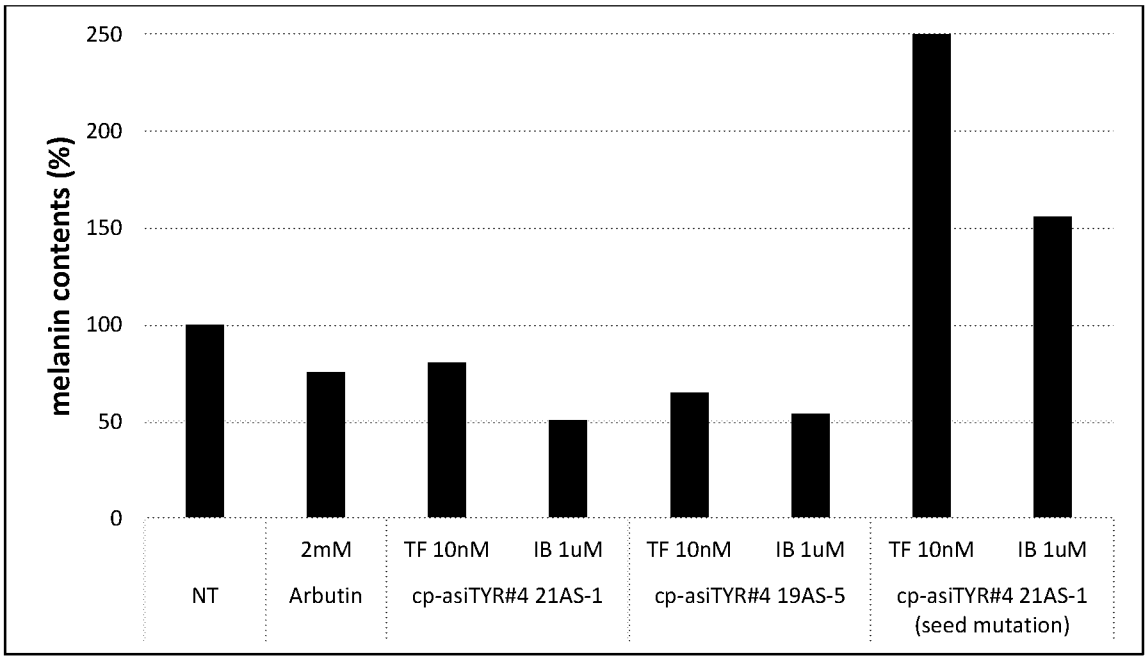

melanin contents (%)
250
200
150
100
50
0
NT
2mM
Arbutin
TF 10nM
IB 1uM
cp-asiTYR#4 21AS-1
TF 10nM
IB 1uM
cp-asiTYR#4 19AS-5
TF 10nM
IB 1uM
cp-asiTYR#4 21AS-1
(seed mutation)

Figure 10

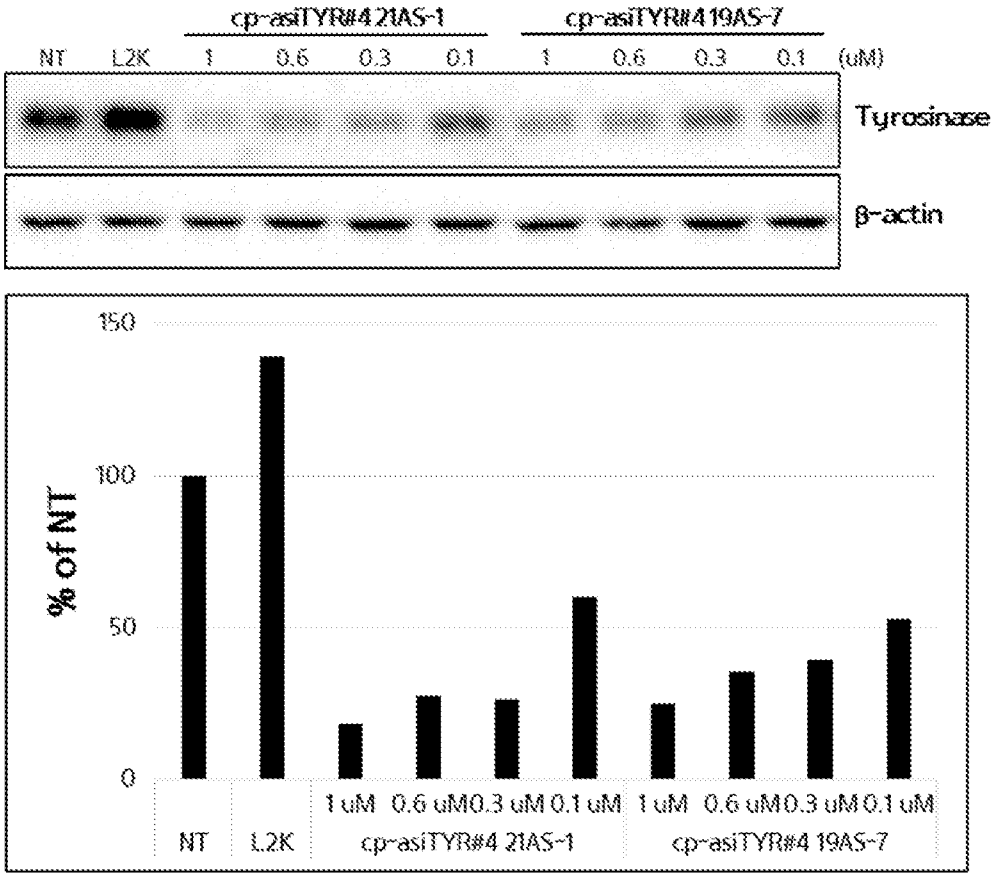

cp-asiTYR#4 21AS-1
cp-asiTYR#4 19AS-7
NT
L2K
1
0.6
0.3
0.1
1
0.6
0.3
0.1
(uM)
Tyrosinase
β-actin
150
100
50
0
% of NT
1 uM
0.6 uM
0.3 uM
0.1 uM
1 uM
0.6 uM
0.3 uM
0.1 uM
NT
L2K
cp-asiTYR#4 21AS-1
cp-asiTYR#4 19AS-7

Figure 11

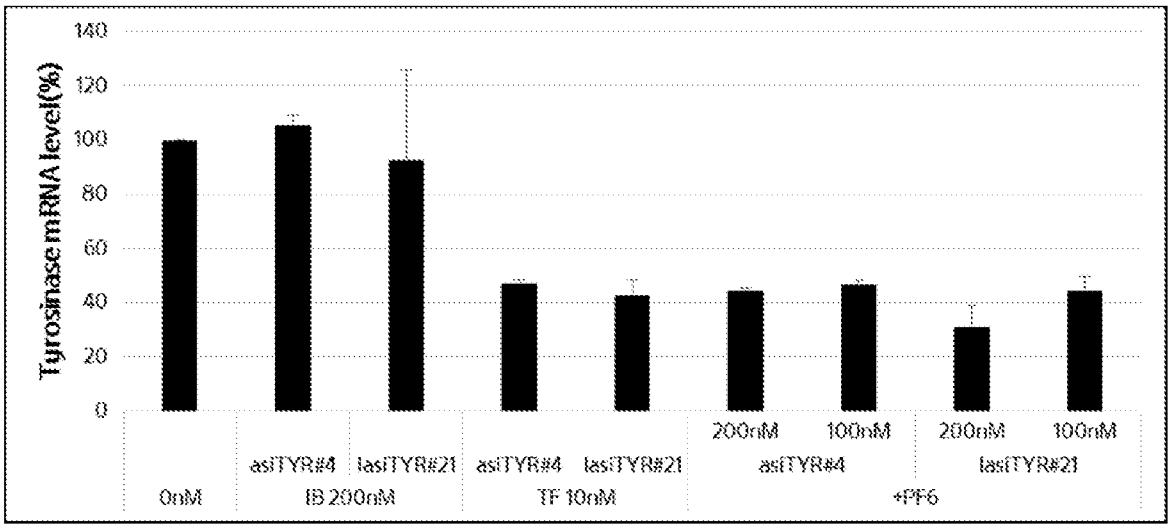
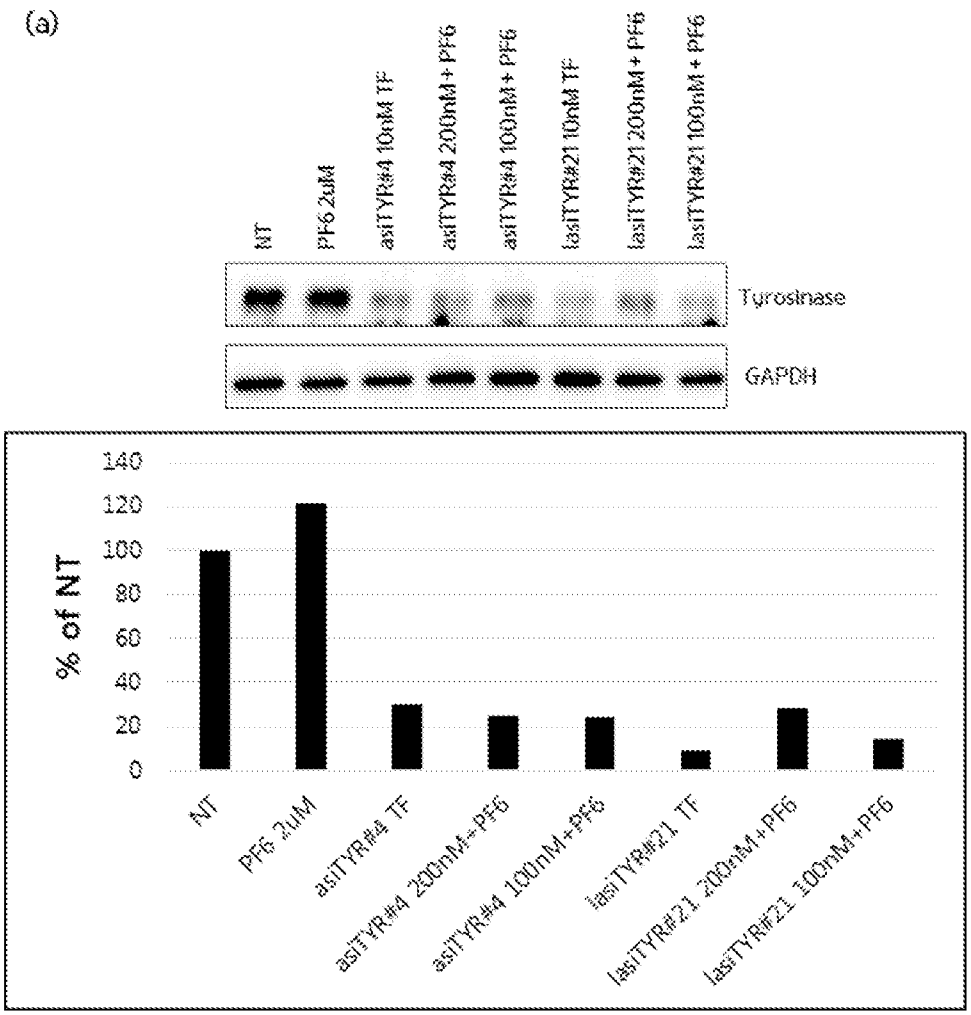

Tyrosinase mRNA level(%)
140
120
100
80
60
40
20
0
0nM
asiTYR#4
lasiTYR#21
IB 200nM
asiTYR#4
lasiTYR#21
TF 10nM
200nM
100nM
asiTYR#4
200nM
100nM
lasiTYR#21
+PF6

Figure 12


(a)
NT
PF6 2uM
asiTYR#4 10nM TF
asiTYR#4 200nM + PF6
asiTYR#4 100nM + PF6
lasiTYR#21 10nM TF
lasiTYR#21 200nM + PF6
lasiTYR#21 100nM + PF6
Tyrosinase
GAPDH
% of NT
140
120
100
80
60
40
20
0
NT
PF6 2uM
asiTYR#4 TF
asiTYR#4 200nM+PF6
asiTYR#4 100nM+PF6
lasiTYR#21 TF
lasiTYR#21 200nM+PF6
lasiTYR#21 100nM+PF6

(b)

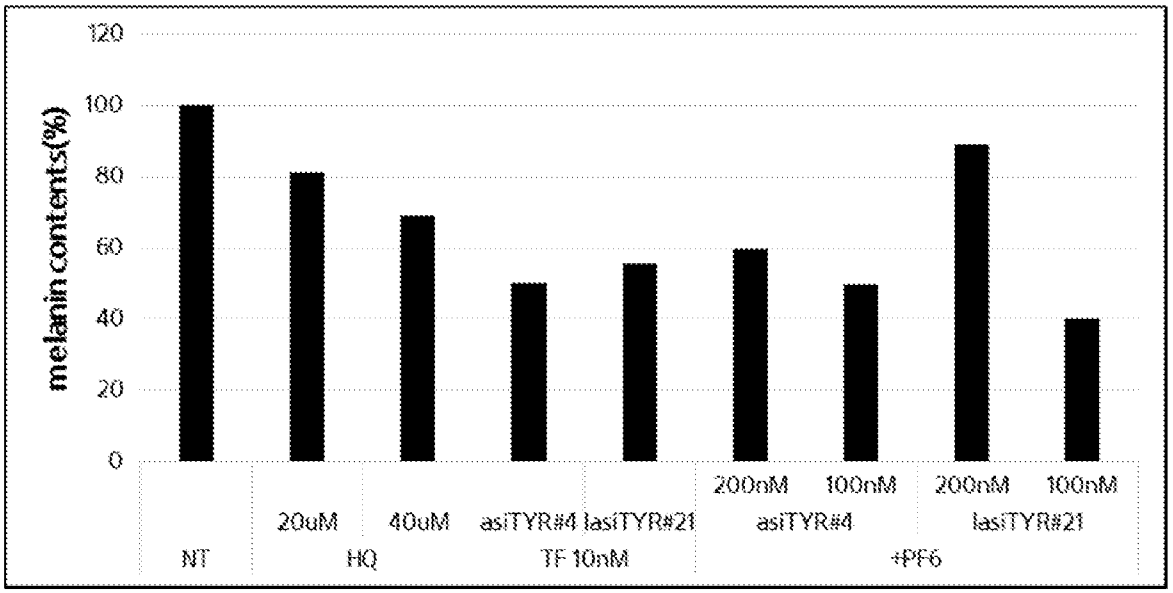
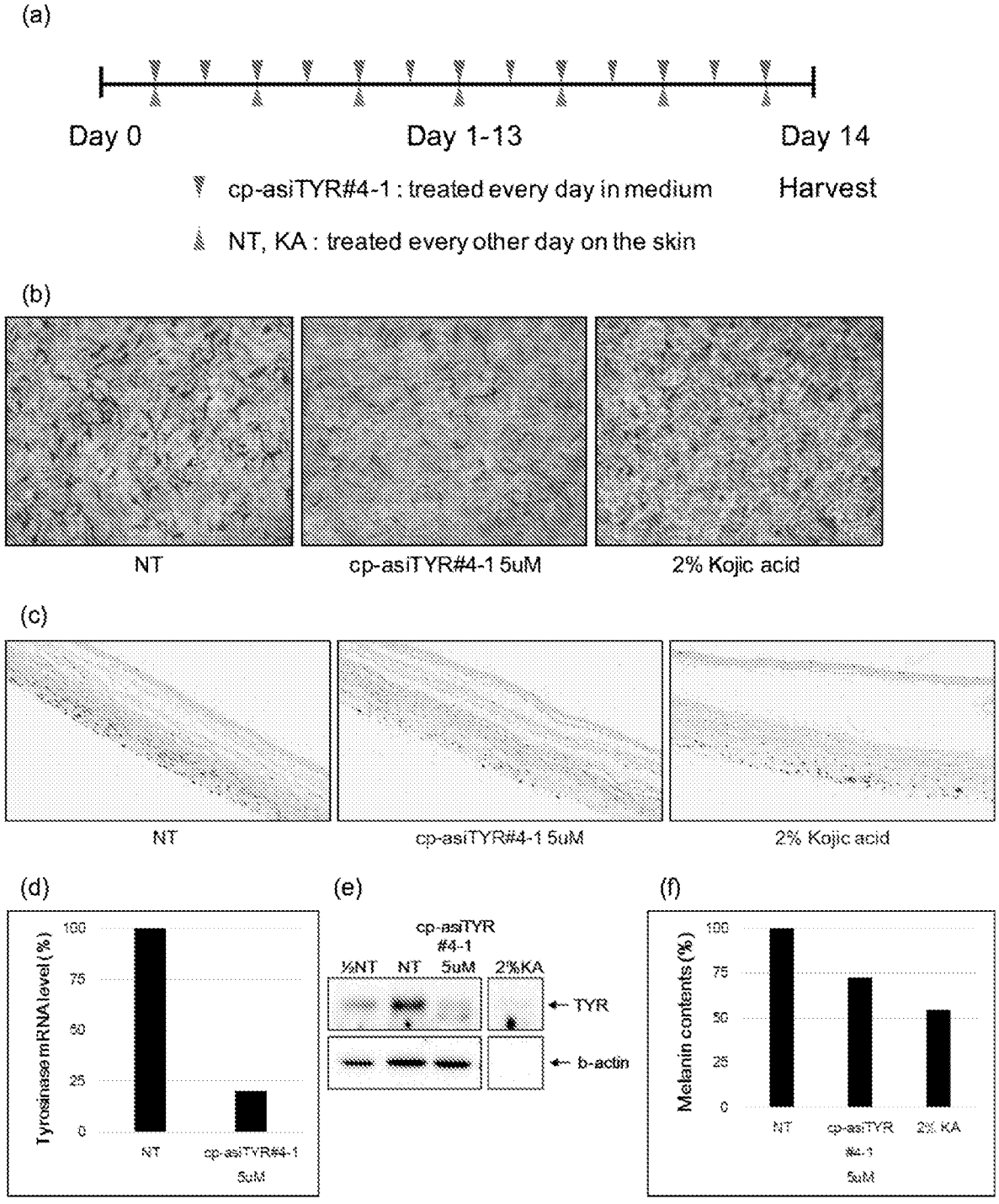

melanin contents(%)
120
100
80
60
40
20
0
NT
20uM
40uM
HQ
asiTYR#4
lasiTYR#21
TF 10nM
200nM
100nM
asiTYR#4
200nM
100nM
lasiTYR#21
+PF6

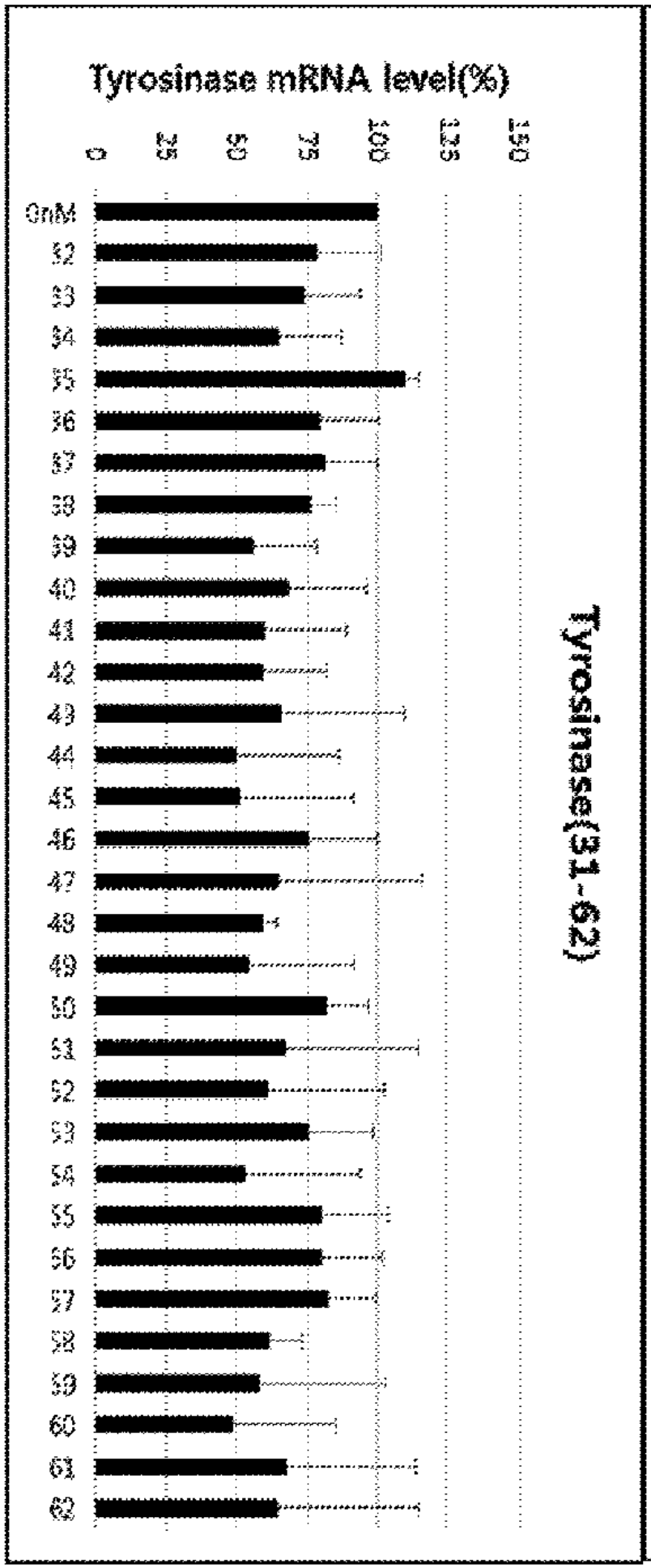
Figure 13

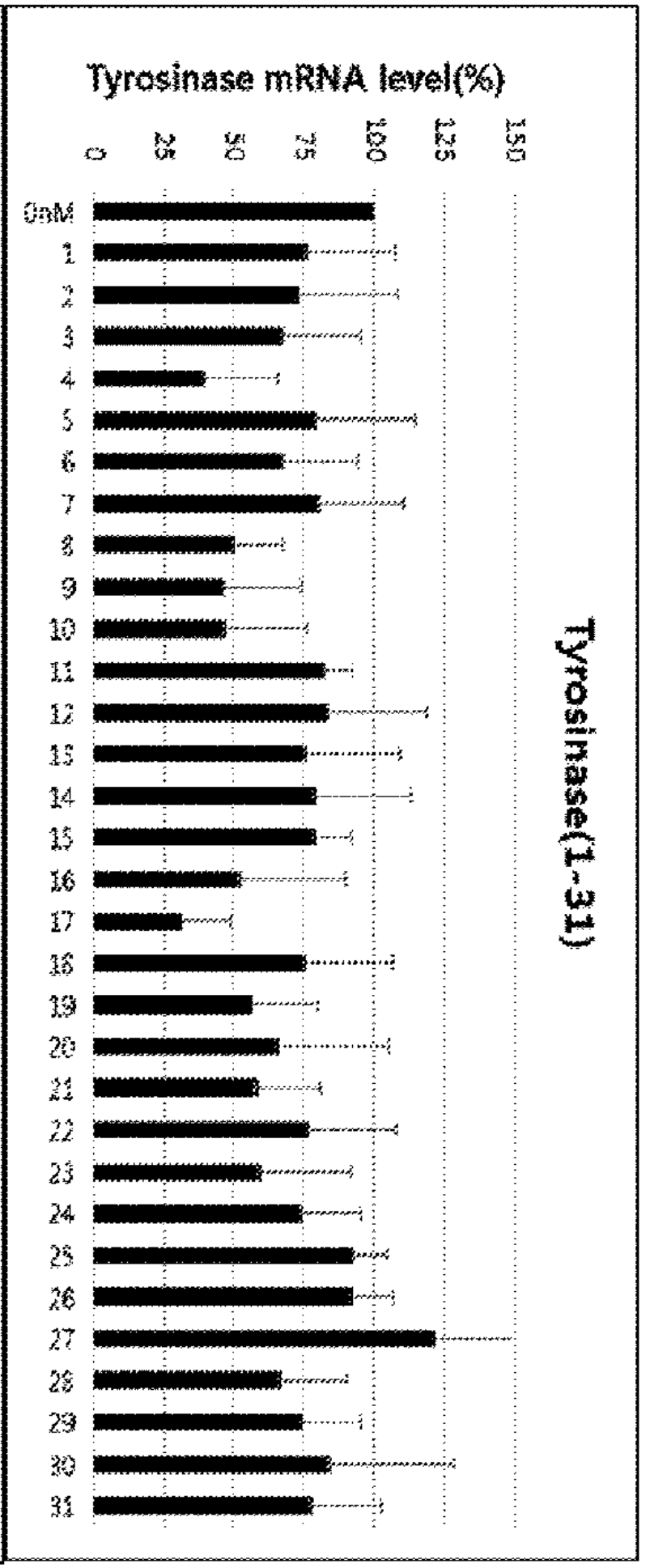
Human Tyrosinase mRNA sequence.

```
 1   atcactgtag tagtagctgg aaagagaaat ctgtgactcc aattagccag ttcctgcaga
61   ccttgtgagg actagaggaa gaatgctcct ggctgttttg tactgcctgc tgtggagttt
121  ccagacctcc gctggccatt tccctagagc ctgtgtctcc tctaagaacc tgatggagaa
181  ggaatgctgt ccaccgtgga gcggggacag gagtccctgt ggccagcttt caggcagagg
241  ttcctgtcag aatatccttc tgtccaatgc accacttggg cctcaatttc ccttcacagg
301  ggtggatgac cgggagtcgt ggccttccgt cttttataat aggacctgcc agtgctctgg
361  caacttcatg ggattcaact gtggaaactg caagtttggc ttttggggac caaactgcac
421  agagagacga ctcttggtga gaagaaacat cttcgatttg agtgccccag agaaggacaa
481  atttttttgcc tacctcactt tagcaaagca taccatcagc tcagactatg tcatccccat
541  agggacctat ggccaaatga aaaatggatc aacacccatg tttaacgaca tcaatattta
601  tgacctcttt gtctggatgc attattatgt gtcaatggat gcactgcttg ggggatctga
661  aatctggaga gacattgatt ttgcccatga agcaccagct tttctgcctt ggcatagact
721  cttcttgttg cggtgggaac aagaaatcca gaagctgaca ggagatgaaa acttcactat
781  tccatattgg gactggcggg atgcagaaaa gtgtgacatt tgcacagatg agtacatggg
841  aggtcagcac cccacaaatc ctaacttact cagcccagca tcattcttct cctcttggca
901  gattgtctgt agccgattgg aggagtacaa cagccatcag tctttatgca atggaacgcc
961  cgagggacct ttacggcgta atcctggaaa ccatgacaaa tccagaaccc caaggctccc
1021 ctcttcagct gatgtagaat tttgcctgag tttgacccaa tatgaatctg gttccatgga
1081 taaagctgcc aatttcagct ttagaaatac actggaagga tttgctagtc cacttactgg
1141 gatagcggat gcctctcaaa gcagcatgca caatgccttg cacatctata tgaatggaac
1201 aatgtcccag gtacagggat ctgccaacga tcctatcttc cttcttcacc atgcatttgt
1261 tgacagtatt tttgagcagt ggctccgaag gcaccgtcct cttcaagaag tttatccaga
1321 agccaatgca cccattggac ataaccggga atcctacatg gttccttta taccactgta
1381 cagaaatggt gatttcttta tttcatccaa agatctgggc tatgactata gctatctaca
1441 agattcagac ccagactctt ttcaagacta cattaagtcc tatttggaac aagcgagtcg
1501 gatctggtca tggctccttg gggcggcgat ggtaggggcc gtcctcactg ccctgctggc
1561 agggcttgtg agcttgctgt gtcgtcacaa gagaaagcag cttcctgaag aaaagcagcc
1621 actcctcatg gagaaagagg attaccacag cttgtatcag agccatttat aaaaggctta
1681 ggcaatagag tagggccaaa aagcctgacc tcactctaac tcaaagtaat gtccaggttc
1741 ccagagaata tctgctggta tttttctgta aagaccattt gcaaaattgt aacctaatac
1801 aaagtgtagc cttcttccaa ctcaggtaga acacacctgt ctttgtcttg ctgttttcac
1861 tcagcccttt taacattttc ccctaagccc atatgtctaa ggaaaggatg ctatttggta
1921 atgaggaact gttatttgta tgtgaattaa agtgctctta ttttaaaaaa ttgaaataat
1981 tttgattttt gccttctgat tatttaaaga tctatatatg ttttattggc cccttcttta
2041 ttttaataaa acagtgagaa atctaaaaaa aaaaaaaaaa aa
```

Figure 14


(a)
Day 0
Day 1-13
Day 14
cp-asiTYR#4-1 : treated every day in medium
Harvest
NT, KA : treated every other day on the skin
(b)
NT
cp-asiTYR#4-1 5uM
2% Kojic acid
(c)
NT
cp-asiTYR#4-1 5uM
2% Kojic acid
(d)
Tyrosinase mRNA level (%)
100
75
50
25
0
NT
cp-asiTYR#4-1 5uM
(e)
cp-asiTYR #4-1
½NT
NT
5uM
2%KA
TYR
b-actin
(f)
Melanin contents (%)
100
75
50
25
0
NT
cp-asiTYR #4-1 5uM
2% KA


Tyrosinase mRNA level(%)
0
25
50
75
100
125
150
0nM
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
Tyrosinase(31-62)


Tyrosinase mRNA level(%)
0
25
50
75
100
125
150
0nM
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
Tyrosinase(1-31)