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(71) **Demandeur/Applicant:**
ORIZURU THERAPEUTICS, INC., JP

(72) **Inventeurs/Inventors:**
HIYOSHI, HIDEYUKI, JP;
YAMAZOE, NORIKO, JP;
TOYODA, TARO, JP;
KONAGAYA, SHUHEI, JP

(74) **Agent:** SMART & BIGGAR LP

(54) **Titre : AGENT DE MATURATION**
(54) **Title: MATURATION AGENT**

(57) **Abrégé/Abstract:**
A purpose of the present invention is to provide a novel method for inducing the differentiation of pluripotent stem cells into an insulin-producing cell population. Provided is a method for producing an insulin-producing cell population, wherein the treatment target is a pancreatic endocrine progenitor cell population and/or a cell population at a subsequent differentiation stage, and the method includes culture in medium containing a compound having tubulin polymerization enhancing activity and/or depolymerization inhibiting activity.

Abstract

An object of the present invention is to provide a novel method for inducing the differentiation of pluripotent stem cells into an insulin-producing cell population. The present invention provides a method for producing an insulin-producing cell population, comprising culturing a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation to be treated in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

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Description

Title of Invention: MATURATION AGENT

Technical Field

[0001]

The present invention relates to a method for producing an insulin-producing cell population from pluripotent stem cells. More specifically, the present invention relates to a method for producing an insulin-producing cell population, comprising treating a pancreatic endocrine progenitor cell population or a cell population at a later stage of differentiation, obtained by the induction of differentiation from pluripotent stem cells with a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[Background Art]

[0002]

Research is underway to induce the differentiation of pluripotent stem cells such as iPS cells or ES cells into insulin-producing cells or pancreatic β cells and to apply the obtained cells to the treatment of diabetes mellitus.

[0003]

Various approaches have been developed and reported so far in order to induce the differentiation of pluripotent stem cells into insulin-producing cell populations (Non Patent Literature 1). However, an insulin-producing cell population obtained by the induction of differentiation comprises various cells in addition to the cells of interest such as insulin-producing cells. In the case of applying an insulin-producing cell population to the treatment of diabetes mellitus, it is very important from a safety standpoint to strictly control the types of cells contained in the cell population. Hence, there has been a strong demand for a novel method for inducing differentiation into an insulin-producing cell population capable of reducing coexisting unintended cells.

Citation List

Non Patent Literature

[0004]

Non Patent Literature 1: Stem Cell Research (2015) 14, 185-197

Summary of Invention

Technical Problem

[0005]

An object of the present invention is to provide a novel method for inducing the differentiation of

pluripotent stem cells into an insulin-producing cell population.

Solution to Problem

[0006]

The present inventors have conducted diligent studies to attain the object and consequently found that an insulin-producing cell population obtained by the induction of differentiation from pluripotent stem cells is subjected to extended culture using a basal medium, whereby even unintended cells (proliferative non-endocrine cells) slightly remaining in the cell population can be detected with high sensitivity.

[0007]

The present inventors have also found that in the process of inducing an insulin-producing cell population from pluripotent stem cells, a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation is cultured in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity (hereinafter, also referred to as a "microtubule inhibitor"), whereby the unintended cells present in the resulting insulin-producing cell population can be reduced.

[0008]

The present invention is based on these novel findings and encompasses the following inventions.

[1] A method for producing an insulin-producing cell population, comprising

culturing a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation to be treated in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[2] The method according to [1], wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is a compound selected from taxoid anticancer agents or a pharmacologically acceptable salt thereof.

[3] The method according to [1] or [2], wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is docetaxel or a pharmacologically acceptable salt thereof.

[4] The method according to any of [1] to [3], wherein the produced insulin-producing cell population comprises 95% or more of CHGA-positive cells.

[5] The method according to any of [1] to [3], wherein the produced insulin-producing cell population comprises 0.1% or less of CHGA-negative and Ki67-positive cells.

[6] The method according to any of [1] to [5], wherein the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of

differentiation to be treated is a cell population produced by the induction of differentiation from pluripotent stem cells.

[7] A culture medium for a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation, comprising a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[8] The culture medium according to [7], wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is a compound selected from taxoid anticancer agents or a pharmacologically acceptable salt thereof.

[9] The culture medium according to [7] or [8], wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is docetaxel or a pharmacologically acceptable salt thereof.

[10] The culture medium according to any of [7] to [9], wherein the culture medium is used for increasing the proportion of CHGA-positive cells and/or for increasing the proportion of insulin-positive and NKX6.1-positive cells.

[10-1] A method for detecting non-endocrine cells in an insulin-producing cell population, comprising culturing the insulin-producing cell population in a medium containing a growth factor.

[10-2] A method for detecting non-endocrine cells in an insulin-producing cell population, comprising culturing the insulin-producing cell population in a medium containing epidermal growth factor (EGF) or a substance having equivalent or similar activity thereto.

[11] A method for detecting non-endocrine cells in an insulin-producing cell population, comprising culturing the insulin-producing cell population in a medium containing epidermal growth factor (EGF).

[12] The method according to [11], wherein the non-endocrine cells are CHGA-negative and PDX1-positive cells and/or CHGA-negative and PDX1-negative cells.

[13] A method for removing non-endocrine cells, comprising

culturing a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation to be treated in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[13-1] A culture medium for an insulin-producing cell population, comprising a growth factor, wherein the culture medium is used for detecting non-endocrine cells in the insulin-producing cell population.

[13-2] A culture medium for an insulin-producing cell population, comprising epidermal growth factor (EGF) or a substance having equivalent or similar activity thereto,

wherein the culture medium is used for detecting non-endocrine cells in the insulin-producing cell population.

[14] A culture medium for an insulin-producing cell population, comprising EGF, wherein the culture medium is used for detecting non-endocrine cells in the insulin-producing cell population.

The present specification encompasses the contents described in the specification, etc. of Japanese Patent Application No. 2021-019120 filed on February 9, 2021 on which the priority of the present application is based.

All publications, patents and patent applications cited herein are incorporated herein by reference in their entirety.

Advantageous Effects of Invention

[0009]

The present invention can provide a novel method for inducing the differentiation of pluripotent stem cells into an insulin-producing cell population. The present invention can produce an insulin-producing cell population in which coexisting unintended cells have been reduced.

Brief Description of Drawings

[0010]

[Figure 1] Figure 1 shows results of analyzing the expression of marker proteins (PDX1 and CHGA) by flow

cytometry in an insulin-producing cell population extended-cultured in a basal medium containing epidermal growth factor (EGF).

[Figure 2] Figure 2 shows results of extended-culturing an insulin-producing cell population obtained by treatment with cisplatin or docetaxel in a basal medium containing EGF, and then analyzing the expression of marker proteins (PDX1 and CHGA) in each resulting cell population by flow cytometry, and a photograph of each cell population thus extended-cultured. The control cell population is an insulin-producing cell population obtained without the use of any of cisplatin and docetaxel, and was extended-cultured in the same manner as above.

[Figure 3] Figure 3 shows results of extended-culturing an insulin-producing cell population obtained by treatment with cisplatin or docetaxel in a basal medium containing EGF, and then analyzing the expression of marker proteins (PDX1 and Ki67) in each resulting cell population by flow cytometry. The control cell population is an insulin-producing cell population obtained without the use of any of cisplatin and docetaxel, and was extended-cultured in the same manner as above.

[Figure 4] Figure 4 shows results of analyzing the expression of marker proteins (INS and NKX6.1; and CHGA and Ki67) by flow cytometry in an insulin-producing cell

population obtained by treatment with docetaxel. The control cell population is an insulin-producing cell population obtained without the use of docetaxel.

[Figure 5] Figure 5 shows (A) results of analyzing the expression of marker proteins (NKX6.1, C-peptide (insulin), PDX1, and CHGA) by flow cytometry in a cell population obtained 7 days after the start of step 6), and (B) results of extended-culturing an insulin-producing cell population obtained by treatment with docetaxel in a basal medium containing EGF for 7 days from 4 days after the start of step 6) or 4 days from 7 days after the start of step 6), and then analyzing the expression of marker proteins (PDX1 and CHGA) in each resulting cell population by flow cytometry. The control cell population is an insulin-producing cell population obtained without the use of docetaxel, and was extended-cultured in the same manner as above.

[Figure 6] Figure 6 shows results of measurement of (A) a human C-peptide concentration in plasma in an immunodeficient NOD/SCID mouse induced to have insulin-deficient diabetes mellitus in which an insulin-producing cell population obtained by treatment with docetaxel (docetaxel(+)) or an insulin-producing cell population prepared without the addition of docetaxel (docetaxel(-)) was subcutaneously transplanted, and (B) a weight of the graft excised 6 month after transplantation.

Description of Embodiments

[0011]

1. Terminology

Hereinafter, the terms described herein will be described.

[0012]

As used herein, "about" or "around" refers to a value which may vary up to plus or minus 25%, 20%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1% from the reference value. Preferably, the term "about" or "around" refers to a range from minus or plus 15%, 10%, 5%, or 1% from the reference value.

[0013]

As used herein, "comprise(s)" or "comprising" means inclusion of the element(s) following the word without limitation thereto. Accordingly, it indicates inclusion of the element(s) following the word, but does not indicate exclusion of any other element.

[0014]

As used herein, "consist(s) of" or "consisting of" means inclusion of all the element(s) following the phrase and limitation thereto. Accordingly, the phrase "consist(s) of" or "consisting of" indicates that the enumerated element(s) is required or essential and substantially no other elements exist.

[0015]

As used herein, "without the use of feeder cell(s)" means basically containing no feeder cells and using no medium preconditioned by culturing feeder cells. Accordingly, the medium does not contain any substance, such as a growth factor or a cytokine, secreted by feeder cells.

[0016]

"Feeder cells" or "feeder" means cells that are co-cultured with another kind of cells, support the cells, and provide an environment that allows the cells to grow. The feeder cells may be derived from the same species as or a different species from the cells that they support. For example, as a feeder for human cells, human skin fibroblasts or human embryonic-stem cells may be used or a primary culture of murine embryonic fibroblasts or immortalized murine embryonic fibroblasts may be used. The feeder cells can be inactivated by exposure to radiation or treatment with mitomycin C.

[0017]

As used herein, "adhered (adherent)" refers to cells are attached to a container, for example, cells are attached to a cell culture dish or a flask made of a sterilized plastic (or coated plastic) in the presence of an appropriate medium. Some cells cannot be maintained or grow in culture without adhering to the cell culture container. In contrast, non-adherent cells can be

maintained and proliferate in culture without adhering to the container.

[0018]

As used herein, "culture" refers to maintaining, growing, and/or differentiating cells in in vitro environment. "Culturing" means maintaining, proliferating, and/or differentiating cells out of tissue or the living body, for example, in a cell culture dish or flask. The culture includes two-dimensional culture (plane culture) and three-dimensional culture (suspension culture).

[0019]

As used herein, "enrich(es)" and "enrichment" refer to increasing the amount of a certain component in a composition such as a composition of cells and "enriched" refers, when used to describe a composition of cells, for example, a cell population, to a cell population increased in the amount of a certain component in comparison with the percentage of such component in the cell population before the enrichment. For example, a composition such as a cell population can be enriched for a target cell type and, accordingly, the percentage of the target cell type is increased in comparison with the percentage of the target cells present in the cell population before the enrichment. A cell population can be enriched for a target cell type by a method of selecting and sorting cells known in the art. A cell

population can be enriched by a specific process of sorting or selection described herein. In a certain embodiment of the present invention, a cell population is enriched for a target cell population at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% by a method of enriching the target cell population.

[0020]

As used herein, "deplete(s)" and "depletion" refer to decreasing the amount of a certain component in a composition such as a composition of cells and "depleted" refers, when used to describe a composition of cells, for example, a cell population, to a cell population decreased in the amount of a certain component in comparison with the percentage of such component in the cell population before the depletion. For example, a composition such as a cell population can be depleted for a target cell type and, accordingly, the percentage of the target cell type is decreased in comparison with the percentage of the target cells present in the cell population before the depletion. A cell population can be depleted for a target cell type by a method of selecting and sorting cells known in the art. A cell population can be depleted by a specific process of sorting or selection described herein. In a certain embodiment of the present invention, a cell population is reduced (depleted) for a target cell population at least

50%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% by a method of depleting a target cell population.

[0021]

As used herein, "purify(ies)" and "purification" refer to removing impurities in a composition such as a composition of cells and making it pure for a certain component and "purified" refers, when used to describe a composition of cells, for example, a cell population, to a cell population in which the amount of impurities is decreased in comparison with the percentage of such components in the cell population before purification and the purity of a certain component is improved. For example, a composition such as a cell population can be purified for a target cell type and, accordingly, the percentage of the target cell type is increased in comparison with the percentage of the target cells present in the cell population before the purification. A cell population can be purified for a target cell type by a method of selecting and sorting cells known in the art. A cell population can be purified by a specific process of sorting or selection described herein. In a certain embodiment of the present invention, the purity of a target cell population is brought by a method of purifying a target cell population to at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% or to the extent at which impurities (including contaminant cells) are undetectable.

[0022]

As used herein, "marker" means a cell antigen or a gene thereof that is specifically expressed depending on a predetermined cell type, such as "marker protein" and "marker gene". Preferably, a marker is a cell surface marker and this allows concentration, isolation, and/or detection of living cells. A marker can be a positive selection marker or a negative selection marker.

[0023]

The detection of a marker protein can be conducted by an immunological assay, for example, ELISA, immunostaining, or flow cytometry using an antibody specific for the marker protein. The detection of a marker gene can be conducted by a method of amplifying and/or detecting nucleic acid known in the art, for example, RT-PCR, microarray, biochip, or the like. As used herein, "positive" for a marker protein means being detected to be positive by flow cytometry and "negative" therefor means being equal to or less than the lower detection limit in flow cytometry. Also, as used herein, "positive" for a marker gene means being detected by RT-PCR and "negative" therefor means being equal to or less than the lower detection limit in RT-PCR.

[0024]

As used herein, "expression" is defined as transcription and/or translation of a certain nucleotide sequence driven by an intracellular promoter.

[0025]

As used herein, "factor having CDK8/19-inhibiting activity" means any substance having the inhibitory activity for CDK8/19. CDK8, in contrast to the other proteins of the same CDK family, is not required for cell proliferation. The inhibition of CDK8 has no great effect under usual conditions. CDK19 and CDK8 are similar to each other. Usually, the inhibition of CDK8 also involves the inhibition of CDK19. In the present invention, conventionally known factor having CDK8/19-inhibiting activity can be used, and such a factor having CDK8/19-inhibiting activity can be found from patent literatures or non patent literatures. Examples thereof include, among compounds described in US2012/0071477, WO2015/159937, WO2015/159938, WO2013/116786, WO2014/0038958, WO2014/134169, JP2015/506376, US2015/0274726, US2016/0000787, WO2016/009076, WO2016/0016951, WO2016/018511, WO2016/100782 and WO2016/182904, compounds (and salts thereof) having CDK8/19-inhibiting activity. More specific examples of the factor having CDK8/19-inhibiting activity include diethyl (E)-(4-(3-(5-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)acrylamido)benzyl)phosphonate, 2-(4-(4-(isoquinolin-4-yl)phenyl)-1H-pyrazol-1-yl)-N,N-dimethylacetamide, 4-((2-(6-(4-methylpiperazine-1-carbonyl)naphthalen-2-yl)ethyl)amino)quinazoline-6-carbonitrile, 4-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-

1H-pyrazol-3-yl)benzene-1,3-diol, 3-(2-(imidazo[1,2-b]pyridazin-6-ylthio)ethyl)-4-(naphthalen-1-ylsulfornyl)-3,4-dihydroquinoxalin-2(1H)-one, (E)-3-(4-(1-cyclopropyl-1H-pyrazol-4-yl)pyridin-3-yl)-N-(4-(morpholinomethyl)phenyl)acrylamide, 8-(2,4-difluorophenoxy)-1-methyl-4,5-dihydro-1H-thieno[3,4-g]indazole-6-carboxamide, 4-((4-fluorophenyl)sulfonyl)-3-(2-(imidazo[1,2-b]pyridazin-6-ylsulfanyl)ethyl)-3,4-dihydroquinoxalin-2(1H)-one, 2-(benzylamino)-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)benzamide, 3-(3-(benzyloxy)phenyl)-1H-pyrrolo[2,3-b]pyridine, 4-(4-(2,3-dihydro-1,4-benzodioxin-6-yl)-1H-pyrazol-3-yl)benzene-1,3-diol, N-butyl-8-(4-methoxyphenyl)-1,6-naphthyridine-2-carboxamide, 8-(4-methylphenyl)-N,N-dipropyl-1,6-naphthyridine-2-carboxamide and salts thereof.

[0026]

The factor having CDK8/19-inhibiting activity is not limited to the compounds described above, and an antisense oligonucleotide or siRNA against CDK8/19 mRNA, an antibody that binds to CDK8/19, a dominant negative CDK8/19 mutant, or the like can also be used as the CDK8/19 inhibitor. The factor having CDK8/19-inhibiting activity or the CDK8/19 inhibitor is commercially available or can be synthesized for use according to a known method.

[0027]

As used herein, "growth factors" are endogenous proteins that promote differentiation and/or proliferation of particular cells. Examples of "growth factors" include epidermal growth factor (EGF), acid fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), insulin-like growth factor 2 (IGF-2), keratinocyte growth factor (KGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), transformation growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), transferrin, various interleukins (for example, IL-1 to IL-18), various colony stimulating factors (for example, granulocyte/macrophage-colony stimulating factor (GM-CSF)), various interferons (for example, IFN-γ, and the like), and other cytokines having effects on stem cells, for example, stem cell factor (SCF), and erythropoietin (Epo).

[0028]

As used herein, "ROCK inhibitors" means substances that inhibit Rho kinase (ROCK: Rho-associated, coiled-coil containing protein kinase) and may be substances that inhibit either of ROCK I and ROCK II. The ROCK inhibitors are not particularly limited as long as they have the aforementioned function and examples include N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide (that may be herein also referred to as Y-27632), Fasudil (HA1077), (2S)-2-methyl-1-[(4-methyl-5-

isoquinolinyl]sulfonyl]hexahydro-1H-1,4-diazepine (H-1152), 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carbamide (Wf-536), N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide (Y-30141), N-(3-{[2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1H-imidazo[4,5-c]pyridin-6-yl]oxy}phenyl)-4-{[2-(4-morpholinyl)ethyl]-oxy}benzamide (GSK269962A), N-(6-fluoro-1H-indazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide (GSK429286A). The ROCK inhibitors are not limited to these and antisense oligonucleotides and siRNA to ROCK mRNA, antibodies that bind to ROCK, and dominant negative ROCK mutants can also be used as the ROCK inhibitors. The ROCK inhibitors are commercially available or can be synthesized according to a known method.

[0029]

As used herein, "GSK3β inhibitors" are substances having the inhibitory activity for GSK3β (glycogen synthase kinase 3β). GSK3 (glycogen synthase kinase 3) is a serine/threonine protein kinase and involved in many signaling pathways associated with the production of glycogen, apoptosis, maintenance of stem cells, etc. GSK3 has the 2 isoforms α and β. "GSK3β inhibitors" used in the present invention are not particularly limited as long as they have the GSK3β-inhibiting activity and they

may be substances having both the GSK3α-inhibiting activity and the GSK3β-inhibiting activity.

[0030]

Examples of GSK3β inhibitors include CHIR98014 (2-[[2-[(5-nitro-6-aminopyridin-2-yl)amino]ethyl]amino]-4-(2,4-dichlorophenyl)-5-(1H-imidazol-1-yl)pyrimidine), CHIR99021 (6-[[2-[[4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]nicotinonitrile), TDZD-8 (4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione), SB216763 (3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione), TWS-119 (3-[6-(3-aminophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yloxy]phenol), kenpaullone, 1-azakenpaullone, SB216763 (3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione), SB415286 (3-[(3-chloro-4-hydroxyphenyl)amino]-4-(2-nitrophenyl)-1H-pyrrole-2,5-dione), and AR-AO144-18, CT99021, CT20026, BIO, BIO-acetoxime, pyridocarbazole-ruthenium cyclopentadienyl complex, OTDZT, alpha-4-dibromoacetophenone, lithium, and the like. The GSK3β inhibitors are not limited to these and antisense oligonucleotides and siRNA to GSK3β mRNA, antibodies that bind to GSK3β, dominant negative GSK3β mutants, and the like can also be used as the GSK3β inhibitors. The GSK3β inhibitors are commercially available, or can be synthesized according to a known method.

[0031]

As used herein, "FGFR1 inhibitors" are substances having the inhibitory activity at least for fibroblast growth factor receptor (FGFR) 1. FGFR1 is a member of the four-pass transmembrane tyrosine kinase family (FGFR1,2,3,4) as a receptor having high affinity for growth factors FGF1 to FGF17. As used herein, "FGFR1 inhibitors" can have at least FGFR1-inhibiting activity and may have the inhibitory activity for other FGFRs. As used herein, "FGFR1 inhibitors" may be, for example, FGFR2 inhibitors, FGFR3 inhibitors, or FGFR4 inhibitors as long as these inhibitors have FGFR1-inhibiting activity. In the present invention, conventionally known FGFR1 inhibitors can be used. More specific examples of the FGFR1 inhibitors include PD-166866 (1-[2-amino-6-(3,5-dimethoxyphenyl)-pyrido(2,3-d)pyrimidin-7-yl]-3-tert-butylurea: CAS No.: 192705-79-6), E-3810 (CAS No.: 1058137-23-7), PD-173074 (CAS No.: 219580-11-7), FGFR4-IN-1 (CAS No.: 1708971-72-5), FGFR-IN-1 (CAS No.: 1448169-71-8), FIIN-2 (CAS No.: 1633044-56-0), AZD4547 (CAS No.: 1035270-39-3), FIIN-3 (CAS No.: 1637735-84-2), NVP-BGJ398 (CAS No.: 1310746-10-1), NVP-BGJ398 (CAS No.: 872511-34-7), CH5183284 (CAS No.: 1265229-25-1), Derazantinib (CAS No.: 1234356-69-4), Derazantinib Racemate, Ferulic acid (CAS No.: 1135-24-6), SSR128129E (CAS No.: 848318-25-2), SSR128129E free acid (CAS No.: 848463-13-8), Erdafitinib (CAS No.: 1346242-81-6), BLU9931 (CAS No.: 1538604-68-0), PRN1371 (CAS No.:

1802929-43-6), S49076 (CAS No.: 1265965-22-7), LY2874455 (CAS No.: 1254473-64-7), Linsitinib (CAS No.: 867160-71-2), Dovitinib (CAS No.: 405169-16-6), Anlotinib (CAS No.: 1058156-90-3), Brivanib (CAS No.: 649735-46-6), Derazantinib (CAS No.: 1234356-69-4), Anlotinib Dihydrochloride (CAS No.: 1360460-82-7), ACTB-1003 (CAS No.: 939805-30-8), BLU-554 (CAS No.: 1707289-21-1), Rogaratinib (CAS No.: 1443530-05-9), BIBF 1120 esylate (CAS No.: 656247-18-6), TG 100572 Hydrochloride (CAS No.: 867331-64-4), ENMD-2076 (CAS No.: 934353-76-1), Brivanib alaninate (CAS No.: 649735-63-7), TG 100572 (CAS No.: 867334-05-2), BIBF 1120 (CAS No.: 656247-17-5), ENMD-2076 Tartrate (CAS No.: 1291074-87-7), TSU-68 (CAS No.: 252916-29-3), Ponatinib (CAS No.: 943319-70-8), Sulfatinib (CAS No.: 1308672-74-3), LY2784544 (CAS No.: 1229236-86-5), Dovitinib lactate (CAS No.: 692737-80-7), SU 5402 (CAS No.: 215543-92-3), FGF-401 (CAS No.: 1708971-55-4), Tyrosine kinase-IN-1 (CAS No.: 705946-27-6), PP58 (CAS No.: 212391-58-7), TG 100801 Hydrochloride (CAS No.: 1018069-81-2), Crenolanib (CAS No.: 670220-88-9), TG 100801 (CAS No.: 867331-82-6), Pazopanib Hydrochloride (CAS No.: 635702-64-6), Pazopanib (CAS No.: 444731-52-6), PD168393 (CAS No.: 194423-15-9), Apatinib (CAS No.: 1218779-75-9), Palbociclib isethionate (CAS No.: 827022-33-3), Foretinib (CAS No.: 849217-64-7), Lenvatinib (CAS No.: 417716-92-8), Tandutinib (CAS No.: 387867-13-2) and salts thereof.

[0032]

The FGFR1 inhibitors are not limited to the compounds described above, and antisense oligonucleotides and siRNA to FGFR1 mRNA, antibodies that bind to FGFR1, and dominant negative FGFR1 mutants can also be used as the FGFR1 inhibitors. The FGFR1 inhibitors are commercially available or can be synthesized according to a known method.

[0033]

As used herein, examples of "serum replacement" include KnockOut(TM) Serum Replacement (KSR: Thermo Fisher Scientific), StemSure(R) Serum Replacement (Wako), B-27 supplement, N2-supplement, albumin (for example, lipid rich albumin), insulin, transferrin, fatty acids, collagen precursors, trace elements (for example, zinc, selenium (for example, sodium selenite)), 2-mercaptoethanol, 3'-thiolglycerol, or mixtures thereof (for example, ITS-G). Preferred serum replacements are B-27 supplement, KSR, StemSure(R) Serum Replacement, ITS-G. The concentration of serum replacement in a medium when added into a medium is 0.01-10% by weight, and preferably 0.1-2% by weight. In the present invention, "serum replacement" is preferably used instead of serum.

[0034]

2. Method for producing insulin-producing cell population, and insulin-producing cell population produced thereby

The present invention provides a method for producing an insulin-producing cell population, comprising treating a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation with a microtubule inhibitor in the process of inducing the differentiation of a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation, obtained by the induction of differentiation from pluripotent stem cells into an insulin-producing cell population, and an insulin-producing cell population prepared thereby.

[0035]

In the present invention, the microtubule inhibitor in the process of inducing the differentiation of a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation, obtained by the induction of differentiation from pluripotent stem cells into an insulin-producing cell population acts on the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of differentiation to give rise to an insulin-producing cell population in which endogenous non-endocrine cells have been reduced.

[0036]

In the present invention, herein, "pluripotency" means the ability to differentiate into tissues and cells having various different shapes and functions and to

differentiate into cells of any lineage of the 3 germ layers. "Pluripotency" is different from "totipotency", which is the ability to differentiate into any tissue of the living body, including the placenta, in that pluripotent cells cannot differentiate into the placenta and therefore, do not have the ability to form an individual.

[0037]

In the present invention, "multipotency" means the ability to differentiate into plural and limited numbers of linages of cells. For example, mesenchymal stem cells, hematopoietic stem cells, neural stem cells are multipotent, but not pluripotent.

[0038]

In the present invention, "pluripotent stem cells" refers to embryonic-stem cells (ES cells) and cells potentially having a pluripotency similar to that of ES cells, that is, the ability to differentiate into various tissues (all of the endodermal, mesodermal, and ectodermal tissues) in the living body. Examples of cells having a pluripotency similar to that of ES cells include "induced pluripotent stem cells" (that may be herein also referred to as "iPS cells"). In the present invention, preferably, pluripotent stem cells are human pluripotent stem cells.

[0039]

Available "ES cells" include murine ES cells, such as various murine ES cell lines established by inGenious, Institute of Physical and Chemical Research (RIKEN), and the like, and human ES cells, such as various human ES cell lines established by National Institutes of Health (NIH), RIKEN, Kyoto University, Cellartis, and the like. For example, available ES cell lines include CHB-1 to CHB-12, RUES1, RUES2, HUES1 to HUES28 from NIH, and the like; H1 and H9 from WiCell Research Institute; and KhES-1, KhES-2, KhES-3, KhES-4, KhES-5, SSES1, SSES2, SSES3 from RIKEN, and the like.

[0040]

"Induced pluripotent stem cells" refers to cells that are obtained by reprograming mammalian somatic cells or undifferentiated stem cells by introducing particular factors (nuclear reprogramming factors). At present, there are various "induced pluripotent stem cells" and iPS cells established by Yamanaka, et al. by introducing the 4 factors Oct3/4, Sox2, Klf4, and c-Myc into murine fibroblasts (Takahashi K, Yamanaka S., Cell, (2006) 126: 663-676); iPS cells derived from human cells, established by introducing similar 4 factors into human fibroblasts (Takahashi K, Yamanaka S., et al. Cell, (2007) 131: 861-872.); Nanog-iPS cells established by sorting cells using expression of Nanog as an indicator after introduction of the 4 factors (Okita, K., Ichisaka, T., and Yamanaka, S. (2007). Nature 448, 313-317.); iPS cells produced by a

method not using c-Myc (Nakagawa M, Yamanaka S., et al. Nature Biotechnology, (2008) 26, 101-106); and iPS cells established by introducing 6 factors in a virus-free way (Okita K et al. Nat. Methods 2011 May; 8(5): 409-12, Okita K et al. Stem Cells. 31 (3) 458-66) may be also used. Also, induced pluripotent stem cells established by introducing the 4 factors OCT3/4, SOX2, NANOG, and LIN28 by Thomson et al. (Yu J., Thomson JA. et al., Science (2007) 318: 1917-1920.); induced pluripotent stem cells produced by Daley et al. (Park IH, Daley GQ.et al., Nature (2007) 451: 141-146); induced pluripotent stem cells produced by Sakurada et al. (Japanese Unexamined Patent Application Publication No. 2008-307007) and the like may be used.

[0041]

In addition, any of known induced pluripotent stem cells known in the art described in all published articles (for example, Shi Y., Ding S., et al., Cell Stem Cell, (2008) Vol 3, Issue 5, 568-574; Kim JB., Scholer HR., et al., Nature, (2008) 454, 646-650; Huangfu D., Melton, DA., et al., Nature Biotechnology, (2008) 26, No. 7, 795-797) or patents (for example, Japanese Unexamined Patent Application Publication No. 2008-307007, Japanese Unexamined Patent Application Publication No. 2008-283972, US2008-2336610, US2009-047263, WO2007-069666, WO2008-118220, WO2008-124133, WO2008-151058, WO2009-006930, WO2009-006997, WO2009-007852) may be used.

[0042]

Available induced pluripotent cell lines include various iPS cell lines established by NIH, Institute of Physical and Chemical Research (RIKEN), Kyoto University and the like. For example, such human iPS cell lines include the RIKEN cell lines HiPS-RIKEN-1A, HiPS-RIKEN-2A, HiPS-RIKEN-12A, and Nips-B2 and the Kyoto University cell lines Ff-WJ-18, Ff-I01s01, Ff-I01s02, Ff-I01s04, Ff-I01s06, Ff-I14s03, Ff-I14s04, QHJI01s01, QHJI01s04, QHJI14s03, QHJI14s04, 253G1, 201B7, 409B2, 454E2, 606A1, 610B1, 648A1, CDI cell lines MyCell iPS Cells (21525.102.10A), MyCell iPS Cells (21526.101.10A), and the like.

[0043]

In the present invention, "pancreatic endocrine progenitor cell population" means a cell population comprising pancreatic endocrine progenitor cells. In the present invention, pancreatic endocrine progenitor cells mean cells characterized by the expression of at least one of the markers chromogranin A (CHGA), NeuroD and NGN3 and no expression of a marker of the pancreas-related hormone system (for example, insulin). The pancreatic endocrine progenitor cells may express a marker such as PAX-4, NKX2.2, Islet-1, or PDX-1.

[0044]

In one embodiment, "pancreatic endocrine progenitor cell population" according to the present invention is a

cell population that corresponds to a culture after the completion of step 5) or a culture in step 6) in the process of inducing the differentiation of pluripotent stem cells into insulin-producing cells as described below in detail.

[0045]

"Pancreatic endocrine progenitor cell population" according to the present invention comprises pancreatic endocrine progenitor cells at a proportion of 30% or more, preferably 40% or more, more preferably 50% or more, further preferably 60% or more, still further preferably 70% or more. The upper limit of the proportion is not particularly limited and is 90% or less, 80% or less, 70% or less, or 60% or less. The proportion can be expressed using two numeric values respectively selected from the numeric values of the upper limit and the lower limit. The proportion is, for example, 30% to 90%, preferably 40% to 80%, more preferably 50% to 80%, further preferably 60% to 70%. The pancreatic endocrine progenitor cell population may include other cells (for example, pancreatic progenitor cells, insulin-producing cells, Ki67-positive cells, and CHGA-negative cells), in addition to the pancreatic endocrine progenitor cells.

[0046]

In the present invention, "cell population at a later stage of differentiation" means a cell population

richer in cells whose stage of differentiation is more advanced than that of pancreatic endocrine progenitor cells, as compared with a pancreatic endocrine progenitor cell population. Examples of "cells whose stage of differentiation more advanced than that of pancreatic endocrine progenitor cells" include insulin-producing cells. In the present invention, insulin-producing cells mean cells characterized by the expression of at least one of the markers insulin and NKX6.1, preferably the expression of both the markers insulin and NKX6.1. The expression level of NGN3 in the insulin-producing cells is preferably at a proportion of less than 1/3 of the maximum expression confirmed in pancreatic endocrine progenitor cells.

[0047]

"Cell population at a later stage of differentiation" according to the present invention comprises insulin-producing cells at a proportion of 30% or more, preferably 40% or more, more preferably 50% or more, further preferably 60% or more, still further preferably 70% or more. The upper limit of the proportion is not particularly limited and is 90% or less, 80% or less, 70% or less, or 60% or less. The proportion can be expressed using two numeric values respectively selected from the numeric values of the upper limit and the lower limit. The proportion is, for example, 30% to 90%, preferably 40% to 80%, more

preferably 50% to 80%, further preferably 60% to 70%. "Cell population at a later stage of differentiation" may include other cells (for example, pancreatic endocrine progenitor cells, insulin-producing cells, Ki67-positive cells, and CHGA-negative cells), in addition to the insulin-producing cells.

[0048]

In one embodiment, "cell population at a later stage of differentiation" according to the present invention corresponds to a cell population of a culture from a period of 3 days, 4 days, 5 days, 6 days, 7 days, 8 days or more before the completion of step 6) or a culture after the completion of step 6) in the process of inducing the differentiation of pluripotent stem cells into insulin-producing cells as described below in detail.

[0049]

The proportion of specific cells in a cell population described herein can be determined on the basis of a known approach capable of calculating the number of cells, such as flow cytometry.

[0050]

It is known that cells having different features depending on the stages of differentiation appear in the process of differentiation of pluripotent stem cells into insulin-producing cells (WO2009/012428 and WO2016/021734). For example, the stages of

differentiation can be broadly classified into pluripotent stem cells, definitive endoderm cells, primitive gut tube cells, posterior foregut cells, pancreatic progenitor cells, pancreatic endocrine progenitor cells, and insulin-producing cells in order from relatively undifferentiated to differentiated forms.

[0051]

The pancreatic endocrine progenitor cell population can be obtained by use of the following known process of inducing differentiation which involves inducing the differentiation of pluripotent stem cells into definitive endoderm cells, primitive gut tube cells, posterior foregut cells, pancreatic progenitor cells, pancreatic endocrine progenitor cells, and insulin-producing cells:

step 1) inducing the differentiation of pluripotent stem cells into definitive endoderm cells;

step 2) inducing the differentiation of the definitive endoderm cells into primitive gut tube cells;

step 3) inducing the differentiation of the primitive gut tube cells into posterior foregut cells;

step 4) inducing the differentiation of the posterior foregut cells into pancreatic progenitor cells;

step 5) inducing the differentiation of the pancreatic progenitor cells into pancreatic endocrine progenitor cells; and

step 6) inducing the differentiation of the pancreatic endocrine progenitor cells into insulin-producing cells.

Hereinafter, each step will be described, though the induction of differentiation into each cell is not limited by these approaches.

[0052]

Step 1) Differentiation into definitive endoderm cells

The pluripotent stem cells are cultured in a medium containing a low dose of activin A to thereby differentiate into definitive endoderm cells.

[0053]

The medium used in this step may be a basal medium for use in the culture of mammalian cells, such as RPMI medium, MEM medium, iMEM medium, DMEM (Dulbecco's modified Eagle medium) medium, Improved MEM Zinc Option medium, Improved MEM/1% B-27 supplement/Penicillin Streptomycin medium, or MCDB131/10 mM Glucose/20 mM Glucose/$NaHCO_3$/FAF-BSA/ITS-X/Glutamax/ascorbic acid/Penicillin Streptomycin medium.

[0054]

The activin A can be contained at a low dose, for example, in an amount of 5 to 100 ng/mL, preferably 5 to 50 ng/mL, more preferably 5 to 10 ng/mL, in the medium.

[0055]

In another embodiment, the concentration of the activin A in the medium is about 0.1 to 100 ng/mL, preferably about 1 to 50 ng/mL, more preferably about 3 to 10 ng/mL.

[0056]

The medium can be further supplemented with a ROCK inhibitor and/or a GSK3β inhibitor.

[0057]

The concentration of the GSK3β inhibitor in the medium is appropriately set depending on the type of the GSK3β inhibitor used. For example, in the case of using CHIR99021 as the GSK3β inhibitor, its concentration is usually 2 to 5 μM, preferably 2 to 4 μM, particularly preferably about 3 μM.

[0058]

The concentration of the ROCK inhibitor in the medium is appropriately set depending on the type of the ROCK inhibitor used. For example, in the case of using Y27632 as the ROCK inhibitor, its concentration is usually 5 to 20 μM, preferably 5 to 15 μM, particularly preferably about 10 μM.

[0059]

The medium can be further supplemented with insulin. The insulin can be contained in an amount of 0.01 to 20 μM, preferably 0.1 to 10 μM, more preferably 0.5 to 5 μM, in the medium. The concentration of the insulin in the medium may be, but is not limited to, the concentration of insulin contained in added B-27 supplement.

[0060]

The culture may be performed by any of two-dimensional culture and three-dimensional culture. The number of cells at the start of culture is not

particularly limited and can be about 50000 to 1000000 cells/cm^2, preferably about 100000 to 800000 cells/cm^2, more preferably about 100000 to 300000 cells/cm^2, for two-dimensional culture. The number of cells at the start of culture is not particularly limited and can be about 10000 to 1000000 cells/mL, preferably about 100000 to 800000 cells/mL, more preferably about 300000 to 600000 cells/mL, for three-dimensional culture. The culture period is 1 day to 4 days, preferably 1 day to 3 days, particularly preferably 3 days.

[0061]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0062]

Alternatively, the definitive endoderm cells according to the present invention can be produced by subjecting the pluripotent stem cells to first culture in a medium under conditions causing action of insulin in the presence of a low dose of activin A and subsequently to second culture in a medium under conditions causing no action of insulin.

[0063]

(1) First culture

"Conditions causing action of insulin" mean conditions that activate an insulin signal transduction

pathway in cells by insulin. Usually, insulin binds to an insulin receptor present on cell membrane surface so that tyrosine kinase incorporated in the receptor is activated for the tyrosine phosphorylation of the insulin receptor substrate protein family (IRS: IRS-1,2,3). As used herein, "activating an insulin signal transduction pathway" refers to causing this series of reactions initiated by the binding of insulin to an insulin receptor.

[0064]

Examples of the conditions causing action of insulin include the case where the medium contains insulin. The insulin can be insulin that can activate an insulin signal transduction pathway in pluripotent stem cells, and may be produced by a recombination method or may be produced through synthesis by a solid-phase synthesis method. Insulin derived from a human, a nonhuman primate, a pig, cattle, a horse, sheep, a goat, a llama, a dog, a cat, a rabbit, a mouse, a guinea pig, or the like can be used. Human insulin is preferred.

[0065]

In the present invention, an insulin mutant, an insulin derivative or an insulin agonist may be used as "insulin" as long as it can activate an insulin signal transduction pathway in pluripotent stem cells. Examples of "insulin mutant" include ones having a polypeptide that consists of an amino acid sequence derived from the

amino acid sequence of insulin by the deletion, substitution, addition or insertion of 1 to 20, preferably 1 to 10, more preferably 1 to 5 amino acids and is capable of activating an insulin signal transduction pathway, or a polypeptide that consists of an amino acid sequence having 80% or more, preferably 90% or more, more preferably 95% or more, most preferably 99% or more sequence identity to the amino acid sequence of insulin and is capable of activating an insulin signal transduction pathway. Amino acid sequences can be compared by a known approach. The comparison can be carried out using, for example, BLAST (Basic Local Alignment Search Tool at the National Center for Biological Information), for example, at default settings. "Insulin derivative" means a polypeptide that consists of an amino acid sequence obtained by the chemical substitution (for example, α-methylation, α-hydroxylation), deletion (for example, deamination), or modification (for example, N-methylation) of one or some of groups of amino acid residues of insulin or an insulin mutant, and is capable of activating an insulin signal transduction pathway, or a substance having a similar effect. "Insulin agonist" means a polypeptide capable of activating an insulin signal transduction pathway by binding to an insulin receptor or a substance having a similar effect, regardless of the structure of insulin.
[0066]

The medium for the first culture can contain the insulin in an amount of 0.01 to 20 μM, preferably 0.1 to 10 μM, more preferably 0.5 to 5 μM. The concentration of the insulin in the medium may be the concentration of insulin contained in added B-27 supplement, but is not limited thereto. As used herein, B-27 supplement containing insulin is also referred to as "B-27(INS+)", and B-27 supplement containing no insulin is also referred to as "B-27(INS-)".

[0067]

The medium can further contain a ROCK inhibitor and/or a GSK3β inhibitor. The concentration of the ROCK inhibitor in the medium is appropriately set depending on the type of the ROCK inhibitor used. For example, in the case of using Y27632 as the ROCK inhibitor, its concentration can be usually 5 to 20 μM, preferably 5 to 15 μM, particularly preferably about 10 μM. The concentration of the GSK3β inhibitor in the medium is appropriately set depending on the type of the GSK3β inhibitor used. For example, in the case of using CHIR99021 as the GSK3β inhibitor, its concentration can be usually 2 to 5 μM, preferably 2 to 4 μM, particularly preferably about 3 μM.

[0068]

The medium can further contain one or more members selected from the group consisting of pyruvate (sodium salt, etc.), L-alanyl L-glutamine, and glucose. The

pyruvate can be contained in an amount of 10 to 1000 mg/L, preferably 30 to 500 mg/L, more preferably 50 to 200 mg/L, particularly preferably about 110 mg/L, in the medium. The L-alanyl L-glutamine can be contained in an amount of 50 to 2000 mg/L, preferably 100 to 1500 mg/L, more preferably 500 to 1000 mg/L, particularly preferably about 860 mg/L, in the medium. The glucose can be contained in an amount of 15 mM or more, preferably 15 to 30 mM, more preferably 15 to 25 mM, particularly preferably about 25 mM, in the medium. The concentrations of the pyruvate, the L-alanyl L-glutamine and the glucose in the medium may be the concentrations of pyruvate, L-alanyl L-glutamine and glucose contained in DMEM medium (DMEM, high glucose, GlutaMAX(TM), pyruvate (Thermo Fisher Scientific)) or other DMEM media, but are not limited thereto.

[0069]

The medium is based on the basal medium and can be supplemented with one or more of the components described above for use. The basal medium is preferably DMEM medium, more preferably DMEM medium containing pyruvate, L-alanyl L-glutamine, and glucose in the amounts described above.

[0070]

The culture period of the first culture can be in a range selected from 6 hours to 48 hours, preferably 12 to 24 hours. The culture temperature is not particularly

limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%. The culture may be performed by any of two-dimensional culture and three-dimensional culture. The number of cells at the start of culture is not particularly limited and can be about 50000 to 1000000 cells/cm^2, preferably about 100000 to 800000 cells/cm^2, more preferably about 100000 to 300000 cells/cm^2, for two-dimensional culture. The number of cells at the start of culture is not particularly limited and can be about 10000 to 1000000 cells/mL, preferably about 100000 to 800000 cells/mL, more preferably about 300000 to 600000 cells/mL, for three-dimensional culture.

[0071]

(2) Second culture

"Conditions causing no action of insulin" mean conditions that do not activate an insulin signal transduction pathway in cells by insulin. "Not activate an insulin signal transduction pathway in cells" not only means that there occurs no activation of the insulin signal transduction pathway but means that there occurs slight activation to an extent that no significant difference is found as compared with the activation of the insulin signal transduction pathway in the absence of insulin. Thus, examples of "conditions causing no action of insulin" include the absence of insulin in the medium,

and, even if insulin is contained in the medium, conditions where the insulin is contained in an amount that causes the slight activation to an extent that no significant difference is found. Alternatively, "conditions causing no action of insulin" also mean that, even if insulin is contained in the medium, the insulin signal transduction pathway is not activated owing to the coexistence of an insulin signaling inhibitor. "Insulin signaling inhibitor" means a component capable of blocking an insulin signal transduction pathway at any position. Examples of such an insulin signaling inhibitor include polypeptides and compounds that bind to or compete with various proteins or the like acting as insulin, an insulin receptor, or a signal transducer and thereby inhibit intermolecular interaction involving these factors. Examples of such an insulin signaling inhibitor include LY294002 [2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one] which competitively inhibits ATP binding to a catalytic subunit of PI3 kinase. The insulin signaling inhibitor is not limited to these, and, for example, an antibody that binds to any of various proteins acting as insulin, an insulin receptor, or a signal transducer, a dominant negative mutant thereof, or an antisense oligonucleotide or siRNA against mRNA of any of various proteins acting as an insulin receptor or a signal transducer can also be used as the insulin signaling inhibitor. The insulin signaling inhibitor is

commercially available or can be synthesized according to a known method.

[0072]

The medium can further contain a ROCK inhibitor and/or a GSK3β inhibitor. The amount of the ROCK inhibitor and/or the GSK3β inhibitor in the medium can be selected from the range described about the first culture and may be the same as or different from the amount used for the first culture.

[0073]

The medium can further contain one or more members selected from the group consisting of pyruvate, L-alanyl L-glutamine, and glucose. The amounts of the pyruvate, the L-alanyl L-glutamine, and the glucose in the medium can be selected from the ranges described about the first culture and may be the same as or different from the amounts used for the first culture.

[0074]

The medium for use in the second culture is based on a basal medium for use in the culture of mammalian cells and can be supplemented with one or more of the components described above for use. The basal medium described about the first culture can be used, and the basal medium may be the same as or different from that used for the first culture. DMEM medium is preferred, and DMEM medium containing pyruvate, L-alanyl L-

glutamine, and glucose in the amounts described above is more preferred.

[0075]

The culture period of the second culture is at least 6 hours and can be in a range selected from preferably 6 to 72 hours, more preferably 24 hours to 72 hours. The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The culture may be performed by any of two-dimensional culture and three-dimensional culture. The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0076]

The media for the first culture and the second culture can contain the low dose of activin A. The amount of activin A contained may be the same or different between the media for the first culture and the second culture.

[0077]

The media for the first culture and the second culture can further contain dimethyl sulfoxide.

[0078]

The proportion of the resulting endocrine cells in step 6) or later can be enhanced by culturing the pluripotent stem cells in the presence of a low dose of activin A or by subjecting the pluripotent stem cells to first culture in a medium under conditions causing action

of insulin in the presence of a low dose of activin A and subsequently to second culture in a medium under conditions causing no action of insulin.

[0079]

Step 2) Differentiation into primitive gut tube cells

The definitive endoderm cells obtained in step 1) are further cultured in a medium containing a growth factor to induce their differentiation into primitive gut tube cells. The culture period is 2 days to 8 days, preferably about 4 days.

[0080]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%. The culture may be performed by any of two-dimensional culture and three-dimensional culture.

[0081]

A basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium may be appropriately supplemented with a serum replacement, a vitamin, an antibiotic, and the like, in addition to the growth factor.

[0082]

The growth factor is preferably EGF, KGF, and/or FGF10, more preferably EGF and/or KGF, further preferably KGF.

[0083]

The concentration of the growth factor in the medium is appropriately set depending on the type of the growth factor used and is usually about 0.1 nM to 1000 μM, preferably about 0.1 nM to 100 μM. In the case of EGF, its concentration is about 5 to 2000 ng/ml (that is, about 0.8 to 320 nM), preferably about 5 to 1000 ng/ml (that is, about 0.8 to 160 nM), more preferably about 10 to 1000 ng/ml (that is, about 1.6 to 160 nM). In the case of FGF10, its concentration is about 5 to 2000 ng/ml (that is, about 0.3 to 116 nM), preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM), more preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM). For example, in the case of using KGF as the growth factor, its concentration is usually 5 to 150 ng/mL, preferably 30 to 100 ng/mL, particularly preferably about 50 ng/mL.

[0084]

Step 3) Differentiation into posterior foregut cells

The primitive gut tube cells obtained in step 2) are further cultured in a medium containing a growth factor, cyclopamine, noggin, and the like to induce their differentiation into posterior foregut cells. The culture period is 1 day to 5 days, preferably about 2 days. The culture may be performed by any of two-dimensional culture and three-dimensional culture.

[0085]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0086]

The basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium may be appropriately supplemented with a serum replacement, a vitamin, an antibiotic, and the like, in addition to the growth factor.

[0087]

The growth factor is preferably EGF, KGF, and/or FGF10, more preferably EGF and/or KGF, further preferably KGF.

[0088]

The concentration of the growth factor in the medium is appropriately set depending on the type of the growth factor used and is usually about 0.1 nM to 1000 μM, preferably about 0.1 nM to 100 μM. In the case of EGF, its concentration is about 5 to 2000 ng/ml (that is, about 0.8 to 320 nM), preferably about 5 to 1000 ng/ml (that is, about 0.8 to 160 nM), more preferably about 10 to 1000 ng/ml (that is, about 1.6 to 160 nM). In the case of FGF10, its concentration is about 5 to 2000 ng/ml (that is, about 0.3 to 116 nM), preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM), more preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM).

For example, in the case of using KGF as the growth factor, its concentration is usually 5 to 150 ng/mL, preferably 30 to 100 ng/mL, particularly preferably about 50 ng/mL.

[0089]

The concentration of the cyclopamine in the medium is not particularly limited and is usually 0.5 to 1.5 μM, preferably 0.3 to 1.0 μM, particularly preferably about 0.5 μM.

[0090]

The concentration of the noggin in the medium is not particularly limited and is usually 10 to 200 ng/mL, preferably 50 to 150 ng/mL, particularly preferably about 100 ng/mL.

The medium may be supplemented with dimethyl sulfoxide.

[0091]

Step 4) Differentiation into pancreatic progenitor cells

The posterior foregut cells obtained in step 3) may be further cultured in a medium containing a factor having CDK8/19-inhibiting activity, preferably a medium containing a factor having CDK8/19-inhibiting activity and a growth factor, to induce their differentiation into pancreatic progenitor cells. The culture period is 2 days to 10 days, preferably about 5 days. The culture may be performed by any of two-dimensional culture and three-dimensional culture. In the case of two-

dimensional culture, according to the previous report (Toyoda et al., Stem cell Research (2015) 14, 185-197), the posterior foregut cells obtained in step 3) are treated with 0.25% trypsin-EDTA and then dispersed by pipetting, and the obtained dispersion is subjected to centrifugal separation. Recovered cells are resuspended in a fresh medium of step 4) and the cell suspension is reseeded to a fresh two-dimensional culture container.

[0092]

The basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium may be appropriately supplemented with a serum replacement, a vitamin, an antibiotic, and the like, in addition to the growth factor.

[0093]

Each of the compounds mentioned above or salts thereof can be used as the factor having CDK8/19-inhibiting activity. The amount of the factor added to the medium is appropriately determined according to the compound or the salt thereof used and is usually about 0.00001 μM to 5 μM, preferably 0.00001 μM to 1 μM. The concentration of the factor having CDK8/19-inhibiting activity in the medium is preferably a concentration that attains inhibitory activity of 50% or more for CDK8/19.

[0094]

The growth factor is preferably EGF, KGF, and/or FGF10, more preferably KGF and/or EGF, further preferably KGF and EGF.

[0095]

The concentration of the growth factor in the medium is appropriately set depending on the type of the growth factor used and is usually about 0.1 nM to 1000 μM, preferably about 0.1 nM to 100 μM. In the case of EGF, its concentration is about 5 to 2000 ng/ml (that is, about 0.8 to 320 nM), preferably about 5 to 1000 ng/ml (that is, about 0.8 to 160 nM), more preferably about 10 to 1000 ng/ml (that is, about 1.6 to 160 nM). In the case of FGF10, its concentration is about 5 to 2000 ng/ml (that is, about 0.3 to 116 nM), preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM), more preferably about 10 to 1000 ng/ml (that is, about 0.6 to 58 nM). For example, in the case of using KGF and EGF as the growth factor, the concentration of EGF is usually 5 to 150 ng/mL, preferably 30 to 100 ng/mL, particularly preferably about 50 ng/mL, and the concentration of KGF is usually 10 to 200 ng/mL, preferably 50 to 150 ng/mL, particularly preferably about 100 ng/mL.

[0096]

Culture on the first day in step 4) may be performed in the presence of a ROCK inhibitor, and culture on the following days may be performed in a medium containing no ROCK inhibitor.

[0097]

The medium may also contain a PKC activator. PdBU (PKC activator II), TPB (PKC activator V), or the like is used as the PKC activator, though the PKC activator is not limited thereto. The concentration of the PKC activator to be added is about 0.1 to 100 ng/ml, preferably about 1 to 50 ng/ml, more preferably about 3 to 10 ng/ml.

[0098]

The medium may also be supplemented with dimethyl sulfoxide and/or activin (1 to 50 ng/ml).

[0099]

In any of the steps, the medium may be supplemented with a serum replacement (for example, B-27 supplement, ITS-G), in addition to the components described above. Also, an amino acid, L-glutamine, GlutaMAX (product name), a non-essential amino acid, a vitamin, nicotinamide, an antibiotic (for example, Antibiotic-Antimycotic (also referred as AA herein), penicillin, streptomycin, or a mixture thereof), an antimicrobial agent (for example, amphotericin B), an antioxidant, pyruvic acid, a buffer, inorganic salts, and the like may be added thereto, if necessary. In the case of adding an antibiotic to the medium, its concentration in the medium is usually 0.01 to 20% by weight, preferably 0.1 to 10% by weight. The culture may be performed by any of two-dimensional culture and three-dimensional culture.

[0100]

In the case of two-dimensional culture, the cell culture is performed by adherent culture without the use of feeder cells. For the culture, a culture container, for example, a dish, a flask, a microplate, or a cell culture sheet such as OptiCell (product name) (Nunc), is used. The culture container is preferably surface-treated in order to improve adhesiveness to cells (hydrophilicity), or coated with a substrate for cell adhesion such as collagen, gelatin, poly-L-lysine, poly-D-lysine, laminin, fibronectin, Matrigel (for example, BD Matrigel (Nippon Becton Dickinson Company, Ltd.)), or vitronectin. The culture container is preferably a culture container coated with type I-collagen, Matrigel, fibronectin, vitronectin or poly-D-lysine, more preferably a culture container coated with Matrigel or poly-D-lysine.

[0101]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0102]

The pancreatic progenitor cells obtained in step 4) can be further purified using a known surface marker glycoprotein 2 (GP2) or the like. The purification can

be performed by a method known per se, for example, using anti-GP2 antibody-immobilized beads.

[0103]

Step 5) Differentiation into pancreatic endocrine progenitor cells

The pancreatic progenitor cells obtained in step 4) are further cultured in a medium containing a growth factor to induce their differentiation into pancreatic endocrine progenitor cells. The culture may be performed by any of two-dimensional culture and three-dimensional culture. In the case of two-dimensional culture, the pancreatic progenitor cells obtained in step 4) are treated with 0.25% trypsin-EDTA and then dispersed by pipetting, and the obtained dispersion is subjected to centrifugal separation. Recovered cells are resuspended in a fresh medium of step 5) and the cell suspension is reseeded to a fresh two-dimensional culture container. The culture period is 2 days to 3 days, preferably about 2 days.

[0104]

The basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium is supplemented with SANT1, retinoic acid, ALK5 inhibitor II, T3, and LDN according to the previous report (Nature Biotechnology 2014; 32: 1121-1133) and may be appropriately further supplemented with a Wnt inhibitor, a ROCK inhibitor, FGF (preferably FGF2), a

serum replacement, a vitamin, an antibiotic, and the like. The medium may be supplemented with dimethyl sulfoxide.

[0105]

The culture is performed by nonadherent culture without the use of feeder cells. For the culture, a dish, a flask, a microplate, a porous plate (Nunc), or the like, or a bioreactor is used. The culture container is preferably surface-treated in order to decrease adhesiveness to cells.

[0106]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0107]

The pancreatic endocrine progenitor cells obtained in step 5) can be further purified using a known surface marker glycoprotein 2 (GP2) or the like. The purification can be performed by a method known per se, for example, using anti-GP2 antibody-immobilized beads.

[0108]

Step 6) Differentiation into insulin-producing cells

The pancreatic endocrine progenitor cells obtained in step 5) are further cultured in a medium containing a FGFR1 inhibitor to induce their differentiation into

insulin-producing cells. The culture period is 10 days to 30 days, preferably about 10 to 20 days.

[0109]

The basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium is supplemented with ALK5 inhibitor II, T3, LDN, γ-secretase inhibitor XX, γ-secretase inhibitor RO, N-cysteine, an AXL inhibitor, and ascorbic acid according to the previous report (Nature Biotechnology 2014; 32: 1121-1133) and may be appropriately further supplemented with a Wnt inhibitor, a ROCK inhibitor, FGF (preferably FGF2), a serum replacement, a vitamin, an antibiotic, and the like. For example, the medium may be supplemented with ALK5 inhibitor II, T3, LDN, γ-secretase inhibitor RO, and ascorbic acid or may be supplemented with T3, ALK5 inhibitor II, $ZnSO_4$, heparin, N-acetylcysteine, Trolox, and R428.

[0110]

The culture may be performed by any of two-dimensional culture and three-dimensional culture. Preferably, the culture is performed by nonadherent culture without the use of feeder cells. For the culture, a dish, a flask, a microplate, a porous plate (Nunc), or the like, or a bioreactor is used. The culture container is preferably surface-treated in order to decrease adhesiveness to cells.

[0111]

The culture temperature is not particularly limited, and the culture is performed at 30 to 40°C (for example, 37°C). The concentration of carbon dioxide in a culture container is on the order of, for example, 5%.

[0112]

The medium can contain a FGFR1 inhibitor in any amount capable of inhibiting FGFR1 activity and can contain the FGFR1 inhibitor in an amount of, for example, 10 μM or less or 5 μM or less, preferably in an amount of less than 5 μM, less than 4 μM, less than 3 μM, or less than 2 μM. The lower limit of the amount of the FGFR1 inhibitor added is not particularly limited and can be 0.1 μM or more, preferably 0.5 μM or more. The amount of the FGFR1 inhibitor added is preferably less than 5 μM and 0.1 μM or more, more preferably less than 5 μM and 0.5 μM or more. The culture in the presence of the FGFR1 inhibitor can be performed for at least 12 hours, preferably 24 hours or longer, 2 days or longer, 4 days or longer, 8 days or longer, 10 days or longer, or 15 days or longer. The culture in the presence of the FGFR1 inhibitor is preferably performed for 4 days or longer. The culture in the presence of the FGFR1 inhibitor can be performed, for example, for about last 4 to 15 days, preferably about last 4 to 7 days, of step 6). The medium may be replaced during the period of treatment with the FGFR1 inhibitor and can be replaced with a medium supplemented with the FGFR1 inhibitor, having the

same or different composition as or from that before the replacement, according to the culture schedule.

[0113]

The culture of the cells in the medium containing the FGFR1 inhibitor can suppress the proliferation of Ki67-positive cells in the resulting insulin-producing cells.

[0114]

The insulin-producing cells obtained in step 6) can be dissociated and recovered using an enzyme such as trypsin. The recovered insulin-producing cells can be cryopreserved until use. Subsequently, the recovered insulin-producing cells can be seeded in an amount of about 500000 to 5000000 cells, preferably about 1000000 to 4000000 cells, more preferably about 2000000 to 3000000 cells, per incubator or well into the medium and subjected to three-dimensional culture to obtain insulin-producing cells in the form of spheroids. Each spheroid consists of about 100 to about 1000 cells, preferably about 200 to about 800 cells, more preferably about 300 to about 500 cells.

[0115]

"Compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity" or "microtubule inhibitor" used in the present invention means a compound having activity of promoting the polymerization of tubulin that forms microtubule to

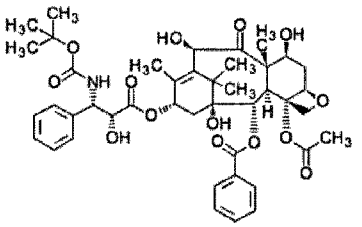
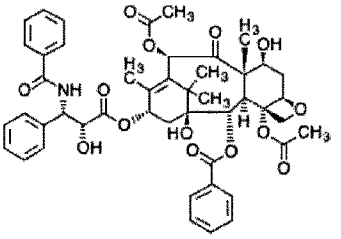
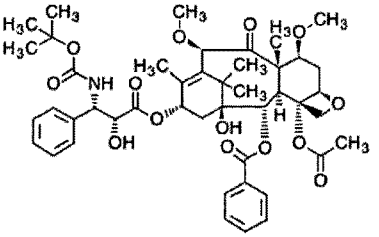
excessively form or stabilize the microtubule, and/or activity of suppressing the depolymerization of tubulin that forms microtubule. The compound preferably has an effect of inhibiting cell division owing to the activity. As used herein, the term "compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity" is used interchangeably with "microtubule inhibitor".

[0116]

"Compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity" or "microtubule inhibitor" that can be used in the present invention is not particularly limited as long as it has the activity. Examples thereof include compounds given below and pharmacologically acceptable salts and solvates thereof. These compounds may have one or more substituents or their substructures (substituents, rings, etc.) may be partially converted as long as the compounds have tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity. Examples of the solvates include, but are not limited to, hydrates and acetone adducts.

[0117]

[Table 1]

Compound	General name: chemical name	Structure
1	**Docetaxel:** (-)-(1S,2S,3R,4S,5R,7S,8S,10R,13S)-4-Acetoxy-2-benzoyloxy-5,20-epoxy-1,7,10-trihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-tert-butoxycarbonylamido-2-hydroxy-3-phenylpropionate	
2	**Paclitaxel:** (-)-(1S,2S,3R,4S,5R,7S,8S,10R,13S)-4,10-Diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino -2-hydroxy-3-phenylpropionate	
3	**Cabazitaxel:** (1S,2S,3R,4S,5R,7S,8S,10R,13S)-4-Acetoxy-2-benzoyloxy-5,20-epoxy-1-hydroxy-7,10-dimethoxy-9-oxotax-11-ene-13-yl (2R,3S)-3-(1,1-dimethylethyl)oxycarbonylamino-2-hydroxy-3-phenylpropionate	

[0118]

In the present invention, "compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity" or "microtubule inhibitor" is preferably a compound that is used as an active ingredient for taxoid anticancer agents or a pharmacologically acceptable salt or a solvate thereof, more preferably docetaxel or a pharmacologically acceptable salt or a solvate thereof.

[0119]

"Compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity" or "microtubule inhibitor" described above is commercially available or can be synthesized for use according to a known method.

[0120]

In the present invention, the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of differentiation, obtained by the induction of differentiation from pluripotent stem cells can be treated with the microtubule inhibitor by the contact of the cell population with the microtubule inhibitor. For example, the treatment with the microtubule inhibitor can be performed by culturing the cell population in a medium supplemented with the microtubule inhibitor. The microtubule inhibitor can be contained in any amount capable of reducing endogenous non-endocrine cells in the finally obtained insulin-producing cell population in the medium, and can be contained in an amount of, for example, 10 μM, 5 μM, 4 μM, 3 μM, 2 μM or less. The lower limit of the amount of the microtubule inhibitor added to the medium is not particularly limited and can be 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 1 μM or more. The range of the amount of the microtubule inhibitor added to the medium can be expressed using two numeric values respectively selected from the numeric values of the upper limit and the lower

limit, and is, for example, 0.1 μM to 10 μM, preferably 0.1 μM to 5 μM, more preferably 1 μM to 3 μM.

[0121]

The culture of the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of differentiation in the presence of the microtubule inhibitor can be performed for any period capable of reducing endogenous non-endocrine cells in the finally obtained insulin-producing cell population. The culture can be performed for, for example, at least 12 hours, preferably 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 10 days, 11 days, 12 days, 15 days or longer. The culture period of the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of differentiation in the presence of the microtubule inhibitor is preferably 3 days, 4 days, 5 days, 6 days, 7 days, 8 days or longer. The medium may be replaced during the period of treatment with the microtubule inhibitor and can be replaced with a medium supplemented with the microtubule inhibitor, having the same or different composition as or from that before the replacement, according to the culture schedule. The basal medium for use in the culture of mammalian cells or the medium for induction of differentiation into insulin-producing cells can be used as culture medium.

[0122]

In the present invention, the pancreatic endocrine progenitor cell population or the cell population at a later stage of differentiation, obtained by the induction of differentiation from pluripotent stem cells can be subjected to the step of differentiation into insulin-producing cells, in addition to being treated with the microtubule inhibitor. As used herein, "in addition to being treated with the microtubule inhibitor" includes the case of performing the step of treatment with the microtubule inhibitor and the step of differentiation at the same time, the case of treating the cell population with the microtubule inhibitor, followed by the step of differentiation, and the case of subjecting the cell population to the step of differentiation, followed by the step of treatment with the microtubule inhibitor. Thus, the medium for use in the treatment with the microtubule inhibitor and the medium for use in the differentiation of the cell population may be separate media, or the medium for use in the step of differentiation may be further supplemented with the microtubule inhibitor.

[0123]

In one embodiment of the present invention, the microtubule inhibitor is contained in a medium in step 6 described above, and allowed to act on the cells, in the process of inducing the differentiation of pluripotent stem cells into insulin-producing cells. Preferably, the

microtubule inhibitor is contained in the medium in step 6 over a period of 3 days, 4 days, 5 days, 6 days, 7 days, 8 days or longer before the completion of step 6 and allowed to act on the cells.

[0124]

In another embodiment of the present invention, the microtubule inhibitor is contained in a medium and allowed to act on the cells after the completion of step 6 described above, in the process of inducing the differentiation of pluripotent stem cells into insulin-producing cells. The basal medium for use in the culture of mammalian cells described in step 1) can be used as culture medium. The medium is supplemented with ALK5 inhibitor II, T3, LDN, γ-secretase inhibitor XX, γ-secretase inhibitor RO, N-cysteine, an AXL inhibitor, and ascorbic acid and may be appropriately further supplemented with a Wnt inhibitor, a ROCK inhibitor, FGF (preferably FGF2), a serum replacement, a vitamin, an antibiotic, and the like. For example, the medium may be supplemented with ALK5 inhibitor II, T3, LDN, γ-secretase inhibitor RO, and ascorbic acid or may be supplemented with T3, ALK5 inhibitor II, $ZnSO_4$, heparin, N-acetylcysteine, Trolox, and R428. Preferably, the microtubule inhibitor is contained in the medium over a period of 3 days, 4 days, 5 days, 6 days, 7 days, 8 days or longer after the completion of step 6 and allowed to act on the cells.

[0125]

The proportion of insulin-producing cells, preferably insulin-positive and NKX6.1-positive cells, in the insulin-producing cell population obtained by the present invention is higher by 5% or more, preferably 10% or more, than the proportion of these cells in an insulin-producing cell population prepared by a conventional method (for example, steps 1) to 6) described above and Nature Biotechnology 2014; 32: 1121-1133). More specifically, the proportion of insulin-producing cells, more specifically, insulin-positive and NKX6.1-positive cells, in the insulin-producing cell population obtained by the present invention is 35% or more, preferably 36% or more, more preferably 37% or more, further preferably 38% or more, still further preferably 39% or more, particularly preferably 40% or more, especially preferably 41% or more. The upper limit of the proportion is not particularly limited and is 70% or less, 60% or less, or 50% or less. The proportion can be expressed using two numeric values respectively selected from the numeric values of the upper limit and the lower limit. The proportion is, for example, 35% to 50%, preferably 36% to 50%, more preferably 37% to 50%, further preferably 38% to 50%, still further preferably 39% to 50%, particularly preferably 40% to 50%, especially preferably 41% to 50%.

[0126]

The proportion of chromogranin A (CHGA)-positive endocrine cells in the insulin-producing cell population obtained by the present invention is higher by 3% or more, preferably 4% or more, more preferably 5% or more, than the proportion of these cells in an insulin-producing cell population prepared by a conventional method (for example, steps 1) to 6) described above and Nature Biotechnology 2014; 32: 1121-1133). More specifically, the proportion of chromogranin A (CHGA)-positive, for example, CHGA-positive and PDX1-positive endocrine cells in the insulin-producing cell population obtained by the present invention is 95% or more, preferably 96% or more, more preferably 97% or more, further preferably 98% or more. The upper limit of the proportion is not particularly limited and is 99% or less. The proportion can be expressed using two numeric values respectively selected from the numeric values of the upper limit and the lower limit. The proportion is, for example, 95% to 99%, preferably 96% to 99%, more preferably 97% to 99%, further preferably 98% to 99%.

[0127]

The insulin-producing cell population obtained by the present invention may include other cells (for example, pancreatic endocrine progenitor cells; other pancreatic hormone-producing cells expressing at least one of the markers glucagon, somatostatin, and pancreatic polypeptide; Ki67-positive cells and CHGA-negative

Claims

[Claim 1]

A method for producing an insulin-producing cell population, comprising

culturing a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation to be treated in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[Claim 2]

The method according to claim 1, wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is a compound selected from taxoid anticancer agents or a pharmacologically acceptable salt thereof.

[Claim 3]

The method according to claim 1 or 2, wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is docetaxel or a pharmacologically acceptable salt thereof.

[Claim 4]

The method according to any one of claims 1 to 3, wherein the produced insulin-producing cell population comprises 95% or more of CHGA-positive cells.

[Claim 5]

The method according to any one of claims 1 to 3, wherein the produced insulin-producing cell population comprises 0.1% or less of CHGA-negative and Ki67-positive cells.

[Claim 6]

The method according to any one of claims 1 to 5, wherein the pancreatic endocrine progenitor cell population and/or the cell population at a later stage of differentiation to be treated is a cell population produced by the induction of differentiation from pluripotent stem cells.

[Claim 7]

A culture medium for a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation, comprising a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[Claim 8]

The culture medium according to claim 7, wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity is a compound selected from taxoid anticancer agents or a pharmacologically acceptable salt thereof.

[Claim 9]

The culture medium according to claim 7 or 8, wherein the compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-

inhibiting activity is docetaxel or a pharmacologically acceptable salt thereof.

[Claim 10]

The culture medium according to any one of claims 7 to 9, wherein the culture medium is used for increasing the proportion of CHGA-positive cells and/or for increasing the proportion of insulin-positive and NKX6.1-positive cells.

[Claim 11]

A method for detecting non-endocrine cells in an insulin-producing cell population, comprising culturing the insulin-producing cell population in a medium containing epidermal growth factor (EGF).

[Claim 12]

The method according to claim 11, wherein the non-endocrine cells are CHGA-negative and PDX1-positive cells and/or CHGA-negative and PDX1-negative cells.

[Claim 13]

A method for removing non-endocrine cells, comprising

culturing a pancreatic endocrine progenitor cell population and/or a cell population at a later stage of differentiation to be treated in a medium containing a compound having tubulin polymerization-promoting activity and/or tubulin depolymerization-inhibiting activity.

[Claim 14]

A culture medium for an insulin-producing cell population, comprising EGF, wherein the culture medium is used for detecting non-endocrine cells in the insulin-producing cell population.

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FIG. 1

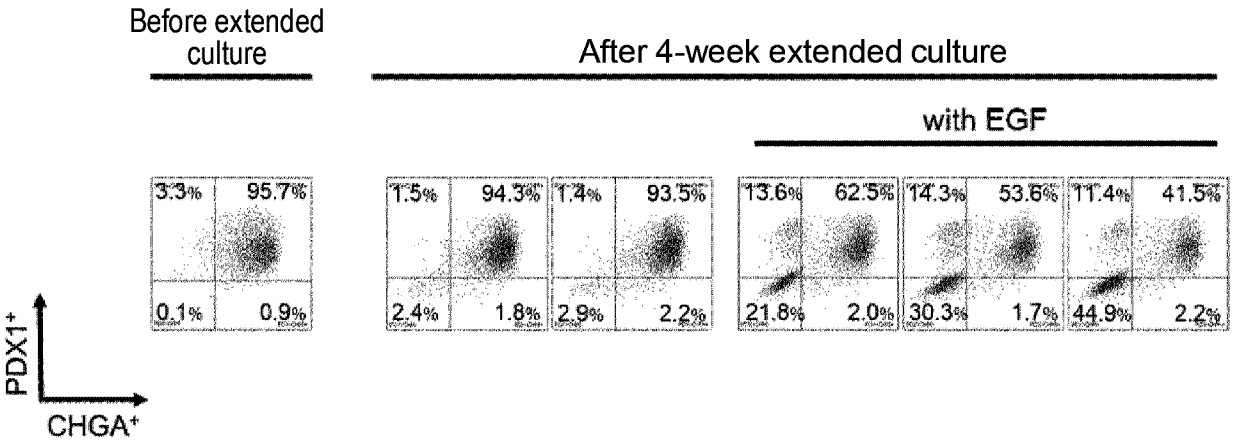
Before extended culture
After 4-week extended culture
with EGF
3.3% 95.7% 0.1% 0.9%
1.5% 94.3% 2.4% 1.8%
1.4% 93.5% 2.9% 2.2%
13.6% 62.5% 21.8% 2.0%
14.3% 53.6% 30.3% 1.7%
11.4% 41.5% 44.9% 2.2%
PDX1+
CHGA+

FIG. 2

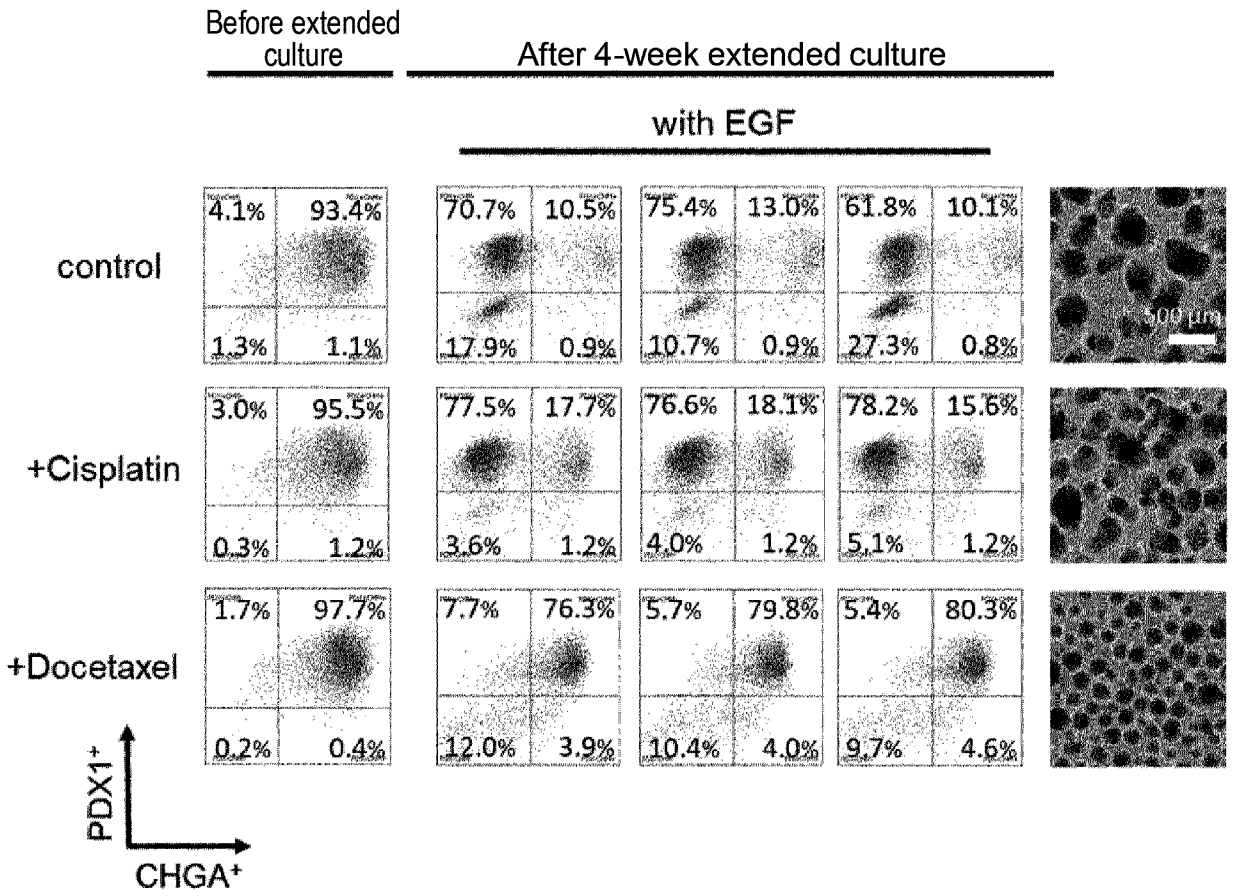
Before extended culture
After 4-week extended culture
with EGF
control
+Cisplatin
+Docetaxel
4.1% 93.4% 1.3% 1.1%
70.7% 10.5% 17.9% 0.9%
75.4% 13.0% 10.7% 0.9%
61.8% 10.1% 27.3% 0.8%
3.0% 95.5% 0.3% 1.2%
77.5% 17.7% 3.6% 1.2%
76.6% 18.1% 4.0% 1.2%
78.2% 15.6% 5.1% 1.2%
1.7% 97.7% 0.2% 0.4%
7.7% 76.3% 12.0% 3.9%
5.7% 79.8% 10.4% 4.0%
5.4% 80.3% 9.7% 4.6%
500 µm
PDX1+
CHGA+

FIG. 3

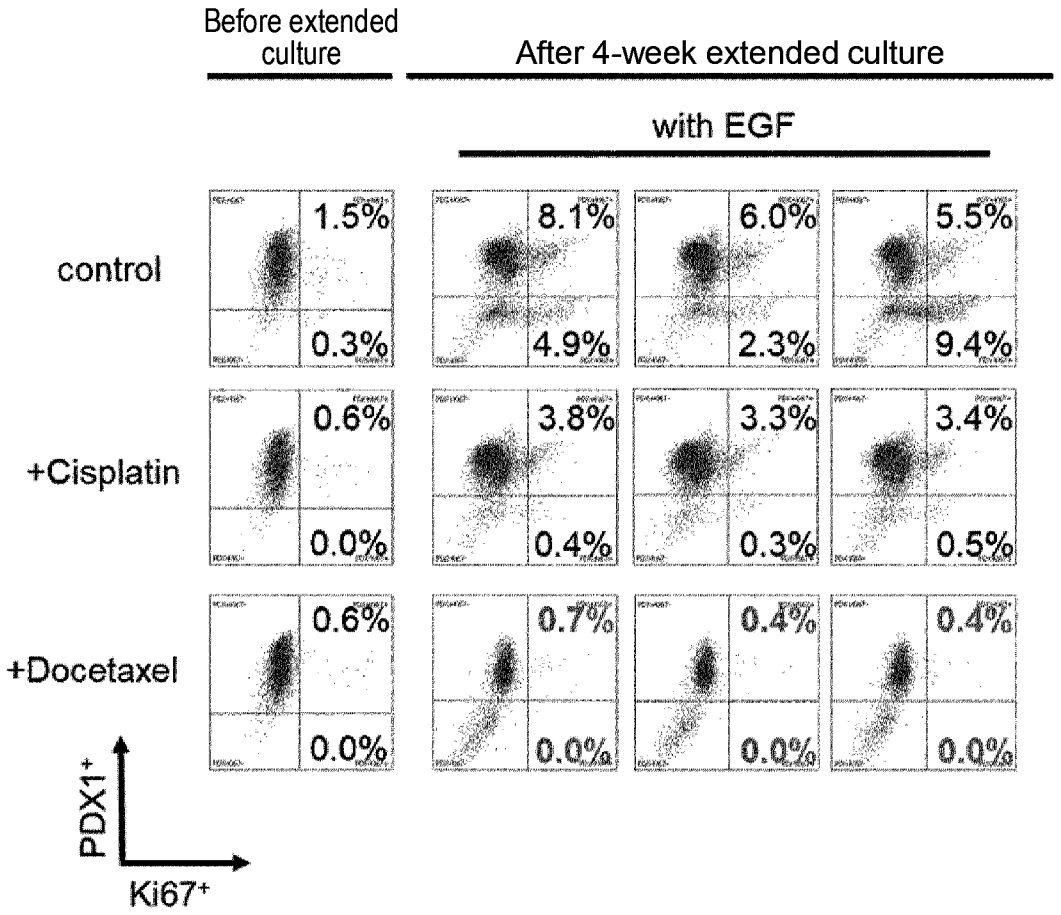

FIG. 4

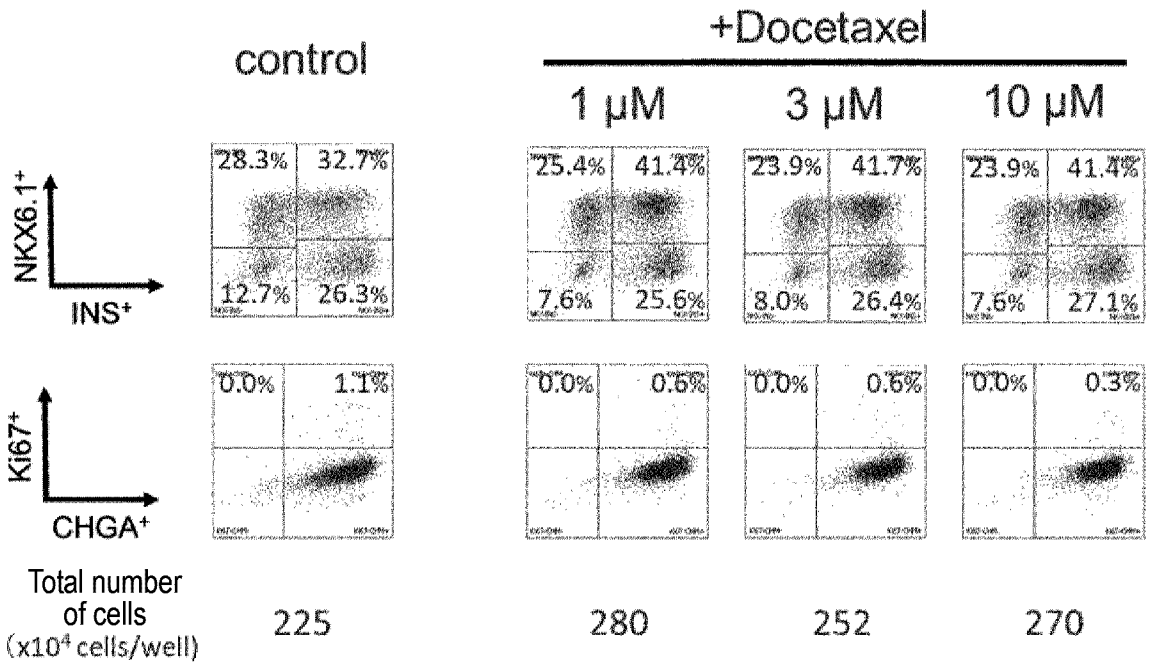

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FIG. 5

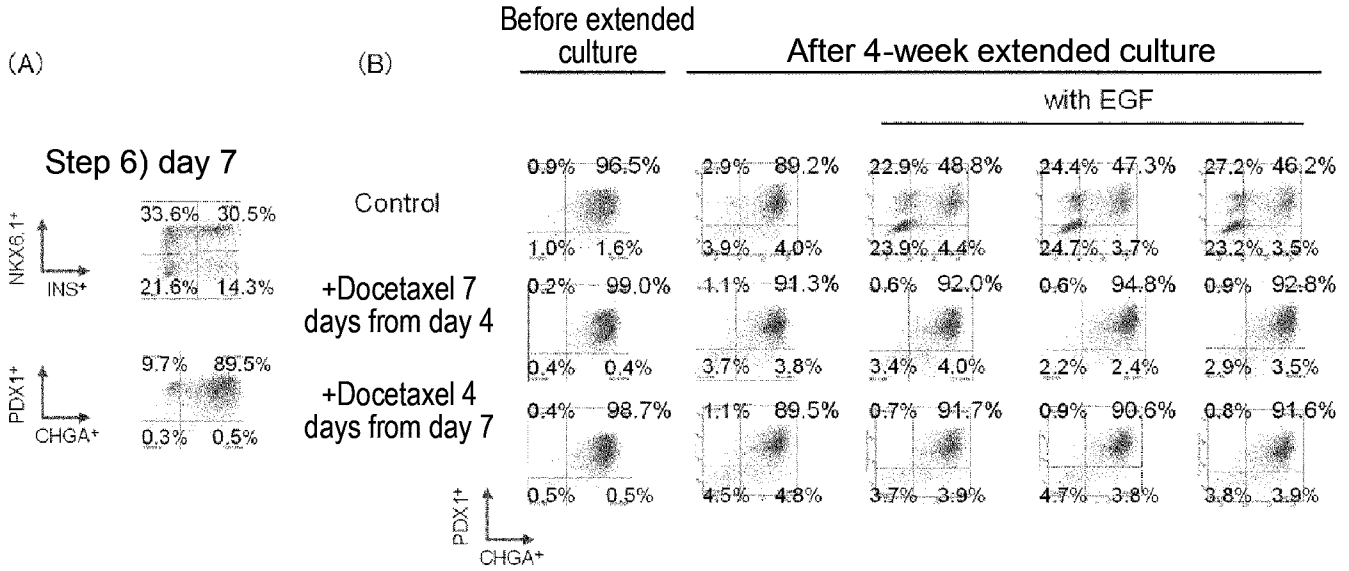
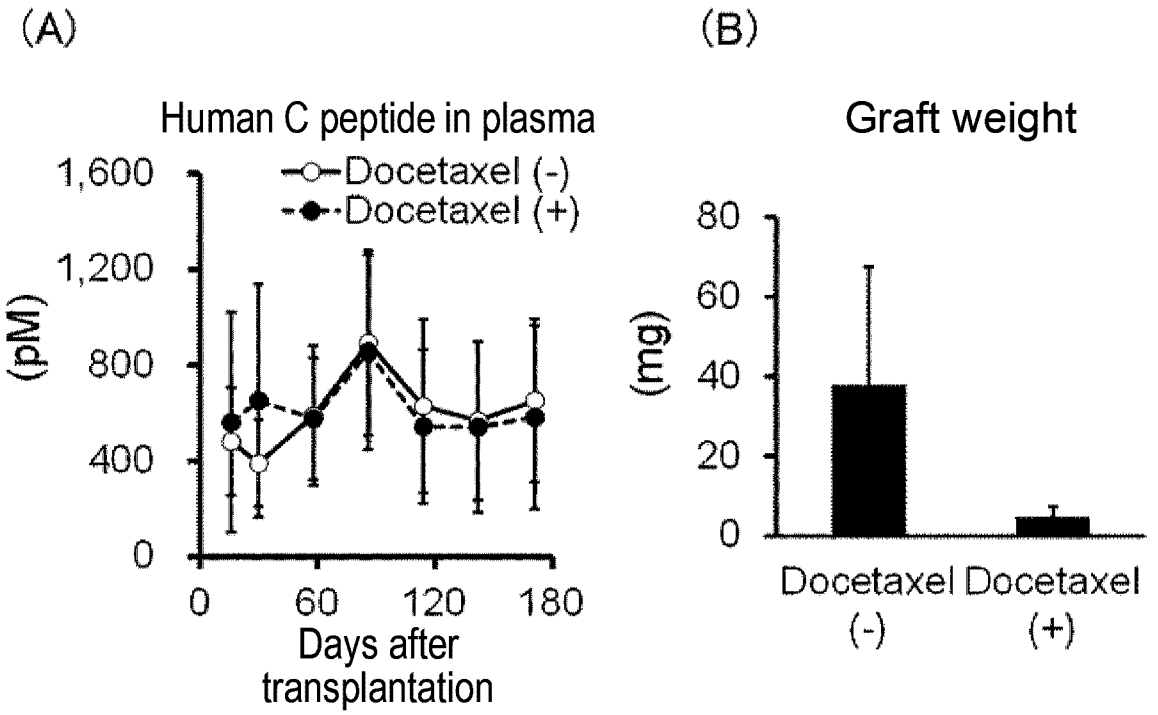


FIG. 6



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