DESCRIPTION

Title of Invention: PROPHYLACTIC OR THERAPEUTIC AGENT FOR DISEASE OR CONDITION ASSOCIATED WITH ANGIOGENESIS, AND VEGF PRODUCTION INHIBITOR

Technical Field

[0001]

The present invention relates to a prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, and a VEGF production inhibitor.

Background Art

[0002]

Angiogenesis, in which new blood vessels are formed, is a physiological function inherent in the body. Angiogenesis that occurs as needed under a regulatory mechanism contributes to wound healing and the like. However, unnecessary angiogenesis resulting from, for example, a problem in the regulatory mechanism is known to be a factor in various diseases or conditions. Examples of such diseases or conditions known in the art include joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis. Among these diseases or conditions, for example, knee osteoarthritis is a progressive and intractable degenerative joint disease, and despite the extremely high prevalence of the disease, no curative therapy is available. Since an increase in the number of patients with knee osteoarthritis is a concern due to the aging of society, there is an urgent need to develop a curative therapy.

[0003]

As described above, there are various diseases or conditions associated with angiogenesis, and development of a method for preventing or treating such diseases or conditions is eagerly desired.

Vascular endothelial growth factor (VEGF) is known to play a central role in angiogenesis, and development of drugs targeting VEGF is underway in various locations.

Citation List

Patent Literature

[0004]

PTL 1: WO 2019/176901

Non-patent Literature

[0005]

NPL 1: Scientific Reports 7: 3584 (2017); miR-29a

Summary of Invention

Technical Problem

[0006]

In one embodiment, an object of the present invention is to provide a novel prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, and a VEGF production inhibitor. In another embodiment, a main object of the present invention is to provide a novel VEGF production inhibitor.

Solution to Problem

[0007]

As a result of investigating the VEGF production-inhibiting effects of a wide variety of miRNAs in synovial sarcoma cells, the present inventors have found that an inhibitory effect was observed for only five miRNAs. As a result of further examining combinations of the five miRNAs, the present inventors have found that a synergistic effect was observed only for a combination of hsa-miR-140-5p and hsa-miR-126-3p. The present invention is based on these novel findings.

[0008]

Accordingly, the present invention provides the following items:

Item 1. A prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-126-3p.

[0009]

Item 2. An agent comprising hsa-miR-140-5p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p.

[0010]

Item 3. An agent comprising hsa-miR-126-3p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p.

[0011]

Item 4. A VEGF production inhibitor comprising hsa-miR-140-5p and hsa-miR-126-3p.

[0012]

Item 5. An agent comprising hsa-miR-140-5p, for inhibiting VEGF production through combined use with hsa-miR-126-3p.

[0013]

Item 6. An agent comprising hsa-miR-126-3p, for inhibiting VEGF production through combined use with hsa-miR-140-5p.

[0014]

Item 7. The agent according to any one of Items 1 to 3, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[0015]

Item 8. The agent according to Item 7, wherein the disease or condition associated with angiogenesis is joint disease.

[0016]

Item 9. The agent according to Item 8, wherein the joint disease is knee osteoarthritis.

[0017]

Item 10. The agent according to any one of Items 1 to 9, wherein

the agent is an injectable preparation.

[0018]

Item 11. A pharmaceutical composition comprising hsa-miR-140-5p and hsa-miR-126-3p, for use in preventing or treating a disease or condition associated with angiogenesis.

[0019]

Item 12. A pharmaceutical composition comprising hsa-miR-140-5p, for use in preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p.

[0020]

Item 13. A pharmaceutical composition comprising hsa-miR-126-3p, for use in preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p.

[0021]

Item 14. A pharmaceutical composition comprising hsa-miR-140-5p and hsa-miR-126-3p, for inhibiting VEGF production.

[0022]

Item 15. A pharmaceutical composition comprising hsa-miR-140-5p, for inhibiting VEGF production through combined use with hsa-miR-126-3p.

[0023]

Item 16. A pharmaceutical composition comprising hsa-miR-126-3p, for inhibiting VEGF production through combined use with hsa-miR-140-5p.

[0024]

Item 17. The pharmaceutical composition according to any one of Items 11 to 13, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[0025]

Item 18. The pharmaceutical composition according to Item 17, wherein the disease or condition associated with angiogenesis is joint disease.

[0026]

Item 19. The pharmaceutical composition according to Item 18, wherein the joint disease is knee osteoarthritis.

[0027]

Item 20. The pharmaceutical composition according to any one of Items 11 to 19, wherein the pharmaceutical composition is an injectable preparation.

[0028]

Item 21. hsa-miR-140-5p and hsa-miR-126-3p for use in preventing or treating a disease or condition associated with angiogenesis.

[0029]

Item 22. hsa-miR-140-5p for use in preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p.

[0030]

Item 23. hsa-miR-126-3p for use in preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p.

[0031]

Item 24. hsa-miR-140-5p and hsa-miR-126-3p for inhibiting VEGF production.

[0032]

Item 25. hsa-miR-140-5p for inhibiting VEGF production through combined use with hsa-miR-126-3p.

[0033]

Item 26. hsa-miR-126-3p for inhibiting VEGF production through combined use with hsa-miR-140-5p.

[0034]

Item 27. The compound according to any one of Items 21 to 23, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder

stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[0035]

Item 28. The compound according to Item 27, wherein the disease or condition associated with angiogenesis is joint disease.

[0036]

Item 29. The compound according to Item 28, wherein the joint disease is knee osteoarthritis.

[0037]

Item 30. The compound according to any one of Items 21 to 29, wherein the compound is administered by injection.

[0038]

Item 31. Use of hsa-miR-140-5p and hsa-miR-126-3p for manufacturing a prophylactic or therapeutic agent for a disease or condition associated with angiogenesis.

[0039]

Item 32. Use of hsa-miR-140-5p for manufacturing an agent for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p.

[0040]

Item 33. Use of hsa-miR-126-3p for manufacturing an agent for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p.

[0041]

Item 34. Use of hsa-miR-140-5p and hsa-miR-126-3p for manufacturing a VEGF production inhibitor.

[0042]

Item 35. Use of hsa-miR-140-5p for manufacturing an agent for inhibiting VEGF production through combined use with hsa-miR-126-3p.

[0043]

Item 36. Use of hsa-miR-126-3p for manufacturing an agent for

inhibiting VEGF production through combined use with hsa-miR-140-5p.

[0044]

Item 37. The use according to any one of Items 31 to 33, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[0045]

Item 38. The use according to Item 37, wherein the disease or condition associated with angiogenesis is joint disease.

[0046]

Item 39. The use according to Item 38, wherein the joint disease is knee osteoarthritis.

[0047]

Item 40. The use according to any one of Items 31 to 39, wherein the agent is an injectable preparation.

[0048]

Item 41. A method for preventing or treating a disease or condition associated with angiogenesis, comprising administering effective amounts of hsa-miR-140-5p and hsa-miR-126-3p to a subject in need thereof.

[0049]

Item 42. A method for inhibiting VEGF production, comprising administering effective amounts of hsa-miR-140-5p and hsa-miR-126-3p to a subject in need thereof.

[0050]

Item 43. The method according to Item 41, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic

retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[0051]

Item 44. The method according to Item 43, wherein the disease or condition associated with angiogenesis is joint disease.

[0052]

Item 45. The method according to Item 44, wherein the joint disease is knee osteoarthritis.

[0053]

Item 46. The method according to any one of Items 41 to 45, wherein hsa-miR-140-5p and hsa-miR-126-3p are administered by injection.

Advantageous Effects of Invention

[0054]

In one embodiment, the present invention can provide a novel prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, and a VEGF production inhibitor. In another embodiment, the present invention can provide a novel VEGF production inhibitor.

[0055]

In contrast, PTL 1 discloses an anti-inflammatory effect caused by osteoarthritis, with an inhibitor of miR-21, which has a nucleotide sequence different from those of hsa-miR-140-5p and hsa-miR-126-3p, or an antagonist of TLR7, 8, and 9 as an active ingredient, but does not describe hsa-miR-126-3p. PTL 1 also states in paragraph [0088] that whether miR-140 is involved in pain in osteoarthritis is unknown. PTL 1 also does not disclose a therapeutic effect on knee osteoarthritis. NPL 1 suggests that miR-29a, which has a nucleotide sequence different from those of hsa-miR-140-5p and hsa-miR-126-3p, provides a joint disorder-protective action (anti-inflammatory effect) through inhibition of excessive angiogenesis and fibrosis in the synovium, but does not disclose hsa-miR-140-5p or hsa-miR-126-3p. NPL 1 also does

not disclose a therapeutic effect on knee osteoarthritis. Accordingly, these effects of the present invention could not have been predicted from the prior art.

Brief Description of Drawings

[0056]

[Fig. 1] shows the results of measuring the amount of VEGF produced per cell using a human synovial sarcoma-derived cell line in Example 2. MOC indicates a group treated with vehicle alone.

[Fig. 2] shows the results of measuring the amount of VEGF produced when hsa-miRNA-126-3p, hsa-miR-140-5p, hsa-miR-107, and hsa-miR-100-5p were used alone or in combination in Example 2.

[Fig. 3] shows the results of measuring the amount of VEGF produced per cell using human synovium-derived cells in Example 3.

[Fig. 4] shows the results of measuring the amounts of VEGF in synovial tissue and joint fluid in knee anterior cruciate ligament transection (ACLT) model rats in Example 4(3).

[Fig. 5] shows the results of a pain test in ACLT model rats in Example 4(4).

[Fig. 6] shows synovitis scores in ACLT model rats in Example 4(5).

[Fig. 7] shows the results of measuring the amount of VEGF produced using a human chondrocyte cell line in Example 5.

[Fig. 8] shows the results of measuring the amount of VEGF produced using a chondrosarcoma cell line in Example 6.

Description of Embodiments

[0057]

Preferred embodiments for carrying out the present invention will be described below. The embodiments described below represent typical embodiments of the present invention, and the scope of the present invention is not limited to these embodiments.

[0058]

1. Prophylactic or Therapeutic Agent for Disease or Condition Associated with Angiogenesis

In one embodiment, the present invention provides an agent comprising hsa-miR-140-5p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p. The present embodiment relates to an agent that comprises hsa-miR-140-5p as an active ingredient and is used in combination with hsa-miR-126-3p.

As used in the present invention, "hsa-miR-140-5p" refers to an miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1 and/or a homolog thereof:

SEQ ID NO: 1. CAGUGGUUUUACCCUAUGGUAG

As used in the present invention, "hsa-miR-126-3p" is an miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2 and/or a homolog thereof:

SEQ ID NO: 2. UCGUACCGUGAGUAAUAAUGCG

Unless otherwise stated, each nucleotide sequence described herein has a 5' end at the left end and a 3' end at the right end. [0059]

The "miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1" may also include a modified miRNA from the viewpoint of, for example, improving cell introduction efficiency and improving stability. Examples of the "miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1" include an miRNA in which at least some of the nucleotides constituting the miRNA are substituted with a methoxy group, a methyl group, an amino group, a thioxo group (=S), a halogen, or the like; an miRNA in which a hydroxy group (-OH) of at least some of the nucleotides constituting the miRNA is substituted with hydrogen (-H); and combinations thereof. The "miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1" may also include an miRNA having none of these modifications. Similarly, the "homolog of the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1", the "miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2", and the "homolog of the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2" may also include those having the above modifications and those having none of the above modifications.

[0060]

In one embodiment of the present invention, unless explicitly stated otherwise, "hsa-miR-140-5p" may also include a nucleotide sequence in which one or several (e.g., one to three, one or two, or one) bases are substituted, added, and/or deleted relative to the nucleotide sequence represented by SEQ ID NO: 1.

Preferred examples of "hsa-miR-140-5p" include one for which, when the VEGF production-inhibiting effect in synovial sarcoma cells is measured in the same manner as in Example 2 except that the "hsa-miR-140-5p" is used in place of the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1, a synergistic effect resulting from combination with the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2 (a significantly higher VEGF production-inhibiting effect than the effects observed when the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1 and the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2 are each used alone) is observed.

[0061]

In one embodiment of the present invention, unless explicitly stated otherwise, "hsa-miR-126-3p" may also include a nucleotide sequence in which one or several (e.g., one to three, one or two, or one) bases are substituted, added, and/or deleted relative to the nucleotide sequence represented by SEQ ID NO: 2.

Preferred examples of "hsa-miR-126-3p" include one for which, when the VEGF production-inhibiting effect in synovial sarcoma cells is measured in the same manner as in Example 2 except that the "hsa-miR-126-3p" is used in place of the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2, a synergistic effect resulting from combination with the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1 (a significantly higher VEGF production-inhibiting effect than the effects observed when the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 1 and the miRNA consisting of the nucleotide sequence represented by SEQ ID NO: 2 are each used alone) is observed.

[0062]

In the present invention, the ratio at which hsa-miR-140-5p and hsa-miR-126-3p are used is not limited, and for example, the amount of hsa-miR-126-3p is preferably 0.01 to 100 mol, more preferably 0.1 to 10 mol, and still more preferably 0.2 to 10 mol per 1 mol of hsa-miR-140-5p.

[0063]

In the present invention, examples of the disease or condition associated with angiogenesis include joint disease, shoulder stiffness, cancer (chondrosarcoma, colon cancer, rectal cancer, non-small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, gastric cancer, ovarian cancer, cervical cancer, malignant glioma, and the like), chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization, age-related macular degeneration, enthesitis (lateral epicondylitis of the humerus, Achilles tendinitis, and the like), baseball elbow, rotator cuff injury, and plantar muscle tendinitis, with joint disease and the like being preferred. As used herein, the term “joint disease” refers to a disease in which an abnormality such as deformation, damage, or inflammation occurs in a joint. The joint is a movable connecting portion that connects bones to each other, and specifically comprises cartilage, synovium, meniscus, ligament, and the like. Examples of the joint disease include diseases of a knee joint, a hip joint, and a finger joint, and the like. Examples of the disease of a knee joint include knee osteoarthritis, pes anserine bursitis, meniscal injury, osteochondritis dissecans of the knee, quadriceps tendinitis, bursitis (prepatellar bursitis), patellofemoral arthropathy, patellar ligament inflammation (patellar tendinitis), Osgood-Schlatter disease, Baker's cyst, rheumatoid arthritis, osteonecrosis of the knee, and heterotopic ossification, with knee osteoarthritis and the like being preferred.

[0064]

In the present invention, hsa-miR-140-5p itself, which is an active ingredient of the present invention, may be used as a prophylactic or therapeutic agent for a disease or condition associated

with angiogenesis, or may be used as a composition in combination with various pharmaceutically acceptable carriers (e.g., an isotonic agent, a chelating agent, a stabilizer, a pH adjuster, a preservative, an antioxidant, a solubilizing agent, and a thickener).

[0065]

Examples of the isotonic agent include sugars such as glucose, trehalose, lactose, fructose, mannitol, xylitol, and sorbitol; polyhydric alcohols such as glycerin, polyethylene glycol, and propylene glycol; and inorganic salts such as sodium chloride, potassium chloride, and calcium chloride.

[0066]

Examples of the chelating agent include edetates such as disodium edetate, calcium disodium edetate, trisodium edetate, tetrasodium edetate, and calcium edetate; ethylenediaminetetraacetate; nitrilotriacetic acid or a salt thereof; sodium hexametaphosphate; and citric acid.

[0067]

Examples of the stabilizer include sodium bisulfite. Examples of the pH adjuster include acids such as hydrochloric acid, carbonic acid, acetic acid, and citric acid; alkali metal hydroxides such as sodium hydroxide and potassium hydroxide; alkali metal carbonates or hydrogen carbonates such as sodium carbonate; alkali metal acetates such as sodium acetate; alkali metal citrates such as sodium citrate; and bases such as trometamol.

[0068]

Examples of the preservative include sorbic acid; potassium sorbate; parahydroxybenzoic acid esters such as methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, and butyl parahydroxybenzoate; quaternary ammonium salts such as chlorhexidine gluconate, benzalkonium chloride, benzethonium chloride, and cetylpyridinium chloride; alkyl polyaminoethylglycine; chlorobutanol; Polyquad; polyhexamethylene biguanide; and chlorhexidine.

[0069]

Examples of the antioxidant include sodium bisulfite, anhydrous

sodium sulfite, sodium metabisulfite, concentrated and mixed tocopherols.

[0070]

Examples of the solubilizing agent include sodium benzoate, glycerin, D-sorbitol, glucose, propylene glycol, hydroxypropyl methylcellulose, polyvinylpyrrolidone, macrogol, and D-mannitol.

[0071]

Examples of the thickener include polyethylene glycol, methylcellulose, ethylcellulose, carmellose sodium, xanthan gum, sodium chondroitin sulfate, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, polyvinylpyrrolidone, and polyvinyl alcohol.

[0072]

In the present invention, hsa-miR-140-5p and/or hsa-miR-140 may be included in the agent of the present invention in a state incorporated into a vehicle or together with a vehicle (e.g., in a state embedded in a vehicle). Examples of the vehicle include a vector and a polymer. Examples of the vector include viral vectors such as an adenoviral vector and a retroviral vector; and non-viral vectors such as a plasmid vector and a bacterial vector. Examples of the polymer include a cationic polymer (e.g., polyethyleneimine nanoparticles). In addition, the "prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-126-3p" according to the present invention also encompasses an agent comprising cells modified to express hsa-miR-140-5p and/or hsa-miR-140; exosomes, membrane vesicles (Evs), or the like prepared from the cells; and the like.

[0073]

In an embodiment of the composition, the content of hsa-miR-140-5p in the composition is not particularly limited and can be appropriately set from conditions such as 99% by mass or more, 95% by mass or more, 90% by mass or more, 70% by mass or more, 50% by mass or more, 30% by mass or more, 10% by mass or more, 5% by mass or more, and 1% by mass or more.

[0074]

In an embodiment of the composition, the upper limit of the content of hsa-miR-140-5p in the composition is not particularly limited and can be appropriately set from conditions such as 99% by mass or less, 95% by mass or less, 90% by mass or less, 70% by mass or less, 50% by mass or less, 30% by mass or less, 10% by mass or less, 5% by mass or less, and 1% by mass or less.

[0075]

In an embodiment of the composition, the composition of the present invention may further include a substance known to be effective in preventing or treating a disease or condition associated with angiogenesis. Examples include platinum preparations such as cisplatin and carboplatin; anticancer agents such as irinotecan, fluorouracil, tegafur, uracil, gemcitabine, cyclophosphamide, methotrexate, and doxorubicin; analgesics such as acetaminophen, NSAIDs, Neurotropin, and opioids (codeine phosphate, morphine, oxycodone, fentanyl, remifentanil, meperidine, tramadol, buprenorphine, pentazocine, and the like); local anesthetics such as Xylocaine and Neovitacain; and hyaluronic acid. These substances can be used singly or in combination of two or more.

[0076]

The dosage form is not particularly limited, and examples include various dosage forms such as oral preparations, comprising tablets, pills, capsules, powders, granules, and syrups; and parenteral preparations, comprising injectable preparations (intravenous injections, intramuscular injections, local injections, and the like), gargles, infusion preparations, external preparations (ointments, creams, patches, and inhalants), and suppositories. Among the above dosage forms, preferred examples include oral preparations and injectable preparations, with injectable preparations and the like being more preferred. These preparations also include embodiments utilizing the above-described exosomes, membrane vesicles, and the like. The agent of the present invention may also be in the form of a syringe in which a pharmaceutical composition is filled in a syringe barrel.

[0077]

In the present embodiment, the dose of the prophylactic or therapeutic agent for a disease or condition associated with angiogenesis varies depending on the administration route, the age, body weight, and symptoms of a patient, and the like, and thus cannot be uniformly specified, but the dose can be set such that a daily dose for an adult, in terms of the amount of hsa-miR-140-5p, is ordinarily 5000 mg or less, 1000 mg or less, 100 mg or less, 10 mg or less, or the like. The lower limit of the dose of the prophylactic or therapeutic agent for a disease or condition associated with angiogenesis is also not particularly limited, and for example, the dose can be set such that a daily dose for an adult, in terms of the amount of hsa-miR-140-5p, is ordinarily 1000 mg or more, 100 mg or more, 10 mg or more, 5 mg or more, or the like. For administration once daily, one preparation may contain this amount, and for administration three times daily, one preparation may contain one-third of this amount.

[0078]

In the present embodiment, the dose of hsa-miR-140 varies depending on the administration route, the age, body weight, and symptoms of a patient, and the like, and thus cannot be uniformly specified, but the dose can be set such that a daily dose for an adult is ordinarily 5000 mg or less, 1000 mg or less, 100 mg or less, 10 mg or less, or the like. The lower limit of the dose of hsa-miR-140 is also not particularly limited, and for example, the dose can be set such that a daily dose for an adult is ordinarily 1000 mg or more, 100 mg or more, 10 mg or more, 5 mg or more, or the like. For administration once daily, one preparation may contain this amount, and for administration three times daily, one preparation may contain one-third of this amount.

[0079]

The prophylactic or therapeutic agent of the present invention for a disease or condition associated with angiogenesis is administered to a patient such as a mammal. Examples of the mammal include a human, a monkey, a mouse, a rat, a rabbit, a cat, a dog, a pig, a cow, a horse, and a sheep, with a human being preferred.

[0080]

In the present invention, an "agent comprising hsa-miR-140-5p,..." encompasses not only an agent comprising hsa-miR-140-5p itself but also a precursor of hsa-miR-140-5p that forms hsa-miR-140-5p in vivo, intracellularly, or the like.

[0081]

As used herein, a "precursor" is an RNA capable of generating a mature miRNA through cleavage, double-strand dissociation, or the like. Accordingly, examples of the precursor include pre-miRNA, pre-miRNA, a double-stranded miRNA consisting of a mature miRNA and an antisense strand thereof (hereinafter also referred to simply as a "double-stranded miRNA"), and combinations thereof. Therefore, in the present invention, examples of the precursor of hsa-miR-140-5p include pri-miRNA of hsa-miR-140-5p, pre-miRNA of hsa-miR-140-5p, a double-stranded miRNA of hsa-miR-140-5p, and combinations thereof.

[0082]

In the present invention, the term "for preventing or treating... through combined use with hsa-miR-126-3p" encompasses not only use of hsa-miR-126-3p itself but also use of a precursor of hsa-miR-126-3p that forms hsa-miR-126-3p in vivo, intracellularly, or the like. In the present invention, examples of the precursor of hsa-miR-126-3p include pri-miRNA of hsa-miR-126-3p, pre-miRNA of hsa-miR-126-3p, a double-stranded miRNA of hsa-miR-126-3p, combinations thereof, and the like.

[0083]

In another embodiment, the present invention provides an agent comprising hsa-miR-126-3p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p. The present embodiment relates to an agent that comprises hsa-miR-126-3p as an active ingredient and is used in combination with hsa-miR-140-5p. Also in the present embodiment, hsa-miR-126-3p itself, which is an active ingredient of the present invention, may be used as a prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, or may be used as a composition in combination with various pharmaceutically acceptable carriers (e.g., an isotonic agent, a chelating agent, a stabilizer, a pH adjuster, a

preservative, an antioxidant, a solubilizing agent, and a thickener). In the present embodiment, the content of hsa-miR-126-3p in the composition is not particularly limited and can be appropriately set from conditions such as 99% by mass or more, 95% by mass or more, 90% by mass or more, 70% by mass or more, 50% by mass or more, 30% by mass or more, 10% by mass or more, 5% by mass or more, and 1% by mass or more.

[0084]

In the present embodiment, the upper limit of the content of hsa-miR-126-3p in the composition is not particularly limited and can be appropriately set from conditions such as 99% by mass or less, 95% by mass or less, 90% by mass or less, 70% by mass or less, 50% by mass or less, 30% by mass or less, 10% by mass or less, 5% by mass or less, and 1% by mass or less.

[0085]

In the present embodiment, the dose of the prophylactic or therapeutic agent for a disease or condition associated with angiogenesis varies depending on the administration route, the age, body weight, and symptoms of a patient, and the like, and thus cannot be uniformly specified, but the dose can be set such that a daily dose for an adult, in terms of the amount of hsa-miR-126-3p, is ordinarily 5000 mg or less, 1000 mg or less, 100 mg or less, 10 mg or less, or the like. The lower limit of the dose of the prophylactic or therapeutic agent for a disease or condition associated with angiogenesis is also not particularly limited, and for example, the dose can be set such that a daily dose for an adult, in terms of the amount of hsa-miR-126-3p, is ordinarily 1000 mg or more, 100 mg or more, 10 mg or more, 5 mg or more, or the like. For administration once daily, one preparation may contain this amount, and for administration three times daily, one preparation may contain one-third of this amount.

[0086]

In the present embodiment, the dose of hsa-miR-140-5p varies depending on the administration route, the age, body weight, and symptoms of a patient, and the like, and thus cannot be uniformly specified, but the dose can be set such that a daily dose for an adult

is ordinarily 5000 mg or less, 1000 mg or less, 100 mg or less, 10 mg or less, or the like. The lower limit of the dose of hsa-miR-140-5p is also not particularly limited, and for example, the dose can be set such that a daily dose for an adult is ordinarily 1000 mg or more, 100 mg or more, 10 mg or more, 5 mg or more, or the like. For administration once daily, one preparation may contain this amount, and for administration three times daily, one preparation may contain one-third of this amount.

[0087]

The definitions and ratio of use of hsa-miR-126-3p and hsa-miR-140-5p in the present embodiment, the fact that precursors of hsa-miR-126-3p and/or hsa-miR-140-5p may be encompassed, components other than hsa-miR-126-3p and hsa-miR-140-5p in an embodiment of the composition, the target disease or condition, and the like are the same as described above.

[0088]

In another embodiment, the present invention provides a prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-126-3p. The present embodiment relates to an agent comprising hsa-miR-140-5p and hsa-miR-126-3p as active ingredients. Also in the present embodiment, hsa-miR-140-5p and hsa-miR-126-3p themselves, which are active ingredients of the present invention, may be used as a prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, or may be used as a composition in combination with various pharmaceutically acceptable carriers (e.g., an isotonic agent, a chelating agent, a stabilizer, a pH adjuster, a preservative, an antioxidant, a solubilizing agent, and a thickener). In the present embodiment, the contents of hsa-miR-140-5p and hsa-miR-126-3p in the composition are not particularly limited and can be appropriately set, in terms of the total amount thereof, from conditions such as 99% by mass or more, 95% by mass or more, 90% by mass or more, 70% by mass or more, 50% by mass or more, 30% by mass or more, 10% by mass or more, 5% by mass or more, and 1% by mass or more.

[0089]

In the present embodiment, the upper limits of the contents of hsa-miR-140-5p and hsa-miR-126-3p in the composition are not particularly limited and can be appropriately set, in terms of the total amount thereof, from conditions such as 99% by mass or less, 95% by mass or less, 90% by mass or less, 70% by mass or less, 50% by mass or less, 30% by mass or less, 10% by mass or less, 5% by mass or less, and 1% by mass or less.

[0090]

In the present embodiment, the "prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-126-3p" encompasses not only an embodiment in which hsa-miR-140-5p and hsa-miR-126-3p are included in a single dosage form, but also a so-called combination preparation in which hsa-miR-140-5p and hsa-miR-126-3p are included in separate dosage forms.

[0091]

The definitions, ratio of use, and doses of hsa-miR-126-3p and hsa-miR-140-5p in the present embodiment, the fact that precursors of hsa-miR-126-3p and/or hsa-miR-140-5p may be encompassed, components other than hsa-miR-126-3p and hsa-miR-140-5p in an embodiment of the composition, the target disease or condition, and the like are the same as described above.

[0092]

2. VEGF Production Inhibitor

According to the present invention, use of hsa-miR-140-5p in combination with hsa-miR-126-3p can inhibit VEGF production and thereby inhibit angiogenesis. Accordingly, in one embodiment, the present invention provides an agent comprising hsa-miR-140-5p, for inhibiting VEGF production through combined use with hsa-miR-126-3p. The present embodiment relates to an agent that comprises hsa-miR-140-5p as an active ingredient and is used in combination with hsa-miR-126-3p.

[0093]

The definitions, ratio of use, and doses of hsa-miR-140-5p and hsa-miR-126-3p in the present embodiment, the fact that precursors of hsa-miR-126-3p and/or hsa-miR-140-5p may be encompassed, components

other than hsa-miR-140-5p and hsa-miR-126-3p in an embodiment of the composition, and the like are the same as described above for the "agent comprising hsa-miR-140-5p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p". The VEGF production inhibitor of the present invention can be used in vivo or in vitro.

[0094]

In one embodiment, the present invention provides an agent comprising hsa-miR-126-3p, for inhibiting VEGF production through combined use with hsa-miR-140-5p. The present embodiment relates to an agent that comprises hsa-miR-126-3p as an active ingredient and is used in combination with hsa-miR-140-5p.

[0095]

The definitions, ratio of use, and doses of hsa-miR-140-5p and hsa-miR-126-3p in the present embodiment, the fact that precursors of hsa-miR-126-3p and/or hsa-miR-140-5p may be encompassed, components other than hsa-miR-140-5p and hsa-miR-126-3p in an embodiment of the composition, and the like are the same as described above for the "agent comprising hsa-miR-126-3p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p". The VEGF production inhibitor of the present invention can be used in vivo or in vitro.

[0096]

In one embodiment, the present invention provides a VEGF production inhibitor comprising hsa-miR-140-5p and hsa-miR-126-3p. The present embodiment relates to an agent comprising hsa-miR-140-5p and hsa-miR-126-3p as active ingredients.

[0097]

The definitions, ratio of use, and doses of hsa-miR-140-5p and hsa-miR-126-3p in the present embodiment, the fact that precursors of hsa-miR-126-3p and/or hsa-miR-140-5p may be encompassed, components other than hsa-miR-140-5p and hsa-miR-126-3p in an embodiment of the composition, and the like are the same as described above for the "prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-

126-3p". The VEGF production inhibitor of the present invention can be used in vivo or in vitro.

[0098]

3. Other Embodiments

Although the present invention has been described above using specific embodiments of the present invention, the present invention is not limited to these embodiments. For example, the present invention may encompass various embodiments, provided that hsa-miR-140-5p and hsa-miR-126-3p are used in combination and that the combination is used for preventing or treating a disease or condition associated with angiogenesis or for inhibiting VEGF production (a kit for preventing or treating a disease or condition associated with angiogenesis, a kit for inhibiting VEGF production, a method for preventing or treating a disease or condition associated with angiogenesis, a method for inhibiting VEGF production, use of hsa-miR-140-5p and/or hsa-miR-126-3p for preventing or treating a disease or condition associated with angiogenesis, use of hsa-miR-140-5p and/or hsa-miR-126-3p for inhibiting VEGF production, and the like).

[0099]

The definitions, ratio of use, and doses of hsa-miR-140-5p and hsa-miR-126-3p in the embodiments, components other than hsa-miR-140-5p and hsa-miR-126-3p in an embodiment of the composition, and the like are the same as described above. An embodiment of the kit may include other components as needed. Examples of the other components include, but are not limited to, a tool for collecting a specimen (e.g., a syringe), a positive control sample, and a negative control sample.

Examples

[0100]

Specific embodiments of the present invention will be described in more detail using Examples, but the present invention is not limited to these Examples.

[0101]

Example 1. miRNA Selection

Candidate miRNAs reported to regulate VEGF mRNA were extracted

using miRTarBase8.0 (Nucleic Acids Research 2020, 8; 48(D1): D148-D154.). Furthermore, miRNA expression data in cartilage, synovium, and blood vessels were obtained from FANTOM (https://fantom.gsc.riken.jp/jp/), and 20 miRNAs exhibiting expression patterns characteristic of the respective tissues were arbitrarily selected. miRIDIAN microRNA Mimic and Negative control#1 (hereinafter, miRNA-mimic: Horizon Discovery Ltd.), which express the selected miRNAs intracellularly, were purchased and used in experiments. The nucleotide sequences of the miRNAs used in the Examples are as follows:

NC(miRIDIAN Negative Control #1 cel-miR-67): UCACAACCUCCUAGAAAGAGUAGA

miR-140-5p: CAGUGGUUUUACCCUAUGGUAG

miR-107: AGCAGCAUUGUACAGGGCUAUCA

miR-126-3p: UCGUACCGUGAGUAAUAAUGCG

miR-27b-3p: UUCACAGUGGCUAAGUUCUGC

miR-199a-3p: ACAGUAGUCUGCACAUUGGUUA

miR-125a-5p: UCCCUGAGACCCUUUAACCUGUGA

miR-199a-5p: CCCAGUGUUCAGACUACCUGUUC

miR-101-3p: UACAGUACUGUGAUAACUGAA

miR-320a: AAAAGCUGGGUUGAGAGGGCGA

miR-128-3p: UCACAGUGAACCGGUCUCUUU

miR-145-5p: GUCCAGUUUUCCCAGGAAUCCCU

miR-126-5p: CAUUAUUACUUUUGGUACGCG

miR-100-5p: AACCCGUAGAUCCGAACUUGUG

miR-146a-5p: UGAGAACUGAAUUCCAUGGGUU

miR-146b-5p: UGAGAACUGAAUUCCAUAGGCU

miR-140-3p: UACCACAGGGUAGAACCACGG

miR-455-5p: UAUGUGCCUUUGGACUACAUCG

miR-148a-3p: UCAGUGCACUACAGAACUUUGU

miR-193b-3p: AACUGGCCCUCAAAGUCCCGCU

miR-193b-5p: CGGGGUUUUGAGGGCGAGAUGA

[0102]

Example 2. Effects of miRNAs and Combined Use in Human Synovial Sarcoma-Derived Cell Line HS-SY-II

The human synovial cell line HS-SY-II (RIKEN Cell Research,

#RCB2231) was seeded in FALCON 24-well plates at 2 x 10^5 cells/well and cultured in DMEM low glucose, pyruvate-containing medium (Gibco #11885) containing 10% FBS (NICHIREI BIOSCIENCES INC) and Antibiotic-Antimycotic Solution (Fujifilm, 161-23181). miRIDIAN microRNA Mimics corresponding to the selected miRNAs (hereinafter, miRNA-mimics: Horizon Discovery Ltd.) were introduced into HS-SY-II cells at a final concentration of 10 nM using Lipofectamin RNAiMax (Thermo fisher), and the cells were cultured in an incubator at 37°C and 5% CO2. On the following day, the medium was replaced, and after further culture for 24 hours under low oxygen Hypoxia) or a normal oxygen concentration (Normoxia), the supernatant was collected, and the amount of VEGF was measured using a Human VEGF SimpleStep ELISA kit (abcam). The number of cells in the 24-well plate was analyzed using CellTiter-Glo (registered trademark) 2.0 Cell Viability Assay (Promega), and the amount of VEGF produced per cell was calculated. miRNA-mimics that significantly inhibited the amount of VEGF produced relative to the Negative control miRNA-mimic-treated group were identified. The results are shown in Fig. 1. Among the 20 miRNA mimics, a tendency to inhibit VEGF production was observed with the miRNA-mimics of five miRNAs hsa-miRNA-126-3p, hsa-miR-140-5p, hsa-miR-107, hsa-miR-100-5p, and hsa-miR-140-3p.

[0103]

Combinations of these miRNAs were examined under conditions in which VEGF production was induced by Hypoxia. The results are shown in Fig. 2. As shown in Fig. 2, the combination of hsa-miR-126-3p and hsa-miR-140-5p exhibited an enhanced inhibitory effect compared with either miRNA alone.

[0104]

Example 3. Combined Effects of miRNAs on Human Synovium-Derived Cells (VEGF Production-Inhibition Test)

Synovial cells were isolated from synovial tissue excised during total knee arthroplasty using 20% FBS DMEM/F12 medium (Gibco #11320) containing 5 mg/ml Collagenase Type I (Worthington #CLS-1), and were cryopreserved using CELLBANKER 1 (ZENOAQ). For the experiment, cells that had been passaged three times in α-MEM GluMAX (Gibco, # 32561)

containing 10% FBS and Antibiotic-Antimycotic Solution (hereinafter, 10% FBS-MEM medium) were used.

Third-passage synovium-derived cells were cultured to subconfluence in 10% FBS-MEM medium and seeded in 12-well FALCON plates at 2 x 10^5 cells/well, and, at the time of seeding, 10 nM hsa-miRNA-126-3p mimic (miR-126-3p), 10 nM miRNA-140-5p mimic alone (miR-140-5p), a combination of 10 nM miRNA-126-3p mimic and 10 nM miRNA-140-5p mimic (Mix), or 20 nM Negative control miRNA mimic (NC) was introduced using Lipofectamin RNAiMax, followed by culture in an incubator at 37°C and 5% CO2. On the day after seeding, the medium was replaced, the medium cultured for 24 to 48 hours after miRNA introduction was collected as the Day 2 culture supernatant, and culture supernatants were similarly collected on Day 3, Day 4, and Day 8. The amount of VEGF was measured using a Human VEGF SimpleStep ELISA kit (abcam). The number of cells in the 12-well plate was analyzed using Cell titer Glo (Promega), and the amount of VEGF produced per cell was calculated. The results are shown in Fig. 3. Relative to the Negative control miRNA-mimic-treated group, the combination of hsa-miR-126-3p and has-miR-140-5p exhibited a stronger inhibitory effect than either miRNA alone.

[0105]

Example 4. Effects of miRNA Mimics on Knee Anterior Cruciate Ligament Transection Model (ACLT)

(1) Preparation of ACLT Model

Thirteen-week-old male Wistar rats were acclimated for two weeks before treatment and then used. Under anesthesia induced by inhalation of isoflurane and oxygen, a 1 cm skin incision was aseptically made using a parapatellar approach to expose the patellar tendon. The knee joint was flexed to 90°, and after the patellar tendon was exposed, the patella was dislocated laterally. After the anterior cruciate ligament in the knee joint was transected with scissors, the medial meniscus was resected with forceps (ACLT treatment). The patellar tendon was repositioned, and the joint capsule and skin were sutured. A group in which only the joint cavity was similarly incised was designated as the Sham-operated group.

[0106]

(2) Intra-Articular Administration of miRNA Mimics

One week after the ACLT treatment, the affected-limb weight-bearing distribution ratio was measured using an INCAPACITANCE METER (LINTON), and the rats were allocated to four groups (n = 5) so that there was no bias in body weight or affected-limb weight-bearing distribution ratio: a Negative control group (NC), an mmu-miR-126-3p mimic single-agent group (miR-126-3p), an mmu-miR-140-5p single-agent group (miR-140-5p), and an mmu-miR-126-3p&mmu-miR-140-5 combined-use group (Mix). The miRNA Mimics were prepared at 2 nmol/30 uL using in vivo jetPEI (Polyplus transfection) in accordance with the instruction manual. In the miR-126-3p&miR-140-5p Mix group, the amount of each miRNA was 1 nmol/30 uL. An incision of approximately 1 cm was made on the medial side of the knee joint, and the patellar tendon was approached using a parapatellar approach. With the knee joint flexed to 90°, the miRNA mimic solution was administered from directly above the patellar tendon toward the inside of the joint using a 29 G needle, the knee joint was slowly returned to an extended position, and the incised skin was sutured. The miRNA Mimic solution was administered intra-articularly at 30 uL once weekly from one week to four weeks after the ACLT treatment. At five weeks after the ACLT treatment, the rats were euthanized, and joint tissues (synovial tissue and cartilage tissue) were collected. For joint tissues used for western blotting, 30 uL of the miRNA Mimic solution was administered intra-articularly twice, at two weeks and four weeks after the ACLT treatment, and the rats were euthanized at five weeks after the ACLT treatment to collect the joint tissues.

[0107]

(3) VEGF Production-Inhibition Test (Western Blotting)

The synovial tissue was lysed in RIPA solution using a bead-type disruption apparatus and centrifuged at 10000 rpm for 5 minutes, and the supernatant was used as a tissue lysate sample. For a joint fluid sample, 30 μL of physiological saline was injected in the same manner as for intra-articular administration, the knee was flexed and extended while the needle tip was maintained in the joint cavity, and all recoverable physiological saline and body fluid in the joint cavity

were collected. The protein concentrations of the samples were measured by the Lowry method, and the proteins in the joint fluid were concentrated by acetone precipitation. Protein in an amount of 300 ng per lane was analyzed by an immunoreaction using WES (ProteinSimple Japan). An anti-VEGF rabbit monoclonal antibody (EPR20705: abcam, ab214424) was used to detect VEGF, correction was performed using a Total protein detection module (ProteinSimple Japan), and the amounts of VEGF in the synovial tissue and joint fluid were compared. The results are shown in Fig. 4. Among the rats subjected to the ACLT treatment, VEGF in the synovial tissue of the Negative control group was increased compared with the Sham group, whereas the increase was significantly inhibited in the miRNA mimic-administered groups.

[0108]

(4) Pain Test

The affected-limb weight-bearing distribution ratio was measured twice weekly from one week to five weeks after the ACLT treatment using an INCAPACITANCE METER (LINTON). The results are shown in Fig. 5. In the NC group, the affected-limb weight-bearing distribution ratio remained at approximately 30% until five weeks after the ACLT treatment, whereas in each single-administration group, the weight-bearing ratio gradually improved after the start of administration and reached 42% at five weeks. In the MIX group, the weight-bearing distribution ratio further improved to 47%.

[0109]

(5) Synovitis Score Evaluation

After fixation of the tissues in 10% buffered formalin, the tissues were decalcified at 4°C, embedded in paraffin, and sectioned. Hematoxylin-eosin staining was performed, and the synovial tissue was examined microscopically. Cartilage degeneration caused by OA was evaluated using the inflammation score classification of Krenn et al. The results are shown in Fig. 6. The synovitis score was increased by the ACLT treatment, but synovitis was inhibited by intra-articular administration of the miRNA-mimics.

[0110]

Example 5. Inhibition of VEGF Production in C28/I2 (Chondrocyte

Cell Line)

The C28/I2 Human Chondrocyte Cell Line (Merck), a human chondrocyte cell line, was seeded in Corning 96-well plates at 3 x 10^3 cells/well in DMEM medium (Gibco #11320) containing 10% FBS (NICHIREI BIOSCIENCES INC) and Antibiotic-Antimycotic Solution (Fujifilm, 161-23181). At the time of seeding, 10 nM hsa-miRNA-126-3p mimic (miR-126-3p), 10 nM miRNA-140-5p mimic alone (miR-140-5p), a combination of 10 nM miRNA-126-3p mimic and 10 nM miRNA-140-5p mimic, or 20 nM Negative control miRNA mimic (NC) was introduced into C28/I2 cells using Lipofectamin RNAiMax, followed by culture in an incubator at 37°C and 5% CO2. Two days later, the medium was removed and replaced with medium containing 10 ng/mL human recombinant IL-1β (R&D) or ordinary medium not containing human recombinant IL-1β, and the supernatant was collected after further culture for 24 hours. The VEGF content in the supernatant was measured using a Human VEGF ELISA kit (abcam). The number of cells in the 96-well plate at the time of collecting the culture supernatant was analyzed using CellTiter-Glo (registered trademark) 2.0 Cell Viability Assay (Promega), and the amount of VEGF produced per cell was calculated. The results are shown in Fig. 7. As shown in Fig. 7, under both conditions without and with addition of IL-1β, the amounts of VEGF produced in the hsa-miRNA-126-3p- or hsa-miR-140-5p-treated groups were significantly lower than that in the Negative control miRNA-mimic-treated group, and the amount was further significantly lower in the combination group than in the single-treatment groups, demonstrating that the combination of the miRNAs enhanced the VEGF production-inhibiting effect also in the human chondrocyte cell line.

[0111]

Example 6. Inhibition of VEGF Production in SW1353 (Chondrosarcoma Cell Line)

The human chondrosarcoma cell line SW1353 (Cell lines service) was seeded in Corning 96-well plates at 3 x 10^3 cells/well in DMEM : F12 (1 : 1) (Gibco #11320) containing 5% FBS (NICHIREI BIOSCIENCES INC) and Antibiotic-Antimycotic Solution (Fujifilm, 161-23181). At the time of seeding, 10 nM hsa-miRNA-126-3p mimic (miR-126-3p), 10 nM miRNA-140-

5p mimic alone (miR-140-5p), a combination of 10 nM miRNA-126-3p mimic and 10 nM miRNA-140-5p mimic, or 20 nM Negative control miRNA mimic (NC) was introduced into SW-1353 cells using Lipofectamin RNAiMax, followed by culture in an incubator at 37°C and 5% CO2. Two days later, the medium was removed and replaced with medium containing 10 ng/mL human recombinant IL-1β (R&D) or ordinary medium not containing human recombinant IL-1β, and the supernatant was collected after further culture for 24 hours. The VEGF content in the supernatant was measured using a Human VEGF ELISA kit (abcam). The number of cells in the 96-well plate at the time of collecting the culture supernatant was analyzed using CellTiter-Glo (registered trademark) 2.0 Cell Viability Assay (Promega), and the amount of VEGF produced per cell was calculated.

The results are shown in Fig. 8. As shown in Fig. 8, under the condition without addition of IL-1β, the amounts of VEGF produced in the miRNAhsa-miRNA-126-3p- and hsa-miR-140-5p-treated groups and the combination group decreased to or below the detection limit relative to the Negative control miRNA-mimic-treated group. Treatment with IL-1β, a cytokine that promotes inflammation, increased the amount of VEGF produced by SW1353 by 3-fold or more, and even under this condition, the amounts of VEGF produced in the miRNAhsa-miRNA-126-3p- and hsa-miR-140-5p-treated groups were significantly lower than that in the Negative control miRNA-mimic-treated group, and the amount was further decreased to or below the detection limit in the combination group, demonstrating that the combination enhanced the VEGF production-inhibiting effect also in the human chondrosarcoma cell line.

CLAIMS

[Claim 1]

A prophylactic or therapeutic agent for a disease or condition associated with angiogenesis, comprising hsa-miR-140-5p and hsa-miR-126-3p.

[Claim 2]

An agent comprising hsa-miR-140-5p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-126-3p.

[Claim 3]

An agent comprising hsa-miR-126-3p, for preventing or treating a disease or condition associated with angiogenesis through combined use with hsa-miR-140-5p.

[Claim 4]

A VEGF production inhibitor comprising hsa-miR-140-5p and hsa-miR-126-3p.

[Claim 5]

An agent comprising hsa-miR-140-5p, for inhibiting VEGF production through combined use with hsa-miR-126-3p.

[Claim 6]

An agent comprising hsa-miR-126-3p, for inhibiting VEGF production through combined use with hsa-miR-140-5p.

[Claim 7]

The agent according to any one of claims 1 to 6, wherein the disease or condition associated with angiogenesis is at least one selected from the group consisting of joint disease, shoulder stiffness, cancer, chronic rheumatoid arthritis, cancer cell proliferation/metastasis, atherosclerosis, neovascular maculopathy, psoriasis, diabetic retinopathy, myopic choroidal neovascularization,

age-related macular degeneration, enthesitis, baseball elbow, rotator cuff injury, and plantar muscle tendinitis.

[Claim 8]

The agent according to claim 7, wherein the disease or condition associated with angiogenesis is joint disease.

[Claim 9]

The agent according to claim 8, wherein the joint disease is knee osteoarthritis.

[Claim 10]

The agent according to any one of claims 1 to 6, wherein the agent is an injectable preparation.